# International Meeting of the Microbiological Society of Korea

# 10.30 (Sun) -11.1 (Tue), 2022

2022

# ICCJEJU, JEJU, Korea

#### Hosted by

• The Microbiological Society of Korea

#### Co-organized by

- CNU Regional Leading Research Center for Microbiome-Brain Disorders
- Global Research Collaboration for Infectious Disease Preparedness KOREA / KRIBB
- Korean Collection for Type Cultures
- Science Research Center for Microbial Survival Systems
- Rznomics Inc.

#### Sponsored by

- Cell Biotech
- CJ Bioscience
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Choy BioLab (CBL) was founded by the distinguished professor Hyonil Choy in Medicine at Chonnam Natl' University, an established salmonella biologist. CBL has modified an avian pathogen, Salmonella gallinarum, for treating tumors without inducing fatal septic shock. The salmonella has been mutated such that expressions of all those genes conferring virulence phenotype encoded in Salmonella Pathogenicity Islands are blocked. The mutated salmonella has been shown to reduce tumor growth in mice by more than 90 percent when injected into the blood stream. The suppression of tumors is presumably by inducing host immunity although the mechanism in detail has yet to be elucidated.

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#### The Microbiological Society of Korea

Rm. 810, The Korea Science Technology Center The first building 22, Teheran-ro 7-gil, Gangnam-gu, Seoul 06130, Republic of Korea E-mail: msk@msk.or.kr, Tel: +82-2-3453-3321/86, Fax: +82-2-3453-3322



# 2022 MSK Organizing Committee

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	Wooseong Kim	(Ewila womans only:)				
Public Affairs C	-		Committee for	Future MSK		
	-	(Kangwon Nat'l Univ.)	Committee for Chair	Jae-Ouk Kim	(IVI)	
<b>Public Affairs C</b> Chair Secretaries	ommittee				(IVI) (Jeonbuk Nat'l Univ.) (KRIBB)	
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KRIBB : Korea Research Institute of Bioscience and Biotechnology POSTECH : Pohang University of Science and Technology KAERI : Korea Atomic Energy Research Institute NNIBR : Nakdonggang National Institute of Biological Resources WIKIM : World Institute of Kimchi

### General Information

#### Registration

Place: ICCJEJU 3rd Floor Lobby Registration fee:

						(KRW)
		Member Non-Mem		nber		
		Regular	Student	Regular	Student	Undergraduate
Early Registration		160,000	90,000	240,000	110,000	40,000
On-Site Registration		210,000	110,000	290,000	130,000	-
	Utilization of Protein Structure Prediction					
<b>a</b>	R Course - Basic					
Paid- Workshop	Beginner's Guide to the Bacterial Genome Analysis	30,000 (each)				-
workshop	R Course - Advanced					
	Statistics for microbiologist					

### Information for Poster Session

#### **Poster Session**

	Schedule	Topics	Presentation
Session 1	Set-up : October 30, 12:00 Removal: October 31, 11:00	B, D, H	Oct 30 (Sun) 18:00-19:30
Session 2	Set-up : October 31, 13:00 Removal: November 1, 12:00	A, C, E, F, G	Oct 31 (Mon) 17:30-19:00

#### **Poster Topics**

- A. Systematics / 미생물분류
- C. Applied Microbiology / 응용미생물
- E. Physiology and Biochemistry / 생리·생화학
- G. Biotechnology / 생물공학

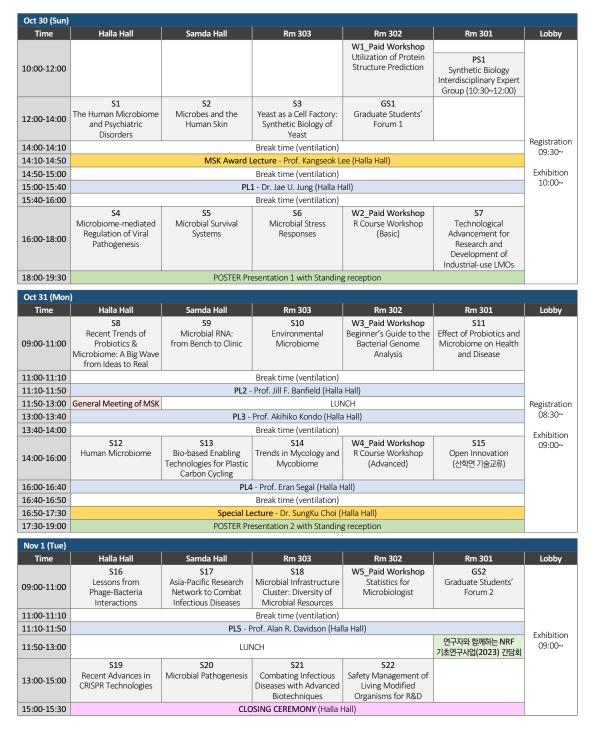
- B. Ecology and Environmental Microbiology / 생태·환경미생물
- D. Immunology and Microbial Pathogenesis / 면역·병원미생물
- F. Genetics / 미생물유전학
- H. Others / 기타

#### **Poster Session Layout**

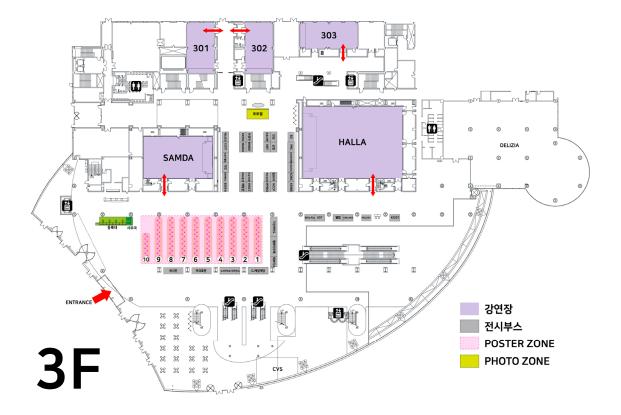
Zone	Poster Session 1	Poster Session 2
1	B001-B032	A001-A032
2	B033-B065	A033-A064
3	B066-B097	A065-A082, C001-C014
4	B098-B116, D001-D014	C015-C046
5	D015-D047	C047-C079
6	D048-D080	C080-C089, E001-E022
7	D081-D113	E023-E040, F001-F014
8	D114-D126, H001-H021	F015-F045, G001
9	H022-H054	G002-G033
10	H055-H072	G035-G045



## I Timetable



### Floor Plans



Halla Hall	MSK Awards Lecture, Plenary Lectures, Special Lecture, Symposia, General Meeting of MSK, Closing Ceremony
Samda Hall	Symposia
Room 301	PS1, Symposia, Graduated Scientists' Forum 2, NRF 간담회
Room 302	Paid Workshops, Graduated Scientists' Forum 1, S22 LMO Session
Room 303	Symposia
3rd Floor Lobby	Registration Desk, Exhibition, Poster



## Scientific Program

#### **Plenary Lectures**



Plenary Lecture 1	Oct 30 (Sun), Halla Hall
Chair: Jin H	yun Ahn (Sungkyunkwan University School of Medicine)
15:00-15:40	
Immunological Sequelae of SARS-CoV-2 Pregna	ancy Infection and Long COVID-19
Jae U. Jung (Cleveland Clinic, USA)	
Plenary Lecture 2	Oct 31 (Mon), Halla Hall
	Chair: Jong-Chan Chae (Jeonbuk National University)
11:10-11:50	
Metagenomic Inference of Acquisition Paths for	or Novel Members of Human Microbiomes
Jill F. Banfield (University of California Berkeley, U	SA)
Plenary Lecture 3	Oct 31 (Mon), Halla Hall
	Chair: Sunghoon Park (UNIST)
13:00-13:40	
Bio-digital Fusion to Establish Biofoundry Tech	nology and Accelerate Cell Factory Development
Akihiko Kondo (Kobe University, Japan)	



PL3

Chair: Heenam Kim (Korea University) 16:00-16:40 Personalized Medicine Based on Deep Human Phenotyping

Oct 31 (Mon), Halla Hall

Nov 1 (Tue), Halla Hall

Eran Segal (Weizmann Institute of Science, Israel)

**Plenary Lecture 4** 

**Plenary Lecture 5** 



Sponsored by CHA University Laboratory of BioantibacterialsChair: You-Hee Cho (CHA University)11:10-11:50The Co-evolution of Anti-CRISPRs and CRISPR-Cas SystemsAlan R. Davidson (University of Toronto, Canada)

Oct 30 (Sun), Halla Hall

*Chair:* Kyu-Ho Lee (Sogang University)

#### MSK Award Lecture

#### AL

#### MSK Award Lecture



14:10-14:50 **Determinants of RNA Function** Kangseok Lee (Chung-Ang University)

#### **Special Lecture**



#### Special Lecture

Oct 31 (Mon), Halla Hall

Chair: GwangPyo Ko (Seoul National University)

16:50-17:30

The Evolution and Progress of the Translational Research and Its Implication in the Clinical Development of the New Therapeutic Agents SungKu Choi (Ildong Pharmaceutical Co.)

#### Workshops



**Utilization of Protein Structure Prediction** 

Oct 30 (Sun), Rm 302

Oct 30 (Sun), Rm 302

단백질 구조 예측 활용

Nam-Chul Ha (Seoul National University)



R Course Workshop (Basic)

#### 16:00-18:00

R을 활용한 미생물 분석 기초: 자료 가공, ggplot, t-test, ANOVA, 상관관계 Tae Kwon Lee (Yonsei University)



W3	Beginner's Guide to the Bacterial Genome Analysis	Oct 31 (Mon), Rm 302
	09:00-11:00	
	초보자를 위한 미생물유전체 분석법	
Y	Sang-Cheol Park (Myongi Hospital)	
W4	R Course Workshop (Advanced)	Oct 31 (Mon), Rm 302
JEAN N	14:00-16:00	
	R을 활용한 미생물 분석 응용	
00	: Clustering (PCA, NMDS), Classification (Decision tree, RandomForest), I	Network 분석
	Tae Kwon Lee (Yonsei University)	
W5	Statistics for Microbiologist	Nov 1 (Tue), Rm 302
	09:00-11:00	
	미생물 학자를 위한 기초 통계 분석	



미생물 학자를 위한 기초 통계 분석 Heejin Jin (Seoul National University)

### NRF 기초연구사업 간담회

#### RT

#### 연구자와 함께하는 NRF 기초연구사업(2023) 간담회

Nov 1 (Tue), Rm 301

Chair: 박숙미 (한국연구재단 기초연구기획실장)

12:00-12:05 간담회 안내 박숙미 (기초연구기획실장)

12:05-12:35 기초연구사업 운영방안 및 기타 안내 김정윤 (기초연구본부장)

12:35-12:55 의견수렴 및 질의응답

12:55-13:00 마무리

Oct 30 (Sun), Rm 301

#### Symposium

PS1

#### Synthetic Biology Interdisciplinary Expert Group / 합성생물학 다학제 전문가회의

Sponsored by Korea Biosafety Clearing House and Dept. of Science Studies, Seoul National University Chair: Gi Cheol Kim (KRIBB KBCH)



PS1-1 10:30-10:55 Issues of Synthetic Biology in CBD and Domestic Discussion Hoon-Gi Kim (Hongik University)



PS1-2 10:55-11:20 Riskscapes of Biotechnology: Case of Synthetic Biology Taemin Woo (Korea Biosafety Clearing House)



PS1-3 11:20-11:45 How to Make Science Research Outcomes to Readable Stories Young-wan Lee [Korea Science Journalists Association (The Chosun Biz)]

패널토의 11:45-12:00 Bong Hyun Sung (KRIBB) Hong-Tak Lim (Seoul National University)



The Human Microbiome and Psychiatric Disorders

Oct 30 (Sun), Halla Hall

Co-organized by CNU Regional Leading Research Center for Microbiome-Brain Disorders

Chair: Min-Soo Kim (Chungnam National University)



Modulatory Roles of Maternal Gut Bacteria in Shaping Offspring's Immune Responses and Neurodevelopmental Phenotypes

Jun R. Huh (Harvard Medical School, USA)

**S1-1** 12:00-12:30





S1-2 12:30-13:00Successful Microbiota Transfer Therapy to Modulate Gut Microbiome and Treat Autism SymptomsDae-Wook Kang (The University of Toledo, USA)



S1-3 13:00-13:30GABA-modulating Bacteria of the Human Gut MicrobiotaKi Hyun Kim (Sungkyunkwan University)



S1-4 13:30-14:00 Human Reference Gut Microbiome to Explore Human Microbial Dark Matter Insuk Lee (Yonsei University)

Co-organized by Skin-Bio Research Cluster, Microbiomics Institute - Chung-Ang University



#### Microbes and the Human Skin

Oct 30 (Sun), Samda Hall

Chair: Woo Jun Sul (Chung-Ang University)



S2-1 12:00-12:30 From Metagenomes to Therapeutics: the Human Skin Microbiome Julia Oh (The Jackson Laboratory, USA)



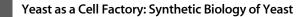
**S2-2** 12:30-13:00 Skin Microbiome Signatures in Atopic Dermatitis John Common (A\*STAR Skin Research Labs, Singapore)



S2-3 13:00-13:30 Elucidating the Molecular Functions of Skin Fungal Secretory Proteases Hao Li (National University of Singapore, Singapore)



S2-4 13:30-14:00 Commensal Microbiota Regulate Regeneration in Damaged Skin Dongwon Kim (Dongseo University)



Oct 30 (Sun), Rm 303

Chair: Eun Ju Yun (Jeonbuk National University)



**S**3

S3-1 12:00-12:25
 Metabolic Engineering of Non-conventional Yeast to Produce Biofuels and Chemicals
 Yong-Su Jin (University of Illinois at Urbana-Champaign, USA)



S3-2 12:25-12:50
Metabolic Engineering of Yeasts for Production of Mycosporine-like Amino Acids, Natural Sunscreen Materials
Ji-Sook Hahn (Seoul National University)



S3-3 12:50-13:15 Yeast Engineering for Fermenting Pectin Sugars Derived from Citrus Waste Soo Rin Kim (Kyungpook National University)

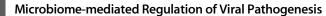


S3-4 13:15-13:40Production of Key Ingredients of Alternative Proteins by Engineered Saccharomyces cerevisiaeSun-Ki Kim (Chung-Ang University)



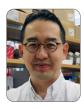
S3-5 13:40-14:00 Gene Regulation by Nuclear Enzyme Aconitase 2 in *Schizosaccharomyces pombe* Soo-Yeon Cho (Chung-Ang University)





Oct 30 (Sun), Halla Hall

Chair: Jinjong Myoung (Jeonbuk National University)



**S4** 

Microbiome Mediated Trained Immunity in Neutrophil Minsoo Kim (University of Rochester Medical Center, USA)

S4-1 16:00-16:30



S4-2 16:30-17:00 Airway Infection Resistance Induced by a Nasal Microbiome Sang Sun Yoon (Yonsei University)



S4-3 17:00-17:30 Gut Microbiota and an Associated Leaky Gut May Affect COVID-19 Severity Heenam Kim (Korea University)



S4-4 17:30-17:50 The Critical Impacts of Tissue-resident T Cells in Long-term Immunity Following the Respiratory Viral Infection

Youngmin Son (Chung-Ang University)

**S**5

#### **Microbial Survival Systems**

Oct 30 (Sun), Samda Hall

Co-organized by Science Research Center for Microbial Survival Systems

Chair: Hyun Ah Kang (Chung-Ang University)



S5-1 16:00-16:25 HPr Prevents FruR-mediated Facilitation of RNA Polymerase Binding to the *fru* Promoter in *Vibrio cholerae* Seung Hwan Lee (Seoul National University)



S5-2 16:25-16:50Mechanism and Regulation of Homologous Recombination and Gene ConversionKeun Pil Kim (Chung-Ang University)

Chair: Che Ok Jeon (Chung-Ang University)



S5-3 16:50-17:15 Heme Auxotrophy in the Most Abundant Freshwater Bacterioplankton Lineage Suhyun Kim (Inha University)



S5-4 17:15-17:40 Arginine-mediated Gut Microbiome Remodeling Promotes Host Pulmonary Immune Defense against Nontuberculous Mycobacterial Infection June-Young Lee (Kyung Hee University)



S5-5 17:40-18:05 CRISPR Diagnosis and Therapeutics with High Accuracy Seung Hwan Lee (Chung-Ang University)

**S6** 

**Microbial Stress Responses** 

Oct 30 (Sun), Rm 303

Co-organized by Chung-Ang University Research Center for Biomolecules and Biosystems (RCBB) Chair: Byoung Sik Kim (Ewha Womans University)



S6-1 16:00-16:25 Chaperone Discovery Changhan Lee (Ajou University)



S6-2 16:25-16:50 Nutrient Starvation Promotes Bacteria Chronic Infection by Regulating Protein Homeostasis Jinki Yeom (Seoul National University)



S6-3 16:50-17:15 The Role of Lipoteichoic Acid in the Beta-lactam Resistance of Methicillin-resistant *Staphylococcus aureus* Taeok Bae (Indiana University School of Medicine-Northwest, USA)





S6-4 17:15-17:40
Secretin-interacting Proteins Prevent an Outer Membrane Permeability Defect during Type IV Pili
Assembly in *Pseudomonas aeruginosa*Hongbaek Cho (Sungkyunkwan University)



S6-5 17:40-18:00Molecular Mechanism of Zinc-dependent Gene Activation by Zur in *Streptomyces coelicolor*Yunchan Choi (Seoul National University)

**S7** 

#### Technological Advancement for Research and Development of Industrial-use LMOs

Oct 30 (Sun), Rm 301

Sponsored by Korea Evaluation Institute of Industrial Technology & Ministry of Trade, Industry and Energy Chair: Gi Cheol Kim (KRIBB KBCH) S7-1 16:00-16:40



Development of Biosafe *Geobacillus* Platform Cells and Enzyme Production Technology for Industrial Uses

Seong-Bo Kim (Yonsei University)



S7-2 16:40-17:20 Strategic Development of Plant Cell-based Expression System for Anti-aging Related Human Growth Factors

Sang Hyun Moh (BIO-FD&C Co., Ltd.)



S7-3 17:20-18:00 Overproduction of Cyclic Dipeptides Which Shows Anti-viral, Anti-fungal, and Anti-bacterial Activities in Yeast

Phun Bum Park (The University of Suwon)

#### **S8**

#### Recent Trends of Probiotics & Microbiome: A Big Wave from Ideas to Real

Oct 31 (Mon), Halla Hall



Sponsored by ILDONG Pharmaceutical Co., Ltd. Chair: Soo Rin Kim (Kyungpook National University) & Dae-Hee Lee (KRIBB) 58-1 09:00-09:30 Commercial Application of Probiotics Isolated in Korea Jungwoo Yang (Ildong Bioscience)



S8-2 09:30-10:00Gut Microbiota Manipulation in Irritable Bowel Syndrome: a Clinician's ViewCheol Min Shin (Seoul National University Bundang Hospital)



S8-3 10:00-10:30
 Cas-sgRNA-Ribonucleoprotein Mediated Genome Engineering in *Leuconostoc citreum* Nam Soo Han (Chungbuk National University)



S8-4 10:30-11:00
Validation of Probiotic Species Identification in Probiotic Products Using Real-time PCR Method Based on Large-scale Genomic Analysis
Hae-Yeong Kim (Kyung Hee University)

**S9** 

Microbial RNA: from Bench to Clinic

Co-organized and Sponsored by Rznomics Inc.



S9-1 09:00-09:30High-resolution Analysis of Human Coronavirus TranscriptomesHyeshik Chang (Seoul National University)

Oct 31 (Mon), Samda Hall

Chair: Donghyuk Kim (UNIST)





S9-2 09:30-10:00Cell-free Systems for Onsite Detection of Viral RNAs and Rapid Discrimination of SNPsJeong Wook Lee (Pohang University of Science and Technology)



S9-3 10:00-10:20
 Initiation tRNA<sup>fMet</sup> Cleavage by Toxin-antitoxin System: Survival Mechanism of Extremophile under
 Extreme Cold Condition
 Eunsil Choi (Pusan National University)



S9-4 10:20-10:50 Development of RNA Editing Therapeutics Based on Tetrahymena Group I Intron Seong-Wook Lee (Dankook University / Rznomics Inc.)

S10

#### **Environmental Microbiome**

Oct 31 (Mon), Rm 303

Chair: Hyo Jung Lee (Kunsan National University)



S10-1 09:00-09:25 Investigation of the Temporal Variation of Bacterial Colonization and Antibiotic Resistance in Microplastic on Marine Environment Keunje Yoo (Korea Maritime and Ocean University)

Co-organized by Skin-Bio Research Cluster, Microbiomics Institute - Chung-Ang University

S10-2 09:25-09:50 Identification and Application of Bacterial and Fungal Denitrifiers to Remove Nitrate from Agricultural Subsurface Drainage

Satoshi Ishii (University of Minnesota, USA)



S10-3 09:50-10:15 Genomic Adaptation to Extreme Environments in Thermoacidophilic Red Algae Hwan Su Yoon (Sungkyunkwan University)



S10-4 10:15-10:40 Unravelling Drivers of Pathogen Emergence: from Selfish Elements to Chromosomes Daniel Croll (University of Neuchâtel, Switzerland)



S10-5 10:40-11:05 *Cutibacterium acnes* in the Gut of the Marine Polychaete *Capitella teleta* Degrading Polycyclic Aromatic Hydrocarbons Jeonghwan Jang (Jeonbuk National University)

S11

#### Effect of Probiotics and Microbiome on Health and Disease Oct 31 (Mon), Rm 301

Chair: Chul Ahn (Cell Biotech) & Ju-Hoon Lee (Seoul National University)

Sponsored by Cell Biotech



S11-1 09:00-09:30 Human Intestinal Organoid as a Platform for Microbiome Research Mi-Young Son (KRIBB)



S11-2 09:30-10:00 ALS Filament Formation by ROS-induced SOD1 and its Inhibition by Tannins Nam-Chul Ha (Seoul National University)



S11-3 10:00-10:30 Effect of Probiotics-produced Bacteriocin on Skin Microbiome of Acne Patients Woo Jun Sul (Chung-Ang University)



S11-4 10:30-11:00 Anti-cancer Role of Probiotic-derived P8 Protein in Colorectal Cancer Byung Chull An (Cell Biotech)



#### S12 Human Microbiome

Oct 31 (Mon), Halla Hall

Chair: Jin-Woo Bae (Kyung Hee University)

S12-1 14:00-14:30 Dynamics in the Human Gut Microbiome Lisa M. Olsson (University of Gothenburg, Sweden)



S12-2 14:30-15:00 *Faecalibacterium prausnitzii* Pathobionts Play a Pivotal Role in the Onset of Atopic Dermatitis Heenam Kim (Korea University)



S12-3 15:00-15:30 Exploring the Association between Microbiome and Asthma Hana Yi (Korea University)



S12-4 15:30-16:00Microbiota-induced Vitamin A Transport and Its Role in the Intestinal ImmunityYe-Ji Bang (Seoul National University College of Medicine)

S13

Bio-based Enabling Technologies for Plastic Carbon Cycling

Oct 31 (Mon), Samda Hall

Chair: Donghyuk Kim (UNIST)

Sponsored by CJ Cheiljedang Corp. / Co-organized by UNIST



S13-1\_Keynote Speaker 14:00-14:45 Biological Production of 3-Hydroxypropionic Acid Sunghoon Park (UNIST)



S13-2 14:45-15:10Biological PET Decomposition; Mechanism, Protein Engineering, and ApplicationsKyung-Jin Kim (Kyungpook National University)

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S13-3 15:10-15:35Biosynthesis of Bio-degradable Plastics by Metabolically Engineered MicroorganismsSi Jae Park (Ewha Womans University)



S13-4 15:35-16:00Discovery and Development of Plastic Biodegradable BiocatalystSoo-Jin Yeom (Chonnam National University)

#### Trends in Mycology and Mycobiome

Oct 31 (Mon), Rm 303

Chair: Hee-Soo Park (Kyungpook National University)



**S14** 

**S14-1** 14:00-14:25 Differential Mycobiome Dysbiosis in CD, UC, and IBS Soo Chan Lee (The University of Texas at San Antonio, USA)



S14-2 14:25-14:50 Roles of Protein Glycosylation in Host Cell Interactions for the Pathogenicity of *Cryptococcus neoformans* Hyun Ah Kang (Chung-Ang University)



S14-3 14:50-15:15 Pathobiological Signaling Circuits of Pan-Drug-Resistant *Candida auris* Yong-Sun Bahn (Yonsei University)



S14-4 15:15-15:40
Convergence of Distinct Secondary Metabolite Biosynthetic Gene Clusters Driving Chemical Innovation in Fungi
Wonyong Kim (Sunchon National University)





#### S14-5 15:40-16:00

Novel Nuclear Localization Sequence of MoHTR1, a Nuclear Effector of the Rice Blast Fungus, is Essential for Translocation to Rice Nucleus and Transcriptional Reprograming of Host Genes You-Jin Lim (Seoul National University)

#### S15 Open Innovation (산학연 기술교류)

Oct 31 (Mon), Rm 301

Chair: Sanghyun Lim (Cell Biotech) & Hyunjin Yoon (Ajou University) S15-1 14:00-14:15 송아지 분변으로부터 분리된 균주를 활용한 설사증 예방 및 완화 제제 박진호 (전북대학교)

S15-2 14:15-14:30 박테리오파지를 이용한 항생제 김민수 (주식회사라이센텍)

S15-3 14:30-14:45 황색포도상구균(MRSA)의 항생제 감수성 신속 진단 장수진 (한국파스퇴르연구소)

S15-4 14:45-15:00 예쁜꼬마선충 감염모델 기반 광범위 세균감염치료물질 발굴법 김우성 (이화여자대학교)

S15-5 15:00-15:15
버섯 유래 멜라닌 탈색 효소를 이용한 피부 미백 기술
전숭종 (동의대학교)

**S15-6** 15:15-15:30 피부미용개선 소재탐색 및 피부마이크로바이옴 조절기술 신학동, 임태규 (세종대학교)

 S15-7
 15:30-15:45

 건강기능성 및 질병 개선 프로바이오틱스 소재화

 최학종 (세계김치연구소)

S15-8 15:45-16:00 유전자변형미생물 LMO 안전성 평가 및 심사 김성보 (연세대학교)

#### S16

#### Lessons from Phage-Bacteria Interactions

Nov 1 (Tue), Halla Hall

Sponsored by CHA University Laboratory of Bioantibacterials

Chair: Alan R. Davidson (University of Toronto, Canada)



S16-1 09:00-09:25 New Mechanisms of Anti-phage Defense Rotem Sorek (Weizmann Institute of Science, Israel)



S16-2 09:25-09:50 How *Pseudomonas aeruginosa* Bacteriophages Evade CRISPR-mediated Demise Joseph Bondy-Denomy (University of California, USA)



**S16-3** 09:50-10:15 Quorum Sensing Anti-activation in the Phage-host Evolutionary Arms Race Karen L. Maxwell (University of Toronto, Canada)



S16-4 10:15-10:35 A Novel Bacterial Determinant for RNA Phage Entry in *Pseudomonas aeruginosa* Hee-Won Bae (CHA University)



**S16-5** 10:35-10:55 *In Silico* Experiments for CRISPR-based Antimicrobials Hyunjin Shim (Ghent University Global Campus)

S17

Asia-Pacific Research Network to Combat Infectious Diseases

Nov 1 (Tue), Samda Hall

Co-organized by Global Research Collaboration for Infectious Disease Preparedness KOREA / KRIBB Chair: Choong-Min Ryu (KRIBB)



S17-1 09:00-09:30

R&D Policy of Ministry of Science and ICT to Strengthen Infectious Disease Response Capabilities Tae-ho Lee (Ministry of Science and ICT)





S17-2 09:30-10:00 Asia-Pacific Infectious Disease Shield: A GloPID-R Regional Hub Project Choong-Min Ryu (KRIBB)



S17-3 10:00-10:30
 International Collaboration of TIDCL (Tropical Infectious Diseases Cooperation Laboratory)
 Ho-Joon Shin (Ajou University School of Medicine)



S17-4 10:30-11:00 Facilitating R&D Based International Network to Tackle Antibiotic Resistance Soojin Jang (Institut Pasteur Korea)



#### Microbial Infrastructure Cluster: Diversity of Microbial Resources Nov 1 (Tue), Rm 303

Co-organized by Korean Collection for Type Cultures

Chair: Song-gun Kim (KRIBB)

#### 09:00-09:10

**Project Introduction: Infrastructure Cluster of Biological Resources** Younghye Kim (Ministry of Science and ICT)



S18-1 09:10-09:32 New Paradigm on Microbial Resources: Plant Microbiome

Yong-Hwan Lee (Seoul National University)



S18-2 09:32-09:54Exploring Fungal Diversity and Their Potential Applications in BioindustriesHyang Burm Lee (Chonnam National University)

# International Meeting of the **M**icrobiological **S**ociety of **K**orea



**S18-3** 09:54-10:16 Korean Culture Collection of Microalgae, and Toxin-producing Dinoflagellates Hyeon Ho Shin (KIOST)



S18-4 10:16-10:38 Establishment of a Public Probiotics Bank and Case Studies for Supporting Industrialization Doo-Sang Park (KRIBB)



S18-5 10:38-11:00 Function of Novel Korean Gut Bacteria in Human Disease Ji-Sun Kim (KRIBB)



#### **Recent Advances in CRISPR Technologies**

S19-1 13:00-13:30

Nov 1 (Tue), Halla Hall

Sponsored by the Korea Research Institute of Bioscience and Biotechnology (KRIBB), which carries out the Korea Bio Grand Challenge Project of the National Research Foundation (NRF) funded by the Ministry of Science and ICT of the Republic of Korea

Chair: Dae-Hee Lee (KRIBB) & Sang Jun Lee (Chung-Ang University)



Systems and Synthetic Biology: Constructing Smart and Programmable Microbes to Address Global Problems

Tae Seok Moon (Washington University in St. Louis, USA)



S19-2 13:30-14:00 CRISPR-Mediated Microbial Genome Editing and New Target Finding Sang Jun Lee (Chung-Ang University)



S19-3 14:00-14:30 CRISPR-based DNA Recording System for Gut Inflammation Dae-Hee Lee (KRIBB)





S19-4 14:30-14:50 Efficient CRISPR Editing with a Hypercompact Cas12f1 and Engineered Guide RNAs Delivered by Adeno-associated Virus Jeong Mi Lee (KRIBB)

#### S20 Microbial Pathogenesis

Nov 1 (Tue), Samda Hall

Chair: Jang Won Yoon (Kangwon National University)



S20-1 13:00-13:25 Modulation of Pathogenicity by Endoribonucleases and Their Regulators in Pathogenic Bacteria Minho Lee (Hallym University)



**S20-2** 13:25-13:50 Bacterial Toxins during Host Infection Eun-Jin Lee (Korea University)



S20-3 13:50-14:15
 A Bacterial Strain Saves Microbial Community: Colistin-degrading Proteases Confer Collective Resistance in Polymicrobial Infection Communities
 Chang-Jun Cha (Chung-Ang University)



S20-4 14:15-14:40 Clostridial Toxin-medicated Gut Inflammation and the Association with Gut Microbiota Eun-Jeong Yoon (Korea Disease Control and Prevention Agency)



S20-5 14:40-15:05 Saccharomyces cerevisiae: A Sexy Yeast with A Prion Problem Moonil Son (Pusan National University)

#### S21

#### Combating Infectious Diseases with Advanced Biotechniques Nov 1 (Tue), Rm 303

Chair: Myung-Ji Seo (Incheon National University)



S21-1 13:00-13:25 Emulating Host-Microbiome Crosstalk in a Microphysiological Human Gut-on-a-chip Hyun Jung Kim (Cleveland Clinic, USA)



S21-2 13:25-13:50 Development of High-throughput Screening Technique for Microbiome Using MALDI-TOF Mass Spectrometry Kun Cho (KBSI)



# S21-3 13:50-14:15

Direct and Rapid Antimicrobial Susceptibility Testing (dRAST) Using Artificial Intelligence to Accelerate Therapeutic Decisions for Blood Stream Infection Sunghoon Kwon (Seoul National University)



S21-4 14:15-14:40 SARS-CoV-2 Aberrantly Elevates Mitochondrial Bioenergetics to Induce Robust Virus Propagation Seong-Jun Kim (Korea Research Institute of Chemical Technology)



S21-5 14:40-15:05 Self-Amplifying mRNA (SAM): Next Generation Expression Platform and Its Application Dal Young Kim (Winstabio Inc.)

**S22** 

#### Safety Management of Living Modified Organisms for R&D

Nov 1 (Tue), Rm 302

Sponsored by KRIBB National Research Safety Headquarters

*Chair:* Taegun Seo (Dongguk University)



S22-1 13:00-14:00 Laws and Regulations for Safety Management of LMOs for R&D Yong ik Kwon (KRIBB NRSH)





S22-2 14:00-15:00 The Method of R&D LMO Safety Management Seung Chul Shin (KRIBB NRSH)

#### **Graduate Students' Forum**

#### GS1 Graduate Students' Forum 1 Oct 30 (Sun), Rm 302 Chair: Moo-seung Lee (KRIBB) & Jang Won Yoon (Kangwon National University) GS1-1 12:00-12:07



Enhancement of the Solubility of Recombinant Proteins by Fusion with a Short-disordered Peptide Suhee Hwang (Chung-Ang University)



GS1-2 12:07-12:14 Genomics and Transcriptomics of Laboratory-evolved Multidrug-resistant Acinetobacter baumannii under Nutritional Stresses Sohyeon Yun (Korea University)



GS1-3 12:14-12:21 A Novel Spore-specific Transcription Factor is Essential for Conidial Maturation and Dormancy in Aspergillus Species Ye-Eun Son (Kyungpook National University)



GS1-4 12:21-12:28 Study of Microbial Carotenoid: A Focus on the Optimization of Bacterioruberin Production and Its **Antioxidant Properties** Chi Young Hwang (Incheon National University)



GS1-5 12:28-12:35 Persistence of Antibiotic Resistance from Agricultural Effluents to Surface Water Revealed by Genome-centric Metagenomics Jin Ju Kim (Chung-Ang University)

# International Meeting of the **M**icrobiological **S**ociety of **K**orea



GS1-6 12:35-12:42 Functional Characterization of 4- $\alpha$ -Glucanotransferase and  $\alpha$ -Amylolytic Enzyme from Hyperthermophilic *Fervidobacterium islandicum* AW-1 Sondor Ganbat (Silla University)



GS1-7 12:42-12:49 Enhancement of Anti-inflammatory and Anti-osteoporosis Effects of Fermented *Abelmoschus Manihot* L. by *Bacillus licheniformis* CP6 Min Kyeong Kim (Silla University)



GS1-8 12:49-12:56 Antimicrobial Spectrum and Characterization of Purified Recombinant Micro Halocin HB384 Derived from Halophiles Bo Hyeon Park (Silla University)



GS1-9 12:56-13:03 Identification of Imidazole Propionate Producing Lactobacillus Bacteria in the Human Gut Mahrukh Butt (Chung-Ang University)



GS1-10 13:03-13:10 Characteristics and Biological Activity of Exopolysaccharide Produced by *Lysobacter* sp. MMG2 Isolated from the Roots of *Tagetes patula* Inhyup Kim (Dongguk University-Seoul)



GS1-11 13:10-13:17 The Effect of Plant-derived Biological Nitrification Inhibitors (BNIs) on the Nitrification in Co-culture of Three Different Ammonia-oxidizing Microorganisms Seongwook Kim (Jeju National University)



GS1-12 13:17-13:24 Transmembrane Helix Stability of Lysis Protein in RNA Phage Lifecycle So-Yeon Kim (CHA University)





#### GS1-13 13:24-13:31

Characterizations of Zinc-ion Dependent Phosphatase (YktC1) and Alcohol Dehydrogenase (GutB1) for 1-Deoxynojirimycin Biosynthesis in Bacillus amyloliquefaciens MBLB0692 Hyo Jung Lim (Incheon National University)



GS1-14 13:31-13:38 Characterization of Integral Membrane Protein, CpImp1, of the Chestnut Blight Fungus, Cryphonectria parasitica Jaehyeon Lee (Jeonbuk National University)

GS2

Graduate Students' Forum 2

Chair: Eui Tae Kim (Jeju National University) & Kyoung-Dong Kim (Chung-Ang University) GS2-1 09:00-09:07 Effects of Human Activities and Environmental Factors on Soil Microbiome of Urban Green Space Yerang Yang (Yonsei University)

Nov 1 (Tue), Rm 301



GS2-2 09:07-09:14 Identification of Multiple dsRNA Mycoviruses of Trichoderma polysporum NFCF205 and Their Effects on Antifungal Activities of T. polysporum Hae-ryeong Yoon (Jeonbuk National University)



GS2-3 09:14-09:21 Predatory Bacteria and Violacein as Alternative Antibiotics under Microgravity Hyochan Jang (Ulsan National Institute of Science and Technology)

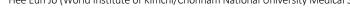


GS2-4 09:21-09:28 BAI22-derived Extracellular Vesicle as a New Synergistic Antibacterial Platform in the Control of **Gram-negative Bacterial Infections** 

Hyejin Cho (Pusan National University)



GS2-5 09:28-09:35 A Prophylactic Effect of Lactic Acid Bacteria on Colorectal Cancer in an Orthotopic Syngeneic Murine Model Hee Eun Jo (World Institute of Kimchi/Chonnam National University Medical School)





GS2-6 09:35-09:42 Primary Transcriptome Analysis on Intra-macrophage *Salmonella* Typhimurium Defined a New Role of LeuO in Virulence Regulation Eunsuk Kim (Ajou University)



#### GS2-7 09:42-09:49

Taxonomic and Functional Diversity of the Chemo-autotrophic Microbiome in the *Pillucina pisidium* (Bivalvia: Lucinidae) Occurring on a Seagrass Meadow in Jeju Island Jong-Seop Shin (Jeju National University)



GS2-8 09:49-09:56 Pullulan Nanoparticles Inhibit the Pathogenicity of *Candida albicans* by Regulating Hypha-Related Gene Expression Sujin Hong (Seoul National University)



GS2-9 09:56-10:03 Quorum Sensing Protein VqmA Senses Glucose-induced Host Signals through HPr in Vibrio vulnificus Gahee Park (Seoul National University)



GS2-10 10:03-10:10 Single-cell Classification in Synthetic Community Using Machine Learning Models and Flow Cytometry In Jae Jeong (Yonsei University)



GS2-11 10:10-10:17 Characterization of a *Vibrio vulnificus* Mutant Deficient in Putative *rcsB* Gene Which Only Present in Clinical Genotype (Biotype 1C) Joon-Gi Kwon (Seoul National University)





GS2-12 10:17-10:24 Maternal High-fat Diet Intake Modulates the Gut Microbial Composition of 16-week-old Rat Offspring Soo-Min Kim (Ewha Womans University Mokdong Hospital)



GS2-13 10:24-10:31 The Field-scale Study to Understand the Performances of Dechlorination Bacteria and Functional Genes in a TCE Contaminated Industrial Site Jeongwon Kim (Korea Maritime and Ocean University)



#### GS2-14 10:31-10:38

Unveiling the Mechanism of Bactericidal Activity by an Engineered Endolysin LNT103 Kyungah Park (Ajou University) MSK2022 International Meeting of the Microbiological Society of Korea

# **Plenary Lectures**

#### PL1

# Immunological Sequelae of SARS-CoV-2 Pregnancy Infection and Long COVID-19

#### Jae Ung Jung

Cancer Biology Department, Infection Biology Program, and Global Center for Pathogen and Human Health Research, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, 44195, USA

<u>Pregnancy</u>: While pregnancy increases the risk for severe COVID19, the immunological implications and consequences of how COVID19 affects maternal-fetal health remains unknown. We present the clinical and immunological landscapes of 93 COVID19 mothers and 45 of their SARS-CoV-2-exposed infants through comprehensive serum proteomics profiling for >1400 cytokines of their peripheral and cord blood specimens, compared to 30 healthy pregnant women and 7 control infants. Prenatal SARS-CoV-2 infection triggered NF- $\kappa$ B-dependent proinflammatory immune activation. Pregnant women with severe COVID19 showed an enhanced IFN- $\lambda$  antiviral signaling, potentially preventing vertical transmission. Furthermore, SARS-CoV-2 infection re-shaped maternal immunity at delivery, promoting late pregnancy- and postpartum-related complications. Finally, COVID19-exposed infants exhibited dysregulated neonatal T cell immunity which might adversely affect immunity and neurodevelopment, while gestational COVID19-exposure conceivably promoted inflammasome-driven neonatal respiratory distress. Our findings demonstrate a COVID19-induced immune rewiring in mother and child.

Long COVID19: It has then become evident that persistent symptoms after COVID19 are not restricted to those who were critically ill or hospitalized but can occur in patients who has mild disease and never needed to be hospitalized. This condition is termed as "post-acute sequelae of SARS-CoV-2 infection (PASC)" or "Long COVID19". Specifically, neurological, or neuropsychiatric symptoms commonly observed in long COVID19 patients include headache, fatigue, brain fog and memory loss. Given the number of individuals of all ages who have been or will be infected with SARS-CoV-2, the public health impact of long COVID19 could be profound. The goal of our study is to comprehensively characterize the biological and clinical mechanisms underlying the pathogenesis of long COVID19. Specifically, integrated multi-omics study of healthy (n=10) and COVID19 (n=62; 30 no-long COVID and 32 long COVID19 with chronic headache) showed striking changes of inflammatory cytokines, metabolites, neurotransmitters, and gene expressions.

# Metagenomic Inference of Acquisition Paths for Novel Members of Human Microbiomes

## Alexander Jaffe<sup>1</sup>, Raphael Meheust<sup>2</sup>, and Jillian F. Banfield<sup>2,3,4\*</sup>

<sup>1</sup>Department of Plant and Microbial Biology, <sup>2</sup>Department of Earth and Planetary Science, <sup>3</sup>Department of Environmental Science, Policy and Management, <sup>4</sup>Innovative Genomics Institute, University of California Berkeley, USA

Interest in microbiomes of the human body continues to increase with improving understanding of the impact of specific organisms and overall microbial community composition on health and disease. Genome-resolved metagenomics approaches have uncovered a diversity of unusual microbiome members, including bacteria from rarely encountered lineages, from groups lacking isolated representatives and unusual phages. Where did they come from? Can the answers to this question inform a more general understanding of the process by which human microbiomes were assembled over animal and human evolution? Of particular interest are Elusimicrobia, Melainabacteria, and Candidate Phyla Radiation bacteria from three lineages: Absconditabacteria, Gracilibacteria, and Saccharibacteria. These bacteria are widely distributed across Earth's habitats and presumably colonized the human body from certain of these natural environments. We investigated migration events and the associated modification of protein inventories and other genomic characteristics that may have been required to overcome potential environmental barriers and allow adaptation to new physiochemical conditions of human body sites. We find bacteria affiliated with several distinct lineages within most of these bacterial phyla underwent habitat transitions into the human body, and all were likely derived from groundwater. Colonization was apparently followed by genome size reduction and gradual, rather than sudden, loss of metabolic versatility. Related groups of alternatively coded bacteriophages (phages) are distributed across diverse animal and human habitats and show protein sets that are animal host type specific. The approach taken here provides a path to more general understanding of the processes by which modern human microbiomes developed.

PL2

## PL3

## Bio-digital Fusion to Establish Biofoundry Technology and Accelerate Cell Factory Development

#### Akihiko Kondo

School of Science, Technology and Innovation, Kobe University, Japan Department of Applied Chemistry, School of Engineering, Kobe University, Japan RIKEN Center for Sustainable Resource Science, Japan

We have developed rapid cell factory construction technology (biofoundry platform), which is an integrated system (DBTL) of advanced technologies such as metabolic design system (Design), rapid breeding technology using long chain DNA-transfected microorganisms (Build), rapid and accurate metabolic evaluation technology (Test), and machine learning or mathematical modelling for further improvement and a new metabolic pathway design (Learn). As metabolic design system, we developed BioProV and M-path, new simulation tools that enable metabolic design for the biosynthesis of unnatural compounds. Gene components such as inducible artificial promoters and terminators were developed to synthesize designed metabolic pathways and gene circuits. To efficiently construct cell factories by re-write genome based on designs, we have developed the platform technologies such as genome editing and a large gene cluster synthesis systems and are going to integrate to set up the automated systems. By tethering the DNA deaminase activity to nuclease-deficient CRISPR/Cas9 system, a genome editing tool that enables targeted point mutagenesis have developed (termed Target-AID or Base Editor). In addition, an efficient DNA assembly method, namely, Ordered Gene Assembly in B. subtilis (OGAB) method have developed. OGAB method can assemble more than 50 DNA fragments to construct up to 100 kb DNA in one-step using B. subtilis. An automated metabolomics analysis system has also been developed to analyse the performance of cell factories in more accurate and high throughput manner. We are applying this biofoundry platform for construction of various cell factories. By BioProV design tool with enzyme engineering technology, we succeeded in expanding the scope of bioproduction targets. Application of constructing artificial metabolic pathways has demonstrated by the C4 unsaturated compound 1,3-butadien synthesis in Escherichia coli. Butadiene biosynthetic pathway is designed in silico, and then realize it by constructing artificial enzyme with rational design.

PL4

## Personalized Medicine Based on Deep Human Phenotyping

## **Eran Segal**

Department of Computer Science, Weizmann Institute of Science, Israel

Recent technological advances allow large cohorts of human individuals to be profiled, presenting many challenges and opportunities. I will present The Human Phenotype Project, a large-scale (>10,000 participants) deep-phenotype prospective longitudinal cohort and biobank that we established, aimed at identifying novel molecular markers with diagnostic, prognostic and therapeutic value, and at developing prediction models for disease onset and progression. Our deep profiling includes medical history, lifestyle and nutritional habits, vital signs, anthropometrics, blood tests, continuous glucose and sleep monitoring, and molecular profiling of the transcriptome, genetics, gut and oral microbiome, metabolome and immune system. Our analyses of this data provide novel insights into potential drivers of obesity, diabetes, and heart disease, and identify hundreds of novel markers at the microbiome, metabolite, and immune system level. Overall, our predictive models can be translated into personalized disease prevention and treatment plans, and to the development of new therapeutic modalities based on metabolites and the microbiome.

## The Co-evolution of Anti-CRISPRs and CRISPR-Cas Systems

# Alan R. Davidson<sup>1,2\*</sup>, Beatrice Fung<sup>1</sup>, Chantel N. Trost<sup>1</sup>, Robert E.J. Wilson<sup>2</sup>, Sabrina Y. Stanley<sup>1</sup>, Jenny Y. Zhang<sup>3</sup>, Adair L. Borges<sup>3</sup>, Joseph Bondy-Denomy<sup>3,4</sup>, and Karen L. Maxwell<sup>2</sup>

<sup>1</sup>Department of Molecular Genetics, University of Toronto, Canada <sup>2</sup>Department of Biochemistry, University of Toronto, Canada <sup>3</sup>Department of Microbiology and Immunology, University of California San Francisco, USA <sup>4</sup>Quantitative Biosciences Institute, University of California San Francisco, USA

CRISPR-Cas systems provide adaptive immunity against invasion by foreign DNA in bacteria and archaea. To counteract CRISPR-Cas systems, mobile genetic elements, such as phages, often encode anti-CRISPRs, proteins that that inhibit CRISPR-Cas systems. First discovered in our lab in 2013, there are now more than 90 families of characterized anti-CRISPRs that block many different types of systems. These anti-CRISPRs inhibit CRISPR-Cas effector complexes through diverse mechanisms, but most involve binding of the anti-CRISPR to the Cas protein components of the complex to mediate steric blocking of an active site. Given the common occurrence of anti-CRISPRs, we expect evolutionary selection for sequence changes in Cas proteins that will allow them to evade the action of anti-CRISPRs. In this talk, I will describe sequence variations existing within the type I-F CRISPR-Cas system in strains of *Pseudomonas aeruginosa* and show that positive selection is acting at amino acid positions frequently bound by anti-CRISPRs. These studies provide insight into the impact of anti-CRISPRs on the diversification of CRISPR-Cas systems.

# **MSK Award Lecture**

## AL

## **Determinants of RNA Function**

#### **Kangseok Lee**

Department of Life Science, Chung-Ang University

RNA transcripts play the role of a messenger and a regulator of gene expression. They also have scaffolding and catalytic functions. My objective here is to discuss what my research group has found out about factors determining (or affecting) the function of various RNA molecules. Specifically, I will discuss about genome-encoded rRNA-mediated specialized ribosomes and endoribonucleases (including their associated factors) that determine stability of mRNA and generation of functional non-coding RNAs.

# **Special Lecture**

SL

# The Evolution and Progress of the Translational Research and Its Implication in the Clinical Development of the New Therapeutic Agents

#### SungKu Choi

Department of Research & Development, Ildong Pharmaceutical Co.

Traditionally, there have been two distinct types of research, basic and clinical. The collaboration of each disciplinary research is crucial to achieving the ultimate goal of enhancing the nation's health. However, there are challenges like the system of academic advancement favoring independent investigators, institutions housing scientists in discrete departments, and the operational obstacles requiring unique resources. Consequently, Balas criticized that it took 17 years to turn 14 percent of original research to the benefit of patient care. Reflecting the environment of siloed research society, translational research, which appeared in the 1990s, has been regarded as a rescuer. The definition of translational research is the field of investigation focused on understanding the scientific and operational principles underlying each step of the translational process. In the presentation, I will share the history, expansion, confusion of staging, and philosophy. Additionally, when translational research is implemented in Korea effectively, requirements will be delivered. Especially leadership, innovation, and focusing on customers will be emphasized.

# Symposium [PS1]

Sponsored by Korea Biosafety Clearing House and Dept. of Science Studies, Seoul National University

## PS1-1

## Issues of Synthetic Biology in CBD and Domestic Discussion

#### Hoon-Gi Kim

College of Liberal Arts, Hongik University

The issues of Synthetic Biology will be covered as the important agenda in the CBD (Convention on Biological Diversity) to be held in Canada in December of 2022. Since July, the project team has organized several meetings consisting of various stakeholders in the preparation for the CBD with the support of KBCH. The meeting was a preliminary form for the MTEG (Multidisciplinary Technical Expert Group) recommended by CBD to be installed in each country, and the discussion material was *Technical Series 100-Synthetic Biology* recently published by the CBD Secretariat.

As a result of the recent meetings, three points have been suggested as follows: First, the concept of Synthetic Biology is too broad and vague. It is difficult to distinguish it from the existing biotechnology, and questionable whether the researchers perceive themselves as Synthetic Biologists. Second, the practical achievements of Synthetic Biology do not seem to be sufficiently available yet. Engineered gene drives are often mentioned as the potential risks affecting biodiversity seriously, but they are in the early stages of development and fall into the category of the existing LMOs. Third, transparent information disclosure and social discussion on research processes as well as final results are needed. For example, it is necessary to label agricultural products made by genome editing technology, and explain actively their benefits and safety to the public.

### PS1-2

## **Riskscapes of Biotechnology: Case of Synthetic Biology**

#### **Taemin Woo**

Korea Biosafety Clearing House, Korea Research Institute of Bioscience and Biotechnology

This paper examines the specific moments of emergence of the new biotechnologies and their biosafety governance with the case of synthetic biology. This paper aims to show that these moments are not merely driven by technological breakthrough, but tightly rooted in the ways we co-produce its regulatory governance. Compare to the social debates that took place actively in the European countries, the emergence of synthetic biology in Korea was rather silent and confined within the techno-bureaucratic discussion. Beyond this observation, this paper employs the concept of the "riskscapes" to analyze plurality of risk perception of each actor including researchers, policymakers, local residents and NGOs. By exploring different discursive and geographical spaces, this paper captures multiple riskscapes of biotechnology in Korea held by different stakeholders, those who empowered and produced their own riskscapes. In this process, the question of novelty was tightly related to the production of different riskscapes.

## PS1-3

## How to Make Science Research Outcomes to Readable Stories

### Young Wan Lee

Senior staff writer, newsroom, Chosunbiz President, Korea Science Journalist Association Adjunct Professor, Moon Sul Graduate School of Future Strategy, KAIST

It is not easy for scientists to communicate their research results to the public. Emphasizing the accuracy of research results tends to make the text difficult to read. Science journalists have the same concerns. Research achievements in advanced scientific fields such as synthetic biology are even more so. I would like to tell researchers that science can develop into an easy and fun story that the general public can be interested in through my own articles that introduces the results of synthetic biology research.

# Symposium [S1]

Co-organized by CNU Regional Leading Research Center for Microbiome-Brain Disorders

# Modulatory Roles of Maternal Gut Bacteria in Shaping Offspring's Immune Responses and Neurodevelopmental Phenotypes

#### Jun R. Huh

Department of Immunology, Harvard Medical School, USA

Along with genetic factors, environmental factors are critical contributors to inflammatory disorders such as inflammatory bowel diseases (IBDs) and neurodevelopmental disorders such as autism. Gut microbes are one of these non-genetic factors that define a threshold to either maintaining a homeostatic balance or developing diseases. Human epidemiological studies support a correlation between exposure to adverse conditions early in life to the development of immune-related and neurodevelopmental diseases. We hypothesize that maternal inflammation and associated prenatal inflammation, which developing fetuses are exposed to, can be an environmental risk factor and promote long-lasting effects on an offspring's immune system and brain development.

A mouse model for maternal immune activation (MIA) that mimics viral-like infection has been successfully used to study the relationship between inflammation in pregnant women and neurodevelopmental disorders in their children. Using this mouse model, we previously demonstrated that a subset of T helper cells (Th17 cells) expressing interleukin 17a (IL-17a) in pregnant mice is essential in driving neurodevelopmental disorder phenotypes in their offspring. Intriguingly, the immune system of those born to pregnant mice exposed to MIA (MIA offspring) also displayed immune-primed phenotypes. For example, when MIA offspring were exposed to another inflammatory stimulus later in life, they exhibited increased levels of inflammatory cytokines, such as IFN-g and IL-17a, compared to a control group. Furthermore, our recent data suggest that MIA offspring develop enhanced susceptibility to colitis-like symptoms upon bacterial infection. We also found that MIA induces changes in the gut microbial community, which underlies immune-primed phenotypes in MIA offspring.

I will discuss our ongoing efforts to dissect gut microbes' effects on influencing offspring's health.

# Successful Microbiota Transfer Therapy to Modulate Gut Microbiome and Treat Autism Symptoms

#### **Dae-Wook Kang**

Department of Civil and Environmental Engineering, the University of Toledo, USA

Last few decades, autism spectrum disorder (ASD) substantially increased globally to be an epidemic level, which greatly impacted our society. However, commonly accepted treatments and even biomarkers for early diagnosis are not available for this complex neurobiological disorder. Based on accumulating evidence that support a link between ASD and the gut microbiome, a microbiota transfer therapy (MTT) was designed by combining fecal microbiota transplant (FMT) with antibiotics, a bowel cleanse, and a stomach-acid suppressant. A pioneering open-label clinical trial for 18 children with ASD showed that this rigorous 10-week long daily therapy significantly improved gastrointestinal and behavioral symptoms, and the improvement was maintained even 2 years after treatment was stopped. With the improvement, MTT increased microbial diversity and beneficial microbes in children with ASD and shifted fecal and plasma profiles. The comprehensive results suggest that modulation in gut microbiome via MTT could be a promising therapy to treat children with ASD. Improvement in ASD behaviors and corresponding changes in gut microbiome reflect a mechanism of the gut-brain connection mediated by the gut microbiota and offer plausible clinical evidence for potential autism biomarkers as well.

## GABA-modulating Bacteria of the Human Gut Microbiota

## Philip Strandwitz<sup>1</sup>, Jon Clardy<sup>2</sup>, Kim Lewis<sup>1</sup>, and Ki Hyun Kim<sup>3\*</sup>

<sup>1</sup>Antimicrobial Discovery Center, Department of Biology, Northeastern University, Boston, MA, USA <sup>2</sup>Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA <sup>3</sup>School of Pharmacy, Sungkyunkwan University

The gut microbiota affects many important host functions, including the immune response and the nervous system. However, while substantial progress has been made in growing diverse microorganisms of the microbiota, 23-65% of species residing in the human gut remain uncultured, which is an obstacle for understanding their biological roles. A likely reason for this unculturability is the absence in artificial media of key growth factors that are provided by neighbouring bacteria in situ. In the present study, we used co-culture to isolate KLE1738, which required the presence of Bacteroides fragilis to grow. Bioassaydriven purification of *B. fragilis* supernatant led to the isolation of the growth factor, which, surprisingly, is the major inhibitory neurotransmitter GABA (y-aminobutyric acid). GABA was the only tested nutrient that supported the growth of KLE1738, and a genome analysis supported a GABA-dependent metabolism mechanism. Using growth of KLE1738 as an indicator, we isolated a variety of GABA-producing bacteria, and found that Bacteroides ssp. produced large quantities of GABA. Genome-based metabolic modelling of the human gut microbiota revealed multiple genera with the predicted capability to produce or consume GABA. A transcriptome analysis of human stool samples from healthy individuals showed that GABA-producing pathways are actively expressed by Bacteroides, Parabacteroides, and Escherichia species. By coupling 16S ribosomal RNA sequencing with functional magnetic resonance imaging in patients with major depressive disorder, a disease associated with an altered GABA-mediated response, we found that the relative abundance levels of faecal Bacteroides are negatively correlated with brain signatures associated with depression.

# Human Reference Gut Microbiome to Explore Human Microbial Dark Matter

## Chan Yeong Kim, Junyeong Ma, and Insuk Lee\*

Department of Biotechnology, Yonsei University

Metagenome sampling bias for geographical location and lifestyle is partially responsible for the incomplete catalog of reference genomes of gut microbial species. Thus, genome assembly from currently under-represented populations may effectively expand the reference gut microbiome and improve taxonomic and functional profiling. We assembled genomes using public whole-metagenomic shotgun sequencing (WMS) data for fecal samples from Korea, India and Japan. We consequently assembled 29,082 prokaryotic genomes from 845 fecal metagenomes for the three under-represented Asian countries and combined them with the Unified Human Gastrointestinal Genome (UHGG) to generate an expanded catalog, the Human Reference Gut Microbiome (HRGM) which contains 232,098 non-redundant genomes for 5414 representative prokaryotic species including 780 that are novel. This is an over 10% increase from the UHGG. The new 780 species were enriched for the Bacteriodaceae family, including species associated with high-fiber and seaweed-rich diets. Importantly, the HRGM significantly improved the taxonomic and functional classification of sequencing reads from fecal samples. Finally, analysis of human self-antigen homologs on the HRGM species genomes suggested that bacterial taxa with high cross-reactivity potential may contribute more to the pathogenesis of gut microbiomeassociated diseases than those with low cross-reactivity potential by promoting inflammatory condition. By including gut metagenomes from previously under-represented Asian countries, Korea, India, and Japan, we developed a substantially expanded microbiome catalog, HRGM. HRGM will facilitate the identification and functional analysis of disease-associated gut microbiota.

# Symposium [S2]

Co-organized by Skin-Bio Research Cluster, Microbiomics Institute - Chung-Ang University

## From Metagenomes to Therapeutics: the Human Skin Microbiome

#### Julia Oh

The Jackson Laboratory for Genomic Medicine, Farmington, Connecticut, USA

The human skin harbors an abundant microbial ecosystem with bidirectional metabolic exchanges supporting symbiotic and commensal functions. Sequence-based analysis of microbial community structure and organization of the human microbiome has yielded valuable insight into the microbial diversity and function of its different body niches. Metagenomic analyses of the diverse skin sites in healthy humans demonstrate that contrasting forces of the skin's biogeography and individual characteristics shape the skin microbiome and the dynamics of its bacteria, fungi, and viruses. However, we have shown that shifts in the ecological properties of the skin microbiome are significantly associated with aging, skin disease, disease severity, and other physiologic host factors such as age or primary immunodeficiency. We have deeply probed skin microbiome composition and function at subspecies resolution, focusing on a keystone skin microbe, Staphylococcus epidermidis. We uncovered an extraordinary within-individual diversity at the strain level that we found can suppress population-level virulence of this opportunistic microbe. We complemented this strain-level genomic investigation with CRISPRi and transcriptomic profiling to probe S. epidermidis gene function in skin environments. Finally, we investigated how these diverse S. epidermidis strains program the skin milieu in 3D skin organoids, identifying strain-specific immune and skin signatures. Taken together, our results highlight the genetic and functional diversity of the skin microbiome at the strain level. Strain diversity is an emerging frontier of understanding hostmicrobiome interactions and therapeutic discovery, as it harbors a tremendous amount of individualand disease-specific genetic and phenotypic diversity.

## **Skin Microbiome Signatures in Atopic Dermatitis**

#### John Common

A\*STAR Skin Research Labs (A\*SRL), Agency of Science Technology and Research, Singapore

Investigating microbial communities present on the skin of atopic dermatitis (AD) patients has led to a deeper understanding of the shifts in community diversity and functional gene pathways. These dynamic shifts across the flare cycle and according to the underlying endotypes of AD provide potential intervention points for topical therapies to reduce infections. We have also recently identified the shared microbiome signatures that exist between children with AD and their caregivers. This supports the inclusion of family members in microbial-based strategies for treating recurrent paediatric AD.

# Elucidating the Molecular Functions of Skin Fungal Secretory Proteases

## Wisely Chua<sup>1,2</sup>, Joleen Goh<sup>3</sup>, Li Fang Koh<sup>3</sup>, Anthony O'Donoghue<sup>4</sup>, John Common<sup>3</sup>, Thomas Dawson Jr.<sup>3</sup>, and Hao Li<sup>1,2\*</sup>

<sup>1</sup>Department of Chemistry, National University of Singapore, Singapore <sup>2</sup>Molecular Engineering Lab, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore

> <sup>3</sup>A\*STAR Skin Research Labs, Agency for Science, Technology and Research, Singapore <sup>4</sup>Skaggs School of Pharmacy, University of California-San Diego, California, USA

The skin is colonized by a rich community of bacteria, fungi and viruses. Community profiling using next generation sequencing revealed that the vast majority of fungi present on the skin belongs to a single family *Malassezia*. Genome annotation of *Malassezia* revealed the presence of a wide variety of secreted hydrolytic enzymes including proteases. However, the functional roles of these extracellular proteases are largely unknown. Utilizing biochemical and genetic approaches, we first defined the functions of the major secreted protease in the highly prevalent *Malassezia globosa* and *Malassezia furfur*. This aspartyl protease, SAP1, has crucial roles in cell adhesion and epidermal colonization. In a cohort of atopic dermatitis patients, we observed an increase in expression of the *M. globosa* homologue of this protease. Furthermore, we observed that the extracellular protease that is highly upregulated in the hyphal state of *M. furfur* and demonstrate that this protease degrades key corneodesmosome proteins. This disruption of the stratum corneum has potentially important consequences for the disease pathology of pityriasis versicolor. Taken together, our work demonstrates that the secreted proteases from the skin commensal *Malassezia* can play different roles in the context of healthy and diseased cutaneous states.

# Commensal Microbiota Regulate Regeneration in Damaged Skin

## Gaofeng Wang<sup>1</sup>, <u>Dongwon Kim<sup>2</sup></u>, and Luis Garza<sup>1\*</sup>

<sup>1</sup>Department of Dermatology, Johns Hopkins School of Medicine, USA <sup>2</sup>Department of Bio-Pharmaceutical Engineering, Dongseo University

The ability of animal to regenerate the original tissue varies dramatically across different phyla, with certain animals like urodele salamanders being able to fully regenerate lost limbs. Although most mammals recover from cutaneous wounds with fibrotic scarring including mice and rabbits are able to bypass this, completely regenerating hair follicles, sebaceous glands, and adipocytes. This phenomenon is known as wound-induced hair neogenesis (WIHN) and is a model of tissue regeneration in adults where hair follicles develop de novo following deep wounding. Previously, we demonstrated that non-coding double stranded RNAs (dsRNAs) released from damaged skin stimulate toll like receptor 3 (TLR3)-mediated signaling and regulate regeneration. Furthermore, TLR3 activation induces intrinsic retinoic acid (RA) synthesis and RA-mediated signaling pathway. Interestingly, the immune system and microbiome are attributed roles in repairing and regenerating structure but their precise interplay is unclear. Here, we assessed the function of skin bacteria in wound healing and WIHN. WIHN levels and stem cell markers correlate with bacterial counts, being lowest in germ-free (GF), intermediate in conventional specific pathogen-free (SPF), and highest in wild type mice, even those infected with pathogenic Staphylococcus aureus. Reducing skin microbiota via cage changes or topical antibiotics decreased WIHN. Inflammatory cytokine IL-1 $\beta$  and keratinocyte-dependent IL-1R-MyD88 signaling are necessary and sufficient for bacteria to promote regeneration. These results demonstrate a role for IL-1eta to control morphogenesis and support the need to reconsider routine applications of topical prophylactic antibiotics.

# Symposium [S3]

# Metabolic Engineering of Non-conventional Yeast to Produce Biofuels and Chemicals

Sangdo Yook<sup>1,2</sup>, Ye-Gi Lee<sup>1,2</sup>, Hyun-Gi Koh<sup>1</sup>, and Yong-Su Jin<sup>1,2\*</sup>

<sup>1</sup>Department of Food Science and Human Nutrition <sup>2</sup>Carl R. Woese Institute of Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Non-conventional yeast strains, such as *Issatchenkia orientalis, Yarrowia lipolytica*, and *Rhodosporidium toruloides*, have received extensive attention due to their potential to produce high-value products under harsh environmental conditions. While efficient and rapid xylose utilization is necessary for the economic conversion of cellulosic hydrolysates, *Y. lipolytica* and *I. orientalis* cannot metabolize xylose at all, and *R. toruloides* assimilate xylose slowly. We have undertaken metabolic engineering and adaptive laboratory evolution approaches to improve the xylose assimilation capacities of *Y. lipolytica*, *I. orientalis*, and *R. toruloides*. We developed a Cas9-based genome editing method and a modular cloning kit for introducing genetic perturbations into the non-conventional yeast strains. Heterologous and endogenous metabolic pathways consisting of xylose reductase (XR), xylitol dehydrogenase (XDH), and xylulokinase (XK) were engineered to increase the rate of xylose assimilation. Also, laboratory evolution and genome re-sequencing were employed to identify mutations eliciting improved xylose utilization. As a result, the titers and productivities of target molecules (ethanol, lactic acid, triacylglycerol, triacetic acid lactone) from cellulosic hydrolysates by the resulting non-conventional yeast strains improved substantially. These results suggest that engineered *Y. lipolytica*, *I. orientalis*, and *R. toruloides* can be employed for the enhanced conversion of lignocellulosic hydrolysates into various biofuels and chemicals.

# Metabolic Engineering of Yeasts for Production of Mycosporine-like Amino Acids, Natural Sunscreen Materials

## Sojeong Kim, Hyunbin Jin, Daeyeol Lee, and Ji-Sook Hahn\*

School of Chemical and Biological Engineering, Seoul National University

Mycosporine-like amino acids (MAAs), having UV-absorbing properties and antioxidant activity, have been gaining attention as cosmetic and pharmaceutical materials. Over 30 MAAs identified so far have cyclohexanone or cyclohexenimine core structure substituted with various amino acids or amino alcohols. Due to the low production yield of MAAs from natural producers such as red alga, production in heterologous microbial hosts is considered a promising alternative. We engineered yeasts, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, to produce MAAs with different structures. By introducing MAA biosynthetic genes from cyanobacteria, MAAs were produced from sedoheptulose 7-phosphate (S7P), an intermediate in the pentose phosphate pathway. S7P is first converted to 4-deoxygadusol (4-DG), and then conjugation of glycine to 4-DG generates mycosporine-glycine (MG). We screened D-Ala-D-Ala ligases conjugating various amino acids, including serine, threonine, and glycine, to MG, and successfully produced shinorine, phorphyra-334, and mycosporine-2-glycine, respectively. Differences between *S. cerevisiae* and *Y. lipolytica* for the production of MAAs will be discussed along with metabolic engineering strategies to improve production.

# Yeast Engineering for Fermenting Pectin Sugars Derived from Citrus Waste

#### Soo Rin Kim

School of Food Science and Biotechnology, Kyungpook National University

Fruit juice wastes represented by citrus peels are high in pectin, a complex polysaccharide consisting mainly of D-galacturonic acid and L-rhamnose with side chains composed of L-arabinose and D-xylose. What these pectic sugars have in common is that industrial microorganisms cannot metabolize them. To develop a platform yeast strain that can metabolize these pectic sugars and produce bio-based chemicals, their heterologous pathways were identified and functionally expressed in *Saccharomyces cerevisiae*. For pathways that cannot stand alone due to redox imbalance issue, appropriate co-substrates were screened. Using an metabolomic approach, their metabolic bottlenecks were identified and resolved by balancing the pathways. Finally, the pectic sugar-metabolizing platform yeast was applied for the production of mucic acid, an industrially important dicarboxylic acid. These results suggest a potential of citrus waste upcycling as a useful biomass for yeast-based biorefinery.

# Production of Key Ingredients of Alternative Proteins by Engineered Saccharomyces cerevisiae

## Hyun-Jae Lee, Gi-Beom Jeon, Young-Oh Lee, and Sun-Ki Kim\*

Department of Food Science & Technology, Chung-Ang University

Food industries are interested in commercializing products formulated with ingredients derived from environmentally sustainable resources. *Saccharomyces cerevisiae* has been widely used food and pharmaceutical industries due to its generally recognized as safe (GRAS) status and well-developed genetic tools. Considering numerous benefits of using a GRAS host for mass production, *S. cerevisiae* has been engineered to produce key ingredients of alternative proteins. This study highlights recent advances in metabolic engineering strategies to enhance production of single cell protein, heme, ovalbumin, and glutathione.

## Gene Regulation by Nuclear Enzyme Aconitase 2 in Schizosaccharomyces pombe

## Soo-Yeon Cho<sup>1,2</sup>, Soo-Jin Jung<sup>2</sup>, Jung-Hye Roe<sup>2\*</sup>, and Kyoung-Dong Kim<sup>1\*</sup>

<sup>1</sup>Department of Systems Biotechnology, Chung-Ang University <sup>2</sup>School of Biological Sciences, Seoul National University

Aconitase, well-known as a mitochondrial TCA cycle enzyme, senses iron deficiency and acts as an iron regulatory protein (IRP) in mammals and metazoa, controlling the expression of mRNA related to iron homeostasis. From prokaryotes to mammals, multiple functions of aconitase have been reported. A recent our studies revealed that aconitase 2 (Aco2) in *Schizosaccharomyces pombe*, a fusion protein of aconitase and a mitochondrial ribosomal protein, exists in the nucleus in addition to mitochondria, and that it is required for protein translation in mitochondria and controls heterochromatin formation in the nucleus. Considering the conserved function of aconitase in various organisms, Aco2 is likely to interact with nucleic acids or transcription factors to regulate gene expression.

To investigate the role of nuclear Aco2, phenotypes of *aco2* mutant devoid of nuclear localization signal (NLS) (*aco2* $\Delta$ *NLS*) were examined. Transcriptome analysis revealed that the mutation caused increase in mRNAs coding for iron uptake transporters, such as Str1, Str3, and Shu1. We found that nuclear Aco2 directly binds to iron uptake mRNAs and help them to be properly degraded by exoribonucleases under iron-sufficient conditions. It is concluded that Aco2 contributes to maintain intracellular iron homeostasis through post-transcriptional regulation of iron transporter genes. In addition, transcriptome analysis result show that gene expressions for nuclear-encoded electron transport chain (ETC) were decreased in *aco2* $\Delta$ *NLS* mutant. Defective respiratory growth was also observed in the mutant, suggesting that nuclear Aco2 may regulate genes involved in cellular respiration. This study newly discovered that *S. pombe* Aco2 regulates genes related to iron homeostasis and cellular respiration. Given the unique features of the moonlighting enzyme and dual targeting, Aco2 is expected to play another important role in maintaining the life of cells.

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# Symposium [S4]

## **Microbiome Mediated Trained Immunity in Neutrophil**

## Ma-Rie Kim<sup>1</sup>, Alissa Trzeciak<sup>2</sup>, Steven R. Gill<sup>2</sup>, Anthony P. Pietropaoli<sup>3</sup>, and Minsoo Kim<sup>2,4\*</sup>

<sup>1</sup>Department of Biomedical Engineering, University of Rochester, Rochester, NY, USA <sup>2</sup>Department of Microbiology and Immunology, <sup>3</sup>Department of Medicine, <sup>4</sup>David H. Smith Center for Vaccine Biology and Immunology, University of Rochester Medical Center, Rochester, NY, USA

Growing evidence from recent outbreaks of respiratory viral infection suggest that the patient lethality is closely linked to exacerbation of robust innate immune responses and systemic inflammation-mediated tissue damage. Thus, the therapeutic strategy to bridle excessive immune damage has been proposed for the severe inflammatory septic conditions associated infections such as SARS CoV-2. Although sepsis is one of the most common causes of hospital death, patient responses to this disease are highly heterogeneous making it difficult to identify the critical pathophysiologic processes that lead to sepsis mortality. Here, we present evidence suggesting that there is a greater level of heterogeneity in activation of neutrophils isolated from healthy donors. Neutrophils are differentially "trained" by the microbiome during homeostasis, leading to altered host responses to inflammation. Unlike mice kept in specificpathogen-free (SPF) facilities, mice housed in nonsterile environmental conditions ("wildling mouse") successfully recapitulate the neutrophil heterogeneity with improved septic survivals. Our data suggest that heterogeneous patient responses to sepsis are induced by the microbiome-mediated trained immunity in neutrophil during homeostasis. Taken together, this study addressed mechanisms regarding how the dysregulated host response to infection threatens patient survival and offers a molecular target for dampening the destructive arms of the hyperinflammatory response while promoting disease resolution and tissue recovery.

## S4-2

## **Airway Infection Resistance Induced by a Nasal Microbiome**

## Gwanghee Kim<sup>1,2</sup> and Sang Sun Yoon<sup>1,2\*</sup>

<sup>1</sup>Department of Microbiology and Immunology, Yonsei University College of Medicine <sup>2</sup>BioMe Inc.

Understanding multi-faceted modes of interactions between microbes, either preexisting or invading, and host tissues is critical to come up with novel strategies for managing infections. Here we illustrate how *Staphylococcus epidermidis*, a predominant nasal commensal, domesticates host airway innate immunity to cope with pathogenic infections. A unique isolate of *S. epidermidis* (termed BM101), when transplanted via intranasal route, completely protected mice against airway infection by PAO1, a laboratory strain of *Pseudomonas aeruginosa*. BM101 persistently colonized mouse airway and induced dramatic increase in populations of neutrophils and monocytes in airway. As a consequence, PAO1 was efficiently eliminated from the mouse airway. Such protective effects of BM101 were determined to be induced by a secretory protein, which we named AIT (Airway Immune Trainer). Mice, when transplanted with the purified AIT, can efficiently manage infections by several other Gram-negative pathogens. Moreover, AIT-pretreated mice maintained the normal health conditions against a lethal influenza virus infection, with viral titers significantly decreased. Together, our results reveal a novel mode of interaction between host airway and a nasal symbiotic microbe or its secretory protein that in turn impacts host infection resistance. Detailed results and potential implications will be discussed.

## S4-3

# Gut Microbiota and an Associated Leaky Gut May Affect COVID-19 Severity

#### **Heenam Stanley Kim**

Division of Biosystems & Biomedical Sciences, College of Health Sciences, Korea University

Coronavirus disease (COVID-19), which has been declared a pandemic, has exhibited a wide range of severity worldwide. Although this global variation is largely affected by socio-medical situations in each country, there is also high individual-level variation attributable to elderliness and certain underlying medical conditions, including high blood pressure, diabetes, and obesity. As both elderliness and the aforementioned chronic conditions are often associated with altered gut microbiota, resulting in disrupted gut barrier integrity, and gut symptoms have consistently been associated with more severe illness in COVID-19 patients, it is possible that dysfunction of the gut as a whole influences COVID-19 severity. Here I summarize the accumulating evidence that supports the hypothesis that altered gut microbiota and its associated leaky gut may contribute to the onset of gastrointestinal symptoms and occasionally to additional multiorgan complications that may lead to severe illness by allowing leakage of the causative coronavirus into the circulatory system.

#### Key messages

- While this still remains to be empirically demonstrated, accumulating evidence supports the hypothesis that altered gut microbiota and its associated leaky gut may contribute to the onset of COVID-19-related gastrointestinal symptoms such as diarrhea and, in severe cases, multiorgan complications.
- Testing for a leaky gut and fecal and plasma viral loads may be useful for diagnosing the seriously ill or for preventing transmission by fecal shedding of the virus.
- Fecal microbiota transplantation (FMT), next-generation probiotics focusing on butyrate-producing gut microbes, or simply increasing the daily intake of dietary fiber may be considered in improving the gut health of COVID-19 patients.

## S4-4

## The Critical Impacts of Tissue-resident T Cells in Long-term Immunity Following the Respiratory Viral Infection

### **Youngmin Son**

Department of Systems Biotechnology, Chung-Ang University

Generally, adaptive immunity including T and B cell responses play a critical role in resolving virus infection in mucosal tissues particularly respiratory tract. Following the clearance of primary viral infection, certain types of T and B cells are observed as memory functional cells both systemically and locally at viral infectious sites. During last two decades, the important effects of tissue-resident memory T and B cells were highlighted. It is well known that the tissue-resident memory cells play an essential role for protection against secondary viral infection rapidly. Recently, we found novel type of tissue resident helper CD4 T ( $T_{RH}$ ) cells which contribute the maintenance and development of tissue-resident memory CD8 T ( $T_{RM}$ ) and B ( $B_{RM}$ ) cells following influenza virus infection at the long-term immunity. Moreover, when the  $T_{RH}$  cells were selectively depleted in the long-term immunity, the number of  $T_{RM}$  and  $B_{RM}$  cells also downregulated and caused an impaired protection against secondary viral infection.

However, at the aged mice model following influenza virus infection, we found dramatically higher number of  $T_{RM}$  cells than young mice. These excessively accumulated in the aged mice in long-term immunity were highly positive correlation of chronic inflammation of lung. We also have observed similar results in the human aged COVID-19 convalescent patients (>60 years of age). These patients had exuberant number of  $T_{RM}$  cells in their the bronchoalveolar lavage fluid (BAL). Critically, the numbers of  $T_{RM}$  cells show dramatic positive correlation with chronic lung inflammation of aged COVID-19 convalescent patients.

Here, we demonstrated the protective function of tissue-resident memory T cells in the long-term immunity following respiratory viral infection and showed the pathological effects which might be involved with those cells. These results suggest that the roles of tissue-resident memory T cells might be changed by depending on the aging environments.

MSK2022 International Meeting of the Microbiological Society of Korea

# Symposium [S5]

Co-organized by Science Research Center for Microbial Survival Systems

# HPr Prevents FruR-mediated Facilitation of RNA Polymerase Binding to the *fru* Promoter in *Vibrio cholerae*

Chang-Kyu Yoon<sup>1,2†</sup>, <u>Seung-Hwan Lee<sup>1†</sup></u>, Jing Zhang<sup>3†</sup>, Hye-Young Lee<sup>4</sup>, Min-Kyu Kim<sup>3\*</sup>, and Yeong-Jae Seok<sup>1,4\*</sup>

<sup>1</sup>School of Biological Sciences and Institute of Microbiology, Seoul National University <sup>2</sup>Research Institute of Basic Science, Seoul National University <sup>3</sup>Radiation Research Division, Korea Atomic Energy Research Institute <sup>4</sup>Department of Biophysics and Chemical Biology, Seoul National University

In most bacteria, utilization of the preferred carbohydrates is primarily mediated by the phosphoenolpyruvate (PEP): carbohydrate phosphotransferase system (PTS). Phosphorylation state-dependent interactions of the PTS components with transcription factors play a major role in the carbon catabolite repression (CCR) mechanism in which glucose is preferred over other carbohydrates. Glucose inhibits the PTS-dependent utilization of fructose and thus is preferred over fructose in *Vibrio cholerae*, but the underlying mechanism remains unknown. Here, we show that, in the presence of glucose, dephosphorylated HPr, a general PTS component, binds to fructose regulator (FruR), and prevents it from facilitating the binding of RNA polymerase to the *fru* promoter. This results in aberrant transcription of the genes encoding the fructose-specific PTS components. Structural analysis reveals that the binding mode of HPr to FruR in *V. cholerae* is similar to that of HPr to its partners in *E. coli*. However, several amino acid residues of FruR important for polar interaction with HPr are only conserved in *Vibrio* species among species in Gammaproteobacteria. Finally, we discuss that the binding mode of HPr to FruR is distinctively different from that of *Bacillus subtilis* HPr binding to CcpA, which participates in CCR in Firmicutes and shows structural similarity to FruR.

# Mechanism and Regulation of Homologous Recombination and Gene Conversion

#### Keun Pil Kim<sup>\*</sup>, Jeong Hwan Joo, and Soogil Hong

Department of Life Science, Chung-Ang University

Meiotic double-strand break (DSB) formation is preceded by a round of pre-meiotic DNA replication such that the duplicated chromosomes are present as a pair of sister chromatids. RecA-homologs, Dmc1 and Rad51, and their accessory proteins involve to complete meiotic DSB repair via recombination that uses a homolog template in homologous chromosome. The biochemical roles of replication protein A (RPA) are well characterized in a wide-range of DNA metabolisms. However, meiosis-specific roles of RPA are poorly characterized. Through physical analyses of meiotic recombination and 3D high-resolution microscopy imaging, we show that RPA-Rad52 association ensures the second-end annealing process that is required for Single-End-Invasion-to-double-Holliday Junction transition and general annealing steps, raising possibility that completion of CO and NCO, normally mediated by Rad52, requires RPA platform and recombinational interplay with Rad52; the protein-interaction activity of RPA is required to promote Dmc1-mediated joint molecule formation, thereby satisfying an interhomolog bias of meiotic recombination; and that the second DSB ends are released to promote ends-apart association and placed on the post-invasion steps, thereby accompanying interhomolog recombinational interaction during early stage of meiotic recombination.

## Heme Auxotrophy in the Most Abundant Freshwater Bacterioplankton Lineage

#### <u>Suhyun Kim</u><sup>1</sup>, Ilnam Kang<sup>2\*</sup>, Jin-Won Lee<sup>3</sup>, Che Ok Jeon<sup>4</sup>, Stephen J. Giovannoni<sup>5</sup>, and Jang-Cheon Cho<sup>1,6\*</sup>

<sup>1</sup>Department of Biological Sciences, Inha University
 <sup>2</sup>Center for Molecular and Cell Biology, Inha University
 <sup>3</sup>Department of Life Science, Hanyang University
 <sup>4</sup>Department of Life Science, Chung-Ang University
 <sup>5</sup>Department of Microbiology, Oregon State University, Corvallis, OR, USA
 <sup>6</sup>Department of Biological Sciences and Bioengineering, Inha University

Heme is a porphyrin ring complexed with iron found in numerous proteins involved in diverse metabolic across all domains of life. Especially, as a cofactor of cytochromes, heme functions as a main electron carrier in all respiratory electron chains. Therefore, it is naturally considered essential for respiring organisms. Several microbial groups called heme-auxotrophic microbes are deficient in the de novo heme biosynthetic pathway and thus are known to require exogenous heme from the environment. However, these heme auxotroph groups are largely limited to pathogens, symbionts, or microorganisms living in nutrient-replete conditions, whereas in free-living organisms the complete absence of heme biosynthesis is extremely rarely reported. Here, we show that the first case of heme auxotrophy found in a predominant and ubiquitous free-living freshwater bacterial group, Actinobacteria acl lineage. We found that two recently cultivated acl isolates require exogenous heme for their growth. Further, genomes of all acl strains isolated to date lack the most enzymes necessary to synthesize heme, indicating that heme auxotrophy is a conserved trait in this lineage. Additionally, analyses of all representative genomes for species clusters of the Genome Taxonomy Database (GTDB) revealed that heme auxotrophy is unexpectedly widespread across abundant but not-yet-cultivated microbial groups, including Patescibacteria, Marinisomatota (SAR406), Actinomarinales (OM1), and Marine group IIb and III of Euryarchaeota. Our findings indicate that heme auxotrophy is a more common phenomenon than previously thought. Heme should be the focus of future research on the metabolic dependency among microorganisms and the role of exchangeable metabolites in structuring diverse ecosystems, and would also be a media component that must be considered when cultivating novel microbes.

# Arginine-mediated Gut Microbiome Remodeling Promotes Host Pulmonary Immune Defense against Nontuberculous Mycobacterial Infection

June-Young Lee<sup>1,2#</sup>, Young Jae Kim<sup>3,4,5,6#</sup>, Jae Jin Lee<sup>7#</sup>, Sang Min Jeon<sup>3,4,5,6#</sup>, Prashanta Silwal<sup>3,4</sup>, In Soo Kim<sup>3,4,5</sup>, Hyeon Ji Kim<sup>3,4,5</sup>, Cho Rong Park<sup>3,4,5</sup>, Chaeuk Chung<sup>4,8</sup>, Jeong Eun Han<sup>1,2</sup>, Jee-Won Choi<sup>1,2</sup>, Euon Jung Tak<sup>1,2</sup>, Ji-Ho Yoo<sup>1,2</sup>, Su-Won Jeong<sup>1,2</sup>, Do-Yeon Kim<sup>1,2</sup>, Warisa Ketphan<sup>7</sup>, Su-Young Kim<sup>9</sup>, Byung Woo Jhun<sup>9</sup>, Jake Whang<sup>10</sup>, Jin-Man Kim<sup>11</sup>, Hyungjin Eoh<sup>7\*</sup>, Eun-Kyeong Jo<sup>3,4,5\*</sup>, and Jin-Woo Bae<sup>1,2\*</sup>

<sup>1</sup>Department of Life and Nanopharmaceutical Sciences and Department of Biology, Kyung Hee University <sup>2</sup>Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University <sup>3</sup>Department of Microbiology, Chungnam National University School of Medicine <sup>4</sup>Infection Control Convergence Research Center, Chungnam National University School of Medicine <sup>5</sup>Department of Medical Science, Chungnam National University School of Medicine <sup>6</sup>Brain Korea 21 FOUR Project for Medical Science, Chungnam National University School of Medicine <sup>7</sup>Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, CA, USA <sup>8</sup>Division of Pulmonary and Critical Care, Department of Internal Medicine, Chungnam National University School of Medicine <sup>9</sup>Division of Pulmonary and Critical Care Medicine, Department of Medicine, Sungkyunkwan University School of Medicine <sup>10</sup>Korea Mycobacterium Resource Center (KMRC) & Basic Research Section, The Korean Institute of Tuberculosis (KIT)

<sup>11</sup>Department of Pathology, Chungnam National University School of Medicine

Nontuberculous mycobacterial pulmonary diseases (NTM-PDs) are emerging as global health threats with issues of antibiotic resistance. Accumulating evidence suggests that gut microbiota and host interaction can provide novel therapeutic candidates for hosts against various infectious diseases. However, little is known about the gut-lung axis in the context of host protective immunity to discover new therapeutics for NTM-PDs. This study was performed to identify gut microbiota and gut-derived metabolites capable of conferring pulmonary immunity to NTM-PDs. Using metabolomics analysis of sera from NTM-PD patients and mouse models, we showed that the levels of L-arginine were decreased

in sera from NTM-PD patients and NTM-infected mice. Oral administration of L-arginine significantly enhanced pulmonary antimicrobial activities with the expansion of IFN-γ-producing effector T cells and a shift to microbicidal (M1) macrophages in the lungs of NTM-PD model mice. Mice that received fecal microbiota transplants from L-arginine-treated mice showed increased protective host defense in the lungs against NTM-PD, whereas L-arginine-induced pulmonary host defense was attenuated in mice treated with antibiotics. Using 16S rRNA sequencing, we further showed that L-arginine administration resulted in enrichment of the gut microbiota composition with *Bifidobacterium* species. Notably, oral treatment with either *Bifidobacterium pseudolongum* or inosine enhanced antimicrobial pulmonary immune defense against NTM infection, even with multidrug-resistant clinical NTM strains. Our findings indicate that L-arginine-induced gut microbiota remodeling with enrichment of *B. pseudolongum* boosts pulmonary immune defense against NTM infection by driving the protective gut-lung axis *in vivo*.

### **CRISPR Diagnosis and Therapeutics with High Accuracy**

#### Seung Hwan Lee

Department of Life Science, Chung-Ang University

CRISPR system is a protein and RNA complex imported from the bacterial immune system, and precisely binds to the target nucleotide sequence using guide RNA to induce target DNA mutations. Using these CRISPR system, people have begun to apply them to various organisms for the purpose of serving as a biological tool or medical treatment, and remarkable results are coming out in the field of molecular diagnosis and gene therapy for human system. However, due to the characteristics of CRISPR system, which recognizes a target nucleotide sequence in a guide RNA dependent manner, many off-targeting problems have been reported. Our study was conducted to develop a gene editing technology with improved accuracy for early disease detection or mutant DNA targeted therapy. The high-accuracy CRISPR technologies developed in this study will be used for personalized medicine in human systems in the near future.

MSK2022 International Meeting of the Microbiological Society of Korea

# Symposium [S6]

Co-organized by Chung-Ang University Research Center for Biomolecules and Biosystems (RCBB)

#### **Chaperone Discovery**

#### Changhan Lee

Department of Biological Sciences, Ajou University

Microorganisms are directly exposed to various stresses in their environment that threaten their survival. In order to cope with these damaging conditions, bacteria need to immediately sense changes in the environment and react rapidly and accordingly. Some types of stressors, such as temperature change, desiccation, and acidity, negatively impact protein stability. Since proteins are essential for various molecular reactions in the cell, protein homeostasis is critically linked to the survival of all living organisms. Chaperones are key players that maintain protein homeostasis. A sophisticated network of chaperones exists in every organism, and these chaperones fulfill various roles including preventing protein aggregation, disaggregating aggregated proteins and aiding in folding. However, the lack of *in vivo* tools to monitor chaperone activity limits the discovery of chaperones. In this talk, I am particularly focused on how to find new chaperones by using a protein folding biosensor.

# Nutrient Starvation Promotes Bacteria Chronic Infection by Regulating Protein Homeostasis

Eunmi Chu<sup>1</sup>, Bora Shin<sup>2</sup>, and Jinki Yeom<sup>1,2,3\*</sup>

<sup>1</sup>Department of Biomedical Science, College of Medicine, Seoul National University <sup>2</sup>Department of Microbiology and Immunology, College of Medicine, Seoul National University <sup>3</sup>Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore

When cells encounter nutrient starvation, they enter the dormant state by decreasing the growth rate. Dormant cells can resuscitate once nutrients become available. Dormancy and resuscitation in bacteria cause chronic infection. Here, we report that pathogenic bacterium, such as Salmonella enterica, regulates a growth state and pathogenesis by controlling the proteolysis of functional proteins under nutrient starvation. First, reduction in functional protein degradation resulted from a decrease in the intracellular concentration of ATP that was nonetheless sufficient to allow the continued degradation of nonfunctional proteins by the same proteases. Furthermore, the return to rapid growth from dormant state requires specific proteins because the proteins for nutrient uptake and stress responses are enriched in rapidly recovered bacteria after dormancy. Second, we uncover that reduced proteolysis of magnesium transporter is required for Salmonella growth inside macrophage. The Salmonella small protein MgtR promotes degradation of magnesium transporters by the protease FtsH. By contrast, the small protein MgtU prevents magnesium transporter MgtB proteolysis thereby furthering magnesium homeostasis inside macrophages. This indicate that small proteins can confer pathogen long-term survival under unfavored environments by reducing proteolysis. Taken together, our findings suggest that protein preservation under nutrient starvation regulates bacteria dormancy and resuscitation that leads to chronic infection.

# The Role of Lipoteichoic Acid in the Beta-lactam Resistance of Methicillin-resistant *Staphylococcus aureus*

#### Majid Shah, Amol Kanampalliwar, and Taeok Bae\*

Department of Microbiology and Immunology, Indiana University School of Medicine-Northwest, USA

*Staphylococcus aureus* is a Gram-positive pathogen causing a wide range of human diseases. In particular, the methicillin-resistant *S. aureus* (MRSA) is resistant to almost all beta-lactam antibiotics, rendering the treatment of bacterial infections more difficult. Although the beta-lactam resistance of MRSA is primarily determined by PBP2a, a transpeptidase whose active site is not accessible to most beta-lactams, other cellular factors also modulate the MRSA beta-lactam resistance. One of such factors is lipoteichoic acid (LTA), an anionic polymer attached to the cell membrane of most Gram-positive bacteria and known to be critical for cell division and viability. The following two LTA stresses are known to sensitize MRSA to beta-lactam antibiotics: the reduction of LTA production and the production of aberrantly large LTA, raising the possibility that anti-LTA therapy can revive the efficacy of beta-lactams in the treatment of MRSA infections. This presentation will provide recent progress in our understanding of how LTA modulates the beta-lactam resistance of MRSA.

# Secretin-interacting Proteins Prevent an Outer Membrane Permeability Defect during Type IV Pili Assembly in *Pseudomonas aeruginosa*

Oh Hyun Kwon<sup>1</sup>, Joel W Sher<sup>2</sup>, Bi-o Kim<sup>1</sup>, You-Hee Cho<sup>3</sup>, and Hongbaek Cho<sup>1\*</sup>

<sup>1</sup>Department of Biological Sciences, College of Natural Sciences, Sungkyunkwan University <sup>2</sup>Department of Microbiology, Harvard Medical School, Boston, USA <sup>3</sup>Department of Pharmacy, College of Pharmacy and Institute of Pharmaceutical Sciences CHA University

Type IV pili (T4P) are important virulence factors involved in host attachment and other aspects of bacterial pathogenesis. In Gram-negative bacteria, the T4P filament is polymerized from pilin subunits at the platform complex in the inner membrane (IM) and exits the outer membrane (OM) through the OM secretin channel. Although it is essential for T4P assembly and function, the OM secretin complexes can potentially impair the permeability barrier function of the OM and allow the entry of antibiotics and other toxic molecules. The mechanism by which Gram-negative bacteria prevent secretin-mediated OM leakage is currently not well understood. Here, we report a discovery of SlkA and SlkB (PA5122 and PA5123) that prevent permeation of several classes of antibiotics through the secretin channel of *Pseudomonas aeruginosa* type IV pili. We found these periplasmic proteins specifically interact with the OM secretin complex and prevent toxic molecules from entering through the channel when there is a problem in the assembly of the T4P IM subcomplexes or when docking between the OM and IM complexes is defective. Thus, our results indicate that the secretin-interacting proteins play an important role in maintaining OM permeability barrier, suggesting they may be attractive targets for potentiators that sensitize Gram-negative pathogens to antibiotics that are normally ineffective at penetrating the OM.

# Molecular Mechanism of Zinc-dependent Gene Activation by Zur in *Streptomyces coelicolor*

#### Yunchan Choi, Junseock Koh, and Jung-Hye Roe\*

School of Biological Science, Seoul National University

Metalloregulators are DNA-binding proteins that undergo conformation changes upon metal binding, which, in turn, affects their DNA binding activity. Zur (Zinc Uptake Regulator) is one of the most widespread zinc-specific transcriptional factors that are responsible for zinc homeostasis. *Streptomyces coelicolor* Zur (ScZur) is unique in that it acts both as a repressor and as an activator of a zinc importer gene (*znuA*) and a zinc exporter gene (*zitB*) respectively. ScZur activates the *zitB* gene by forming oligomers toward the upstream sequence of *zitB* promoter in a zinc concentration dependent manner.

Here, we elucidated a molecular mechanism of zinc-dependent gene activation by Zur in *S. coelicolor*. Given that zinc promotes Zur oligomerization toward the upstream region of *zitB* promoter, the zinc binding characteristics of Zur was analyzed by isothermal titration calorimetry (ITC), and confirmed the presence of an additional zinc binding site. Mutational analysis identified an amino acid residue that contributes to an additional zinc binding site critical for zinc ion binding on an additional zinc binding site. Moreover, the residue also significantly contributed to DNA binding activity and oligomerization property of Zur. The zinc-dependent behavior or Zur demonstrates a mechanism by which zinc ions binding to multiple regulatory zinc sites finely tunes both DNA-binding activity and oligomerization property of a metalloregulator.

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# Symposium [S7]

Sponsored by Korea Evaluation Institute of Industrial Technology & Ministry of Trade, Industry and Energy

#### S7-1

# Development of Biosafe *Geobacillus* Platform Cells and Enzyme Production Technology for Industrial Uses

Seong-Bo Kim<sup>1\*</sup>, Dong-Woo Lee<sup>2</sup>, Sang-Jae Lee<sup>3</sup>, and Sung Haeng Lee<sup>4</sup>

<sup>1</sup>Bio-Living Engineering Major, Yonsei University <sup>2</sup>Department of Biotechnology, Yonsei University <sup>3</sup>Major in Food Biotechnology, Silla University <sup>4</sup>Department of Cellular and Molecular Medicine, Chosun University

Genus Geobacillus is an aerobic and facultative aerobic bacteria that grow under a temperature range of 30 to 70°C, with an optimum of 55 to 65°C, and many studies on industrial application of Geobacillusderived enzymes have been reported for advantage of thermo-tolerant characteristics. In particular, Geobacillus stearothermophilus is the only strain noticed in the QPS (Qualified Perception of Safety, European Food Safety Authority) list among Geobacillus genus strains, and it is valued when considering its safe use as an enzyme for food application. Recently, KFDA (Korean Food and Drug Association) determined that the contained use of self-cloned microorganism, cloned only for homozygous recombination not using antibiotics resistance genes, are classified as non-LMO category. In general, the commercial use of LMO microorganism have to be approved by the safety review committee operated by KFDA, this newly occurred non-LMO classification is highly considered as a new opportunity to reduce a lot of time and cost for safety regulation. From this background, the research to construct a Self-Cloned Tool Box derived from *Geobacillus stearothermophilus* was promoted. In this study, the strain derived from domestic resources are newly isolated and identified, and strains showing fast growth rate and enzyme production are selected and culture-optimized for fermentation. Natural gene sources (plasmid, promoter, origin) possessed by screened strains are selected and recombined to construct an optimal vector system for enzyme production. Using these newly constructed self-cloned strains, it is expected to secure a high-temperature continuous production processes based on solid and liquid fermentation condition.

#### **\$7-2**

# Strategic Development of Plant Cell-based Expression System for Anti-aging Related Human Growth Factors

#### Sang Hyun Moh

BIO-FD&C Co., Ltd.

Plants are emerging as an affordable and safe production platform for diverse recombinant proteins beyond traditional biological factories, such as microbial and mammalian cell cultures. Heterogeneous production of recombinant proteins has been performed stably or transiently in plants using Agrobacteria. Nowadays, a variety of proteins including vaccine, antibodies, and pharmaceuticals are produced by transient expression in plant tissues, in a process known as agro-infiltration. However, a cellular stress response is often triggered in the plant cells upon agroinfiltration which block the expression of target genes. To control this regulatory bottleneck, construction of an appropriate expression system is essential to produce proper amount of the proteins. Here, we show that an efficient protein expression system is developed by modulating several parameters such as codon optimization, coexpression of gene silencing suppressor, fusion with soluble tag, and promoter in conjunction with inducer optimization in the expression cassette that resulted in increase in protein yield and protein quality/functionality. Besides, we demonstrate radiofrequency inducible system is efficient in plant-based protein production. Our results represent that, for the successful production of high-value proteins, it is necessary to design production processes compatible with physiology of gene expression in plants, which can further be used for the applications including pharmaceutical and cosmetic development.

#### S7-3

# Overproduction of Cyclic Dipeptides Which Shows Anti-viral, Anti-fungal, and Anti-bacterial Activities in Yeast

#### Gwi Hwi Shin, Yang Gyun No, Ji Eun Shin, Da Ol Im, Rui Liu, and Phun Bum Park<sup>\*</sup>

Department of Bioscience and Biotechnology, University of Suwon

Cyclic dipeptides (CDPs) are secondary metabolites produced by lactic acid bacteria and have antibacterial, anti-fungal, and anti-viral activities. Especially pro-leu, and pro-phe cyclic dipeptides have antibacterial, anti-fungal, and anti-viral activities. The gene for the cyclic dipeptide synthetase was cloned by using the peptide sequencing approach in the native protein gel and the activity of the cyclic dipeptide synthetase was confirmed in the *E. coli* over-expression cell line. For the over-production of CDPs, the cyclic dipeptide synthetase gene was cloned into the pYES 2 vector and expressed in yeast. For the maximum production of CDPs, the culture time, addition of various concentration of nitrogen source, and addition of various condition carbon source were investigated. MSK2022 International Meeting of the Microbiological Society of Korea

# Symposium [S8]

Sponsored by ILDONG Pharmaceutical Co., Ltd.

#### **Commercial Application of Probiotics Isolated in Korea**

#### Jungwoo Yang

Ildong Bioscience, Pyeongtaek, Gyeonggi-do

Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Typically, *Bifidobacterium (adolescentis, animalis, bifidum, breve* and *longum*) and *Lactobacillus (acidophilus, casei, fermentum, gasseri, johnsonii, paracasei, plantarum, rhamnosus,* and *salivarius*) compose a core group of well-studied probiotic genera. On the basis of numerous clinical trials, systematic reviews and meta-analyses, probiotics can be ascribed having certain health outcomes such as in the treatment and prevention of gastrointestinal diseases, enhancement of the immune response, and reduction of serum cholesterol. Sufficient evidence has accumulated to support the concept of health outcomes based on various mechanisms of probiotic action including acid/short chain fatty acids production, regulation of intestinal transit, normalization of perturbed microbiota, competitive exclusion of pathogens, vitamin synthesis and gut barrier reinforcement. In this presentation, several important issues for commercial application of probiotics will be discussed: 1) To assess the safety of probiotics including antimicrobial resistance and cytotoxicity to a murine mouse model; 2) To exemplify functional studies of probiotics in Ildong Bioscience; and 3) To manufacture probiotics for enhancing stability under harsh conditions.

## Gut Microbiota Manipulation in Irritable Bowel Syndrome: a Clinician's View

#### Cheol Min Shin

Internal Medicine, Seoul National University Bundang Hospital

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders seen by primary care physicians. Pathophysiology of IBS is complicated, which makes its diagnosis and treatment challenging. To date, there have been several studies of gut microbiota composition in IBS patients, which showed inconsistent results. Nevertheless, alternations in gut microbiota in IBS (or dysbiosis) might promote the development and maintenance of IBS symptoms. From this background, manipulation of the gut microbiota to improve IBS symptoms has evolved as a novel treatment strategy recently. The low fermentable oligosaccharides, disaccharides, monosaccharides and polyol (FODMAP) diet is widely used in the dietary management of IBS. Non-absorbable antibiotics such as rifaximin has been shown to relieve abdominal symptoms in IBS patients with diarrhea or abdominal bloating. Also, it has been suggested that probiotics can relieve various abdominal symptoms in IBS. To date, the proposed mechanisms of action underlying the beneficial effects of probiotics include competitive exclusion of pathogenic microorganisms, inhibition of pathogen adhesion, production of anti-microbial substances, and modulation of the immune system. Fecal microbiota transplantation (FMT) has recently shown promising results regarding symptom reduction and gut microbiota manipulation, especially in case of severe IBS. However, more studies are necessary to clarify the role of FMT as a treatment for IBS in clinical practice.

### Cas-sgRNA-Ribonucleoprotein Mediated Genome Engineering in *Leuconostoc citreum*

#### Nam Soo Han

Brain Korea 21 Center for Bio-Health Industry, Department of Food Science and Biotechnology, Chungbuk National University

Lactic acid bacteria (LAB) have been used not only for traditional food fermentations, probiotics, potential therapeutics, but also as and cell factories for producing beneficial substances. Studies to explore the gene function of LAB, to improve their probiotic properties, to eliminate their undesired characteristics, and even to confer new desirable properties are still ongoing. To date, efficient and accurate gene editing approaches based on CRISPR-Cas9 technology and its variants have been developed for LAB. However, the delivery of Cas9 and sgRNA still remain as challenge due to the large size of Cas9 and requirement of viral vectors, which pose safety concerns for therapeutic genome editing. Cas9-sgRNA-ribonucleoprotein complex is an attractive non-viral formulation for CRISPR-mediated gene editing due to develop the Cas RNP mediated genome engineering in lactic acid bacteria. To achieve the goal, we developed RNP based genome engineering technology, in which, preassembled RNP targeting dextransucrase gene were transformed into competent cells of *Leuconostoc citreum* by electroporation. As result, mutants with significantly reduced EPS production were successfully obtained on sucrose-containing agar medium. This study highlights that DNA-free genome editing tools using preassembled Cas9 and sgRNA can be efficiently used to knock-out target gene in *L. citreum*.

# Validation of Probiotic Species Identification in Probiotic Products Using Real-time PCR Method Based on Large-scale Genomic Analysis

#### Hae-Yeong Kim

Department of Food Science and Biotechnology, Kyung Hee University

The health benefits of probiotics are strain-specific, and hence it is important to declare the correct information in probiotic products. However, several studies have found significant inaccuracies with respect to the mentioned components on the label claim. This emphasizes the need for a consistent novel method for performing quality checks in probiotic and dairy products. In this study, a real-time PCR method based on pan-genome analysis was developed to identify commercial probiotic and dairy products more rapidly and accurately than sequencing methods. Twenty-five species or subspecies that can be primarily used for probiotic strains were selected as the target. Assessment of whole genome sequences revealed that the genomes of some Lacticaseibacillus, Lactobacillus, and Bifidobacterium species genomes deposited in GenBank were misclassified. To mine molecular markers, genetic marker genes were identified by comparing genomic sequences present in the target genome but not present in the pangenome of other genomes. All genetic markers exhibited 100% specificity for reference strains and successfully constructed the criterion for the guantified standard curve in real-time PCR. Probiotic and dairy products marketed worldwide were investigated to verify the information claimed on the label. Real-time PCR results showed that most products reflected the bacterial species declared in the label claim, whereas some products did not match or showed the presence of missing species. There was a discrepancy in the nomenclature of closely related species for these products. Real-time PCR method developed in this study for accurately verifying the labeling of probiotic and dairy products would be useful for quality control and safety.

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# Symposium [S9]

Co-organized and Sponsored by Rznomics Inc.

# High-resolution Analysis of Human Coronavirus Transcriptomes

Hanju Lee<sup>1,2</sup>, Dongwan Kim<sup>2,3</sup>, Ari Hong<sup>1,2</sup>, Jayoung Ku<sup>2,3</sup>, Uni Park<sup>4</sup>, Jeong-Sun Yang<sup>5</sup>, Jun Won Kim<sup>5</sup>, Joo-Yeon Lee<sup>5</sup>, Nam-Hyuk Cho<sup>4</sup>, V. Narry Kim<sup>2,3</sup>, and Hyeshik Chang<sup>1,2,3\*</sup>

<sup>1</sup>Interdisciplinary Program in Bioinformatics, Seoul National University <sup>2</sup>Center for RNA Research, Institute for Basic Science <sup>3</sup>School of Biological Science, Seoul National University <sup>4</sup>Department of Microbiology and Immunology, Seoul National University College of Medicine <sup>5</sup>Center for Infectious Diseases Research, Korea National Institute of Health

Seven different human coronaviruses have been identified since the first human common cold virus, B814, was discovered in 1965: 229E, NL63, OC43, HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2. Coronaviruses use discontinuous transcription to produce multiple types of subgenomic messenger RNAs (sgmRNAs) by skipping particular regions during transcription. The donor and acceptor sites of template switching contain transcription regulatory sequences (TRSes), which control discontinuous transcription. The stochastic template switching shapes the viral transcriptome and proteome. In addition, it frequently initiates recombination between unexpected sites within a viral genome or between different viral strains infecting the same host cell. Using three complementary methods, including short-read poly(A)+ and total RNA sequencing, nanopore direct RNA sequencing, we sequenced the transcriptomes of cells infected with one of six human coronaviruses. Here we show the conserved and divergent regulations applied to sgmRNAs of human coronaviruses by comparing transcript structures, expression levels, and epitranscriptomic changes.

# Cell-free Systems for Onsite Detection of Viral RNAs and Rapid Discrimination of SNPs

#### Jeong Wook Lee<sup>1,2</sup>

<sup>1</sup>Department of Chemical Engineering, POSTECH <sup>2</sup>School of Interdisciplinary Bioscience and Bioengineering, POSTECH

Cell-free systems provide flexibility to characterize biological components and modulate molecular devices. This capability is often harnessed to extend cell-free systems for a variety of purposes. In this talk, I will introduce two cell-free transcription systems. One is to characterize biological components, and the other is to develop molecular reaction cascades to detect RNA in a one-step reaction. Characterization of biological components in a non-model organism is often challenging due to the limited genetic engineering tools. We use CRISPR/Cas12a to detect output RNAs transcribed from promoter candidates in cyanobacteria cell-free transcription system. We demonstrate cyanobacteria cell-free transcription for the first time and confirmed a positive correlation between the in vitro and in vivo transcription performance. Furthermore, we generate a synthetic promoter library and investigate the characteristics of promoter subregions by using the assay. The second example is about the cell-free transcription system used in an on-site RNA detection method. The assay relies on a sustained isothermal reaction cascade producing an RNA aptamer that binds to a fluorogenic dye. The RNA aptamer is transcribed by the T7 RNA polymerase from the ligation product of a promoter DNA probe and a reporter DNA probe that hybridized with the target single-stranded RNA sequence via the SplintR ligase. With nasopharyngeal SARS-CoV-2 samples, the assay reached positive and negative predictive values of 95 and 100%, respectively. We also demonstrate that the assay can rapidly detect a range of viral and bacterial RNAs and discriminate SNPs between the substrains. Overall, we show that cell-free systems can be easily used and designed to resolve academic and practical issues. We will continue to expand cell-free systems for various applications, including therapeutics development, parts mining, etc.

# Initiation tRNA<sup>fMet</sup> Cleavage by Toxin-antitoxin System: Survival Mechanism of Extremophile under Extreme Cold Condition

Eunsil Choi<sup>1,2</sup>, Hyerin Jeon<sup>1</sup>, and Jihwan Hwang<sup>1,2\*</sup>

<sup>1</sup>Department of Microbiology, Pusan National University <sup>2</sup>Microbiological Resource Research Institute, Pusan National University

Toxin-antitoxin (TA) systems are ubiquitous genetic modules among bacteria and composed of a toxin interfering with a wide range of cellular processes and its cognate antitoxin, which counteracts the activity of the toxin. Initially identified as plasmid maintenance elements, they have been shown to be involved in the formation of persister cells and inhibit some phage infections. They also participate in the adaptation of bacteria to unfavorable conditions. The Bosea sp. PAMC 26642 used in this study was isolated from the Arctic lichen Stereocaulon sp.. There are twelve putative type II TA loci in the genome of Bosea sp. PAMC 26642. Of these, nine functional TA systems have been shown to be toxic in Escherichia coli. The toxin inhibits growth, but this inhibition is reversed when the cognate antitoxin genes are co-expressed, indicating that these putative TA loci were bona fide TA modules. Only the BoVapC1 (AXW83 01405) toxin, a homolog of VapC, showed growth inhibition specific to low temperatures, which was recovered by the co-expression of BoVapB1 (AXW83 01400). Microscopic observation and growth monitoring revealed that the BoVapC1 toxin had bacteriostatic effects on the growth of E. coli and induced morphological changes. RNA analyses showed that the BoVapC1 toxin had a ribonuclease activity on the initiator tRNA<sup>fMet</sup>, implying that degradation of tRNA<sup>fMet</sup> might trigger growth arrest in *E*. coli. Furthermore, the BoVapBC1 system was found to contribute to survival against prolonged exposure at 4°C. This is the first study to identify the function of TA systems in cold adaptation.

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# Development of RNA Editing Therapeutics Based on Tetrahymena Group I Intron

#### Seong-Wook Lee

Department of Bioconvergence Engineering, Research Institute of Advanced Omics, Dankook University and R&D Center, Rznomics Inc.

The Tetrahymena group I intron-based trans-splicing ribozyme enables to sense and reprogram target RNA into gene of interest. We are developing the ribozyme-based RNA editing strategy through RNA replacement or repair as a gene therapeutic strategy for incurable human diseases including malignant, degenerative, and hereditary diseases. We have modified and optimized ribozymes for application as therapeutic modalities by developing them to have high target specificity and efficacy, targeting fidelity, and least off-target capacity in cells. This RNA editing technology has unique features that differentiate it from other nucleic acid-based therapeutics as follows: (1) A single substance has a cumulative effect of suppressing target RNA expression and simultaneously expressing a therapeutic gene. (2) Safety can be improved by selectively inducing therapeutic gene expression only in cells/tissues in which the target gene is expressed. (3) Therapeutic gene expression can be regulated proportionally to endogenous cellular target RNA levels. (4) Gene editing occurs at the RNA level, not the genomic level, thus minimizing genomic toxicity and eternal genome changes. (5) Indications with multiple mutation sites can be targeted and treated through downstream replacement of the target site in RNA. (6) Safety can be conferred by acting as a mechanism of the ribozyme itself, without the need for intrinsic cellular mechanism or external proteins. We have built pipelines targeting indications with highly unmet medical needs for which these characteristics may be the most competitively applied. In this presentation, I will introduce in detail the characteristics of group I intron-based RNA editing approaches and focus on recent progress of our leading pipelines, specifically for (1) malignant diseases including hepatocellular carcinoma and glioblastoma and (2) inherited degenerative ocular disease called Retinitis Pigmentosa.

MSK2022 International Meeting of the Microbiological Society of Korea

# Symposium [S10]

Co-organized by Skin-Bio Research Cluster, Microbiomics Institute - Chung-Ang University

# Investigation of the Temporal Variation of Bacterial Colonization and Antibiotic Resistance in Microplastic on Marine Environment

#### Hyunsu Kim and Keunje Yoo\*

Department of Environmental Engineering, Korea Maritime and Ocean University

Microplastic pollution in the marine environment has become a major global issue that threatens marine ecosystem and human health. Biofilm-forming bacteria form a specific community on microplastic surfaces and can absorb harmful pollutants, such as antibiotic resistance bacteria and/or gene from the surrounding environment. Nevertheless, the dynamic changes in the microbial community and antibiotic resistance accumulation in marine microplastics remain largely unknown. Thus, in this study, four types (PE, PP, PS and PVC) of microplastics were incubated in the marine environment for three periods (46, 63, and 102 days) in Busan city, South Korea. As a result of morphology analysis using Scanning Electron Microscopy (SEM), the biofilm density on the MP surface was relatively higher in PE and PP than PS and PVC. Moreover, the microbial community based on 16S rRNA genes showed that although Proteobacteria, Bacteroidetes, and Firmicutes, which are known as biofilm-forming bacteria were dominant in all microplastic samples, their proportions were different according to polymer type and exposure time. In addition, there was significant differences in the diversity of the bacterial diversity according to the polymer types (P < 0.05). The qPCR results indicated that total abundance of antibiotic resistance genes (ARGs) in PVC samples were the highest  $(1.18 \times 10^8 \text{ copies per 16S rRNA})$  compared with other polymers. Moreover, microplastic samples were a relatively 2-5 times higher abundance of ARGs than sea water samples. Among the identified ARGs, Sul1 and Sul2 genes (sulfonamide) were predominant in all polymer types, significantly increasing during incubation (p <0.05). These findings provide important insights into understanding biofilm-forming bacteria and the marine ecological risk of ARGs in the plastisphere. [Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science, ICT & Future Planning) (No.2020R1C1C1008951).]

# Identification and Application of Bacterial and Fungal Denitrifiers to Remove Nitrate from Agricultural Subsurface Drainage

#### Satoshi Ishii<sup>1,2</sup>

<sup>1</sup>Department of Soil, Water, and Climate, University of Minnesota, USA <sup>2</sup>BioTechnology Institute, University of Minnesota, USA

Agricultural subsurface drainage is commonly used in the Midwest U.S. and Europe to improve soil conditions for plant growth. However, it has also increased the amount of nutrients, especially nitrate, released from fields into surrounding waterways, causing eutrophication. One approach to remove nitrate from subsurface drainage water is to install denitrification bioreactors at the end of the drainpipes before water is discharged to ditches or streams. Although woodchip bioreactors have demonstrated success in nitrate removal in many field locations, low water temperature during the cold seasons significantly limits bioreactor performance. We have been trying to improve the nitrate removal of denitrification bioreactors at cold temperatures by using biotechnology and applied microbiology approaches. We first identified cold-adapted denitrifiers in woodchip bioreactors by comparing bioreactor microbiomes amended with and without nitrate. The N cycle gene abundance was also examined by using high-throughput nitrogen cycle-associated gene quantification (NiCE chip). The cold-adapted denitrifying bacteria were then isolated and inoculated to the field-scale bioreactors (i.e., bioaugmentation). We also injected labile carbon to enhance microbial activities (i.e., biostimulation). While bioaugmentation and biostimulation enhanced nitrate removal, the effects did not last long most likely due to the washout of bacteria from the reactors. To solve this problem, we also isolated cold-adapted, cellulose-degrading, and nitrate-reducing fungi from woodchip bioreactors. Field testing of these fungal strains has yet to be done, but fungi might be better retained in the bioreactors due to their hyphal networks. These approaches are promising to mitigate nitrate pollution in agricultural subsurface drainage.

# Genomic Adaptation to Extreme Environments in Thermoacidophilic Red Algae

#### Chung Hyun Cho and Hwan Su Yoon\*

Department of Biological Sciences, Sungkyunkwan University

Life near hot springs has a critical impact on cellular components due to high temperature, acidic, and heavy-metal rich environments. Cyanidiophyceae (Rhodophyta) is a photosynthetic eukaryote that thrives in extreme conditions around the hot spring regions of the world. Based on a few distinct characteristics (e.g., phylogenetic position, morpho-ecological traits, energy production system), Cyanidiophyceae are divided into two major groups, Cyanidiales and Galdieriales. According to previous genomic studies, exotic prokaryotic genes have been discovered in the nuclear genomes of these groups allowing them to adapt to polyextreme environments. Following the foregoing, Cyanidiophyceae species have been proposed as an excellent eukaryotic model for studying the relationship between environmental adaptability and genome evolution. Here, we reconstructed 20 chromosomes of two Cyanidiales species, Cyanidiococcus and Cyanidium, and a pseudochromosome-level assembly of the Galdieriales genome with a precise gene modeling procedure based on multiple platform-derived transcriptome data. Our comprehensive analysis, which includes previously inaccessible Cyanidium genome, provides a detailed view of entire cyanidiophycean evolution, not only gained through HGTs, but also a few other genomic evidence, such as subtelomeric gene amplification of functional genes and the elimination of superfluous eukaryotic traits. Furthermore, our findings may shed new light on cyanidiophycean adaptation strategies in extreme environments, as well as why different species in each lineage prefer different microhabitats.

### Unravelling Drivers of Pathogen Emergence: from Selfish Elements to Chromosomes

#### Daniel Croll

Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel, Switzerland

Adaptation in fungal pathogens attacking crops procedes often at speeds that easily overwhelm our ability to identify resistant cultivars and develop new antifungals. Hence, fungal pathogens pose severe risks to global food security. Recent outbreaks and recurrent breakdowns of fungicide efficacy are clear indications of this threat. Understanding the molecular basis of pathogen adaptation is critical to define more sustainable containment strategies. Here, I will show how studying variation within emerging pathogen species enables insights into pathogen adaptation and future trajectories. For this, we perform joint analyses of thousands of genome sequences, genome-wide transcription and phenotypic trait variation data. I will highlight how dynamic regions in pathogen genomes make the most significant contributions to recent pathogen adaptation. Finally, I will argue how understanding constraints on pathogen adaptation provides an avenue for more sustainable crop production.

## *Cutibacterium acnes* in the Gut of the Marine Polychaete *Capitella teleta* Degrading Polycyclic Aromatic Hydrocarbons

Jeonghwan Jang<sup>1\*</sup>, Valery E. Forbes<sup>2</sup>, and Michael J. Sadowsky<sup>3</sup>

<sup>1</sup>Division of Biotechnology, Jeonbuk National University <sup>2</sup>Department of Ecology, Evolution, and Behavior, University of Minnesota, USA <sup>3</sup>BioTechnology Institute, University of Minnesota, USA

Capitella teleta is an opportunistic sediment-feeding marine polychaete frequently found with high density in organically enriched sediment of estuarine environments such as sites contaminated with sewage or petroleum. The invertebrate is known to degrade various polycyclic aromatic hydrocarbons (PAHs) from oil spill, however, it is unclear whether and how the worm's gut microbiota contribute to PAHs degradation. To elucidate the role of the gut microbiota in C. teleta, worms incubated in marine sediment microcosms were investigated for their microbiome changes according to the microcosm conditions. Although direct evidence showing that the gut microbiota contribute to PAHs degradation was not found, we successfully isolated strains of Cutibacterium acnes from the worm's gut, in which the bacterial genus was observed to be a member of the core microbiota in C. teleta. Thirteen C. acnes strains isolated from worms were predominantly belonged to phylotype IB group and produce propionate and vitamin B<sub>12</sub> in vitro, which are essential microbial metabolites usable by the host. Presence of C. acnes in C. teleta was consistent despite of antibiotic treatment and different culture history in geographically distant laboratories for worms. Collectively, C. acnes is suggested to be truly a member of the worm's core functional microbiota selectively favored by physiology and gut environment of the host. This is the first interesting report of the C. acnes presence in the C. teleta gut for host-microbe mutual symbiosis since C. acnes has been mainly studied as an opportunistic pathogen on human skin or body parts.

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# Symposium [S11]

Sponsored by Cell Biotech

## Human Intestinal Organoid as a Platform for Microbiome Research

## Hana Lee<sup>1§</sup>, Kwang Bo Jung<sup>1§</sup>, Ohman Kwon<sup>1§</sup>, Ye Seul Son<sup>1</sup>, Dae-Soo Kim<sup>1\*</sup>, Doo-Sang Park<sup>2\*</sup>, and <u>Mi-Young Son<sup>1\*</sup></u>

<sup>1</sup>Korea Research Institute of Bioscience and Biotechnology (KRIBB), Stem Cell Convergence Center <sup>2</sup>Department Korean Collection for Type Cultures, Biological Resource Center, KRIBB

The small intestine (SI) is a complex organ with multiple histological and functional structures that promote efficient nutritional absorption, control intestinal microorganisms, and provide protection and defense against pathogens and toxins. Several studies have shown that a stepwise differentiation process can efficiently produce human intestinal organoids (hIOs), a three-dimensional (3D) structure of epithelial cells derived from human pluripotent stem cells (hPSCs). Recently, we described an in vitro maturation technique for generating adult-like, mature 3D hIOs from hPSCs that closely resemble the in vivo tissue structure and cellular diversity. In this study, we examined the function of Lactobacillus spp. and evaluated its probiotic properties from the perspective of human intestinal maturation and functional development using the hPSC-derived hIO system, which is a physiologically relevant developmental and disease model of the human intestine. We identified a novel strain of Limosilactobacillus reuteri, isolated from the feces of a healthy infant, and showed that L. reuteri-derived metabolites have beneficial effects on intestinal maturation, as they induced the transition of immature hIOs into adult-like mature hIOs and improved the intestinal development of infant mice. Experiments in a hIO-based intestinal inflamed model revealed that the cell-free supernatant from L. reuteri comprising biologically active metabolites was important for intestinal protection against cytokine-induced intestinal epithelial injury. Our findings indicated that novel L. reuteri strain has potential to become a useful agent for treatment for disorders of early gut development and prevention of intestinal barrier dysfunction.

# ALS Filament Formation by ROS-induced SOD1 and its Inhibition by Tannins

## Yeongjin Baek and Nam-Chul Ha\*

Department of Agricultural Biotechnology and Research Institute of Agricultural Life Sciences, Seoul National University

Eukaryotic Cu, Zn-superoxide dismutase (SOD1) is primarily responsible for cytotoxic filament formation in amyotrophic lateral sclerosis (ALS) neurons. Two cysteine residues in SOD1 form an intramolecular disulfide bond. This study determined the crystal structure of the double mutant (C57D/C146D) SOD1 that mimics the overoxidation of the disulfide-forming cysteine residues. The double mutant SOD1 produced more contagious filaments than wild-type protein, promoting filament formation of the wild-type SOD1 proteins. Importantly, we further found that HOCI treatment to the wild-type SOD1 proteins facilitated their filament formation. We next screened natural compounds inhibiting filament formation of the SOD1 protein. Four compounds belonging to the soluble tannin family inhibited the filament formation of SOD1. The compounds also inhibited filament formation of the mutant SOD1 protein. Combined with these findings, we propose a feasible mechanism for SOD1 filament formation in ALS from the wild-type SOD1, where tannins inhibit this filament formation process. Our findings will help develop drugs or functional food materials preventing neurodegenerative diseases caused by protein filaments, such as Alzheimer's disease.

## Effect of Probiotics-produced Bacteriocin on Skin Microbiome of Acne Patients

#### Woo Jun Sul

Department of Systems Biotechnology, Chung-Ang University

Dysbiosis of the skin microbiome is considered to be the cause of acne, with a particular focus on *Cutibacterium acnes. Enterococcus faecalis* SL-5, a LAB strain isolated from the human gut, is known to have substantial antimicrobial activity against gram-positive bacteria, especially *C. acnes.* Therefore, we identified that the extract of *E. faecalis* SL-5 would improve the condition of acne skin patients, and we tried to research the changes in the skin microbiome accordingly. Twenty patients were enrolled in this randomized, placebo-controlled, split-face comparative study. We performed DNA extraction with skin surface samples and sebum samples, and after sequencing using the Illumina MiSeq platform, microbiome analysis was performed through QIIME2 pipeline. In the case of skin surface samples, phylogenetic diversity decreased after applying a lotion containing *E. faecalis* extract compared to before application. This phenomenon was more significant in the test site than in the control site. However, there was no significant difference in the sebum samples. In addition, when the skin condition was assessed as improved by the investigator's assessment, the PD was significantly lower in the skin surface samples than in the non-improvement samples, as was the case in the sebum sample. It could be expected that some microbes among skin microbes were removed by bacteriocin contained in *E. faecalis* extract, leaving only species at a close distance in the phylogenetic tree.

## Anti-cancer Role of Probiotic-derived P8 Protein in Colorectal Cancer

### Byung Chull An, Jun Young Ahn, Yongku Ryu, and Myung Jun Chung\*

R&D Center, Cell Biotech, Co., Ltd.

Probiotics are beneficial microbes that inhabit human intestine. These microbes have shown beneficial effects in several diseases, including colorectal cancer (CRC). Probiotics may serve as drug delivery systems for the secretion of proteins, such as P8, with anti-CRC effects, indicating that probiotics that secrete biotherapeutics may overcome the limitations of existing anti-cancer chemical drugs.

We have developed a novel approach to screen for useful biotherapeutic agents secreted by probiotics. Furthermore, probiotics can be designed as drug delivery systems to deliver biotherapeutic agents. We had previously described the use of probiotics to deliver a *Lactobacillus rhamnosus*-derived therapeutic protein, P8, which suppressed CRC proliferation. The *L. rhamnosus* gene encoding P8 was incorporated into *Pediococcus pentosaceus* SL4, generating a genetically engineered *P. pentosaceus* SL4 secreting P8 (PP-P8). We found that P8 could enter the cytosol of DLD-1 cells through endocytosis, translocate into the nucleus, and result in anti-proliferation and anti-migration.

The present study analyzed the anti-cancer mechanism of actions (MOAs) of P8. We first identified the initial targets, GSK3 $\beta$  and Smad1, for P8 mediated anti-cancer activities using P8 pull-down of DLD-1 cell lysates. We also investigated signal trasductions which were mediated P8-GSK3 $\beta$  or P8-Smad1.

To our knowledge, this study is the first to provide evidences about the P8-GSK3 $\beta$ -cell cycle arrest and P8-Smad1-EMT regulatory networks in CRC, suggesting that P8 is a potential new biotherapeutic agents. Moreover, evaluation of the toxicity of the *Pediococcus pentosaceus* SL4-based P8 delivery system (PP-P8) provides valuable data on the safety of P8 and the probiotic-based delivery system, suggesting that P8 may the first agent originating from a probiotic that can be used in anti-cancer drug development.

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# Symposium [S12]

## **Dynamics in the Human Gut Microbiome**

## Lisa Olsson<sup>1\*</sup>, Valentina Tremaroli<sup>1</sup>, and Fredrik Bäckhed<sup>1,2,3</sup>

<sup>1</sup>The Wallenberg Laboratory, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden <sup>2</sup>Region Västra Götaland, Department of Clinical Physiology, Sahlgrenska University Hospital, Gothenburg, Sweden <sup>3</sup>Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

The human gut is home to trillions of bacteria that can complement our own genetic functions, which we have not evolved, and thus contribute to host metabolism and physiology. The gut microbiome starts to develop from birth and co-develop with the rest of our physiology and immune system. The ecosystem of the gut is dynamic just like any other ecosystem on earth. Compare to other environments on our body the microbiome in the gut is specific for that environment, and all children follow the same trajectory in their gut metagenome development. At the same time the gut microbiome composition in adults is specific to the individual and the composition in non-disease individual is mostly stable over time. However, 23% of the variation in the microbiota can be assigned to the dynamic within the individual over time. When assessing the microbiome contribution to health or disease including the contribution of the community stability and these dynamics will be important for identifying reliable markers of functional changes in the gut microbiome.

## Faecalibacterium prausnitzii Pathobionts Play a Pivotal Role in the Onset of Atopic Dermatitis

#### **Heenam Stanley Kim**

Division of Biosystems & Biomedical Sciences, College of Health Sciences, Korea University

The gut microbiota plays a pivotal role in human physiology and their malfunction has been linked to chronic diseases, including atopic dermatitis and inflammatory bowel diseases. We have been investigating the interrelationship between the aberrant gut microbiota and atopic dermatitis. We recently discovered that subspecies-level misbalance in one of the main gut bacteria, *Faecalibacterium prausnitzii*, underlies the onset and/or progression of atopic dermatitis. *F. prausnitzii* has long been known as one of the most beneficial species in the human gut. However, we isolated subspecies of *F. prausnitzii* that act as pathobionts, enrichment of which in the gut is associated with the disease. In a murine model, the atopic-disease potential caused by the *F. prausnitzii* pathobionts in dams were inter-generationally transferred to their pups, demonstrating the phenomenon similar to the development of atopic dermatitis in human infants. Increased incidences of atopic dermatitis over the past decades are largely due to lifestyle changes and misbalance in the *F. prausnitzii*-driven gut microbiology is in its early stage, it will be of tremendous significance in the future to understand the onset and the progression of atopic dermatitis and various chronic diseases.

## **Exploring the Association between Microbiome and Asthma**

## Sungmi Choi<sup>1</sup>, Kyoung-Hee Sohn<sup>2</sup>, Young-Chan Kim<sup>3</sup>, Hye-Ryun Kang<sup>3\*</sup>, and Hana Yi<sup>1,4\*</sup>

<sup>1</sup>Interdisciplinary Program in Precision Public Health, Korea University <sup>2</sup>Department of Internal Medicine, Kyung Hee University Hospital <sup>3</sup>Department of Internal Medicine, Seoul National University Hospital <sup>4</sup>School of Biosystems and Biomedical Sciences, Korea University

Asthma is a heterogeneous, complex disease comprising distinct airway inflammation phenotypes and endotypes. Among the many explanations for these heterogeneities, the microbiome has come into the spotlight as one of the major players regulating inflammation. However, the previous findings on asthma microbiome were not consistent among studies and the key microbiota associated with asthma remain elusive. Overcoming those challenges will provide more insight into the pathogenesis of asthma. Therefore, we aimed to understand the characteristics of the asthmatic microbiome comprehensively by analyzing and integrating the multi-omics data. The induced sputum, feces, and blood were collected from healthy participants and asthma patients and analyzed for the bacteriome, virome, functional metagenome, and transcriptome. The association between host genetic factor and the lung microbiome in asthma was investigated using peripheral blood transcriptomics. The result uncovered three transcriptomic clustering is present in the tested asthma patients, but the lung bacteriome did not differ depending on the host genetic clusters. This may be due to that the immune response induced by the microbiome may be limited to the local environment. The gut bacteriome analyses revealed that gut microbiome affect the endotypes of asthma supporting the gut-lung axis hypothesis, though further investigation is required to uncover the mechanisms mediating bidirectional crosstalk between the gut microbiome and host immune system. The result of the lung bacteriome and virome discovered potential biomarkers for better diagnosis and new therapeutic targets in asthmatic patients. Asthma severity was associated with reduction in the abundance of the bacterial and viral biomarkers in asthmatic airways and the increased polymicrobial interactions centered on the biomarkers. The possibility of therapeutic intervention and diagnostic usage of the newly discovered microbial species is under way through mechanistic study.

## Microbiota-induced Vitamin A Transport and Its Role in the Intestinal Immunity

#### Ye-Ji Bang

Department of Microbiology and Immunology, Seoul National University College of Medicine

Vitamin A and its derivative retinol are essential for the development of intestinal adaptive immunity. Retinoic acid (RA)-producing myeloid cells are central to this process, but how myeloid cells acquire retinol for enzymatic conversion to RA is unknown. Here, we show that serum amyloid A (SAA) proteins, retinol binding proteins induced in intestinal epithelial cells by the microbiota, deliver retinol to myeloid cells. We identify LDL receptor-related protein 1 (LRP1) as an SAA receptor that facilitates endocytosis of SAA-retinol complexes and promotes retinol acquisition by RA-producing intestinal myeloid cells. Consequently, SAA and LRP1 are essential for vitamin A-dependent immunity, including T and B cell homing to the intestine and immunoglobulin A production. Our findings identify a key mechanism underpinning vitamin A's effects on the immune system and provide molecular insight into how the microbiota promotes intestinal immunity.

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# Symposium [S13]

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## **Biological Production of 3-Hydroxypropionic Acid**

Nam Hoai Nguyen<sup>1</sup>, Somasundar Ashok<sup>1</sup>, Hang-Duk Roh<sup>1</sup>, Thuan Phu Nguyen-Vo<sup>2</sup>, and Sunghoon Park<sup>2\*</sup>

> <sup>1</sup>Biotechnology Laboratory, NOROO Holdings Co., Ltd. <sup>2</sup>School of Energy and Chemical Engineering, UNIST

We present biological production of 3-HP from crude glycerol at high titer (>100 g/L), rate (>3 g/L/h) and yield (>0.95 mol/mol glycerol or >0.7 mol 3-HP carbon/mol substrate carbon) on 100 L pilot-scale. *Pseudomonas* sp. which can aerobically synthesize coenzyme  $B_{12}$ , an essential and expensive cofactor, was used as host, and heterologous 3-HP synthesis pathway composed of the  $B_{12}$ -dependent glycerol dehydratase (GDHt) and NAD<sup>+</sup>-dependent aldehyde dehydrogenase (ALDH) was expressed under the control of dynamic promoters which are upregulated (30—150 fold) by the target product 3-HP. The activities of GDHt and ALDH were maintained at a high level till the end of fermentation, without serious inactivation caused by oxygen, highly toxic intermediate 3-hydroxypropionaldehyde (3-HPA), and/or the target product 3-HP. Furthermore, the 3-HP inducible promoters were extensively engineered to improve their strength while maintaining the inducibility by 3-HP, and the 3-HP synthesis pathway was incorporated into the chromosome for industrial use of the recombinant. When grown in industrial culture medium, the resulting recombinant *P. denitrificans* showed high activity and stability and the abovementioned TRY could be achieved at low cell density < 5 g/L within 36 h.

## Biological PET Decomposition; Mechanism, Protein Engineering, and Applications

#### Kyung-Jin Kim

School of Life Sciences, Kyungpook National University

The worldwide production and use of plastics have led to the accumulation of non-biodegradable plastic waste in landfills and ocean, resulting in serious environmental problems. Poly(ethylene terephthalate) (PET) composed of terephthalic acid and ethylene glycol linked by ester bond is one of the most commonly used plastics for fiber and packaging materials. Although various microbial hydrolases including cutinases, lipases, and carboxylesterases have been reported to degrade PET, the biodegradation of high crystallinity PET under ambient temperature remains infeasible. Recently, the bacterium Ideonella sakaiensis 201-F6 was isolated and it uses PET as a carbon source at moderate temperature (30°C) using PET hydrolase (PETase) and monohydroxyethyl terephthalate hydrolase (MHETase). In this study, the extensive structural and biochemical studies on IsPETase and IsMHETase suggest the mechanism of PET degradation. Furthermore, we developed the variants with remarkably enhanced thermal stability and highly improved PET degradation ability based on structure-based protein engineering.

## Biosynthesis of Bio-degradable Plastics by Metabolically Engineered Microorganisms

#### Si Jae Park

Department of Chemical Engineering and Materials Science, Ewha Womans University

Biodegradable plastics synthesized by microorganisms have been suggested as one of the promising solutions to solve plastic waste problems since complete biodegradation after use of plastics may be ultimate solution to remove plastic accumulation caused by improper disposal of non-degradable plastics. Also, bio-based plastics synthesized from renewable resources can be regarded as carbon-neutral materials that can reduce CO<sub>2</sub> emission, thus, global warming problems caused by green house gas emission can also be solved by using these kinds of plastics. Development of bioprocesses for the production of bio-based plastics have much examined by employing natural and metabolically engineered microorganisms as host strains, in which bio-based plastics are synthesized as final products in biological manner or precursors produced by bioprocesses for bio-based plastics are used for further chemical process for the synthesis of polymers.

In this presentation, the strategies for bio-based production of biodegradable polymers are mainly discussed introducing polyhydroxyalkanoates as main examples.

## Discovery and Development of Plastic Biodegradable Biocatalyst

#### Soo-Jin Yeom

School of Biological Sciences and Technology, Chonnam National University

Plastic contamination currently threatens a wide variety of ecosystems and presents damaging repercussions and negative consequences for many wildlife species. Sustainable plastic waste management is an important approach to environmental protection and a necessity in the current life cycle of plastics. In this regard, plastic biodegradation by microorganism is most notable. This presentation includes current plastic pollution trends, screening of plastic biodegradable new bacteria and the research hypothesis is that our common area is already surrounded with a bunch of plastics, so it could be possible to screen plastic-degrading bacteria, although a trash-contaminated uncommon environment might have many plastic-degrading bacteria. In addition, we provide proposal to use hypothetical P450 enzymes as potent trigger biocatalysts to biodegrade polyethylene (PE) via in-chain hydroxylation into smaller products of linear aliphatic alcohols and alkanoic acids based on cascade enzymatic reactions. Furthermore, we propose adopting P450 into plastic-eating synthetic bacteria for PE biodegradation. This strategy can be applicable to other dense plastics, such as polypropylene and polystyrene.

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# Symposium [S14]

## Differential Mycobiome Dysbiosis in CD, UC, and IBS

## Eun Young Huh<sup>1,2</sup>, Kijung Kim<sup>3</sup>, Courtney Smith<sup>2</sup>, Chang Hwan Choi<sup>3</sup>, and Soo Chan Lee<sup>1\*</sup>

<sup>1</sup>South Texas Center for Emerging Infectious Diseases (STCEID), Department of Molecular Microbiology and Immunology, The University of Texas at San Antonio, San Antonio, Texas, USA

<sup>2</sup>Current Address: Department of Maxillofacial Injury and Disease, Naval Research Unit - San Antonio (NAMRU-SA), Texas, USA

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<sup>3</sup>Department of Internal Medicine, College of Medicine, Chung-Ang University

Inflammatory bowel diseases (IBDs) are characterized by chronic patchy inflammation on any part of the GI tract (Crohn's disease) or continuous (non-patchy) inflammation on the colon and rectum (ulcerative colitis). And irritable bowel syndrome (IBS) is associated with abdominal pain and abnormal bowel movement. There has been rising interest in the roles of enteric microbiome in these gastrointestinal diseases. This study characterizes and analyzes the intestinal bacteriome and mycobiome in adult South Korean patients with Crohn's disease (CD) (n=35) or ulcerative colitis (n=78) or IBS (n=92) along with healthy individuals (n=119). Gut microbiome composition was analyzed with Illumina sequencing of the V4 hypervariable region of the 16S rDNA gene for bacteria and internal transcribed spacer 1 (ITS1) for fungi amplified from stool DNA samples from the patients.

In both types of IBD's, a significant decrease in bacterial alpha diversity (species richness) and a significant increase in fungal alpha diversity were observed compared to HC using the Chao1 estimator of diversity. In IBS, interestingly, both bacterial and fungal alpha diversities were decreased. When species components were considered (beta diversity), there are alterations both in bacterial and fungal beta diversities in UC and IBS. However, in CD, only bacterial beta diversity was found altered.

Taxa analysis revealed disease-associated fungi, in which *Candida albicans* was abundant in CD, *Saccharomyces mikatae* is in UC, and *C. tropicalis* in IBS. Interestingly, abundance of three bacterial species, *Lactobacillus ruminis, Bifidobacterium adolescentis*, and *Akkermansia muciniphila* appeared to be decreased in the diseases. These in silico correlations were further translated into *in vitro* interactions, where *L. ruminis* inhibited the growth of *C. albicans, C. tropicalis*, and *S. mikatae* and *A. muciniphila* inhibited the growth of *C. albicans* and *C. tropicalis* but enhances the growth of *S. mikatae*. One of the symptoms in IBD and IBS is increased gut barrier permeability, which leads to invasion of microbes and/or their effector molecules through the epithelial layers to trigger host immune responses. Monolayers of the CaCo-2 human epithelial colorectal adenocarcinoma cells were used to measure the permeability,

where *C. albicans* increased the permeability in the epithelial cell monolayers. Interestingly, the presence of *L. ruminis* reversed the effect of *C. albicans* on the epithelial monolayers permeability. We further elucidated that the increased *in vitro* permeability of the monolayers and *in vivo* penetrations of the fungus from the gut to the blood vessel are mediated by the toxin candidalysin. Higher abundance of *Mucor* was found in CD and the fungus was also found to induce increased permeability in the epithelial monolayers. These findings suggest that fungi play roles in development and/or progression of the GI tract diseases and the presence or higher abundance of probiotic bacteria such as *L. ruminis* is important to mitigate the effects of fungi on the diseases.

## Roles of Protein Glycosylation in Host Cell Interactions for the Pathogenicity of *Cryptococcus neoformans*

#### Hyun Ah Kang

Department of Life Science, Chung-Ang University

Post-translational glycosylation contributes to the function and immunogenicity of many cell surface proteins. The human pathogenic yeast Cryptococcus neoformans has serotype-specific high mannosetype N-glycans with or without  $\alpha$  1,2-xylose residue. In contrast to the other human-pathogenic yeast, Candida albicans, the outer mannose chains of N-glycans are dispensable for the virulence of C. neoformans. However, the systematical analysis of a set of alg mutant strains with defects in lipid-linked N-glycan assembly revealed that an intact core N-glycan structure is required for C. neoformans pathogenicity. The alg null mutants producing truncated core N-glycans are defective in inducing host cell death after phagocytosis, which is triggered as a mechanism of pulmonary escape and dissemination of C. neoformans, thus becoming inactive in causing fatal infection. C. neoformans has assembles two types of O-linked glycans on its proteins: Ktr3-mediated major O-glycans without xylose and Cap6mediated minor O-glycans containing xylose residues. The ktr3 $\Delta$  cap6 $\Delta$  double mutant strain, in which the extension of O-glycans in the Golgi is completely blocked, is completely avirulent in a mouse infection model and displays decreased activities in mediating host cell interaction at several steps during infection processes, including adhesion to lung epithelial cells, proliferation within macrophages, and transcytosis of the blood-brain barrier. It is notable that O-glycan extension is required for the host immune response, partly by involvement in trafficking of ergosterol, which is considered as a trigger of macrophage pyroptosis. Considering no human homolog for Cap6 and Ktr3, these fungal specific-mannosyltransferases are promising targets for the development of antifungal agents.

## Pathobiological Signaling Circuits of Pan-Drug-Resistant *Candida auris*

#### Yong-Sun Bahn

Department of Biotechnology, Yonsei University

The fungus Candida auris is considered an emerging human pathogen of global concern. Most clades of C. auris cause invasive candidiasis, which has been reported in Asia, South Africa, and South America. In a healthcare setting, C. auris can form biofilms on the surface of medical devices or on the surface of patient's skin. Infection is of particular concern as C. auris is resistant to many of the existing antifungal drugs, including azoles. The US Centers for Disease Control and Prevention (CDC) recommends treating C. auris infections with echinocandins, but some strains are also resistant to these. Because of these problems, the mortality rate from candidemia is exceptionally high, ranging from 30% to 60%. Despite such a growing concern of pan-resistant C. auris infection, the pathogenicity of this ascomycetous fungal pathogen and the signaling circuitries governing its resistance to antifungal drugs are largely unknown. Therefore, here we analyzed the pathobiological functions of cAMP/protein kinase A (PKA) signaling pathway in C. auris, which play conserved roles in the growth and virulence of fungal pathogens. We show that adenylyl cyclase Cyr1 and PKA have pleiotropic roles in growth, morphogenesis, stress responses, antifungal drug and disinfectant resistance, and ploidy shifts of C. auris. Notably, however, we observed that the  $tpk1\Delta$   $tpk2\Delta$  mutant generally exhibited more disrupted phenotypes than the  $cyr1\Delta$  mutant, and we suggest Tpk1 and Tpk2 have both cAMP-dependent and -independent roles in this pathogen. Most surprisingly, we observed that hyperactivation, not inhibition, of the cAMP/PKA pathway reduced virulence of C. auris. In conclusion, this study provides comprehensive insights into the role of the cAMP/ PKA pathway in drug resistance and pathogenicity of C. auris and suggests a potential therapeutic option for treatment of C. auris-mediated candidemia.

## Convergence of Distinct Secondary Metabolite Biosynthetic Gene Clusters Driving Chemical Innovation in Fungi

Rundong Liu<sup>1</sup>, Jun Hyuck Lee<sup>2</sup>, Hyun Park<sup>3</sup>, Jae-Seoun Hur<sup>1\*</sup>, and <u>Wonyong Kim<sup>1\*</sup></u>

<sup>1</sup>Korean Lichen Research Institute, Sunchon National University <sup>2</sup>Research Unit of Cryogenic Novel Material, Korea Polar Research Institute <sup>3</sup>Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University

An endophytic fungus, Phoma sp. NG-25, produces a set of dibenzofurans, including a potent antifungal agent, cercosporamide, and a well-known lichen metabolite, usnic acid. Here, we identified a biosynthetic gene cluster (BGC) involved in the biosynthesis of cercosporamide and usnic acid, which consists of two BGCs of apparently distinct origins. Functional analyses of individual genes in the BGC determined their biosynthetic roles. Interestingly, a truncated polyketide synthase (PKS), homologous to VrtA in viridicatumtoxin BGCs found in Aspergillus and Penicillium species, was truly functional and responsible for the biosynthesis of the carbamoyl side of cercosporamide. Deletion of VrtA did not affect usnic acid production, while deletion of another PKS, homologous to MPAS found in lichen species abolished production of both cercosporamide and usnic acid. Phylogenetic analyses of VrtA and MPAS indicated that a VrtA gene ancestor has diverged into several lineages, one of which appears to have converged with a usnic acid BGC, which resulted in chemical diversity producing a set of dibenzofurans. The cercosporamide BGC contained three transcription factors: one plays as a negative regulator and the others as positive regulators. One of the two positive regulators was homologous to VrtR2 in viridicatumtoxin BGCs and have a role in activation of genes homologous to Vrt genes (A/B/I and J) in the cercosporamide BGC. Interestingly, the VrtR2 was positively and negatively regulated by the other two transcription factors, suggesting that the regulatory system has been successfully rewired toward cercosporamide biosynthesis since the BGC convergence. The cercosporamide BGC also contained a second copy of protein kinase C (PKC2), the authentic molecular target of cercosporamide. Mutant strains lacking PKC2 became highly sensitive to exogenously-applied cercosporamide, indicating that PKC2 is a self-resistant gene to protect themselves from cercoporamide. This study illuminated how fungi evolved to have diverse repertoires of natural products and demonstrated BGC convergence as a novel mechanism driving chemical innovation in fungi.

## Novel Nuclear Localization Sequence of MoHTR1, a Nuclear Effector of the Rice Blast Fungus, is Essential for Translocation to Rice Nucleus and Transcriptional Reprograming of Host Genes

You-Jin Lim<sup>1</sup>, Hyunjun Lee<sup>2</sup>, Gobong Choi<sup>3</sup>, Seongbeom Kim<sup>2</sup>, and Yong-Hwan Lee<sup>1,2,3,4\*</sup>

 <sup>1</sup>Research Institute of Agriculture and Life Sciences, Seoul National University
 <sup>2</sup>Department of Agricultural Biotechnology, Seoul National University
 <sup>3</sup>Interdisciplinary Program in Agricultural Genomics, Seoul National University
 <sup>4</sup>Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Plant Immunity Research Center, Seoul National University

Plant pathogens secrete effectors to modulate the host immune system. Among the effectors secreted into plant cells, nuclear effectors are translocated in the host nuclei and interact with proteins and DNA to regulate defense mechanisms. Nuclear localization sequence (NLS) is the most well-known factor in transporting proteins from cytoplasm to nucleus. NLS is classified into classical and non-classical NLS according to the composition of sequence residues and participation of importin. However, molecular mechanism on NLS-associated transportation and the roles of NLS in the regulation of host gene expression are not well understood. We previously reported that MoHTR1, a nuclear effector of the rice blast fungus, Magnaporthe oryzae, is translocated to rice nuclei but not in fungal nuclei. To identify plantspecific NLS, we used NLS prediction tools and found non-classical NLS (PGRSKKE) in MoHTR1. We further identified that RxKK residues was important for the nuclear localization of MoHTR1. Nuclear effector candidates which have RxKK sequence were also localized in rice nuclei. Addition of MoHTR1 NLS altered the localization of cytoplasmic effectors of *M. oryzae* from cytoplasm to nucleus in the host. SUMOylation was involved in the secretion and translocation of MoHTR1 to BICs and host nuclei. In addition, MoHTR1 NLS is associated with reprograming of host gene expression including nucleosome assembly, DNA binding, and hormone response. Taken together, our findings will provide novel insights on the plantspecific NLS and the roles of nuclear effector NLS in pathogen-host interactions.

MSK2022 International Meeting of the Microbiological Society of Korea

# Symposium [S15]

# 송아지 분변으로부터 분리된 균주를 **활용한 설사증 예방 및 완화 제제**

연구자 박진호(전북대학교 수의과대학) | 기술분야 바이오 의약, 건강기능성 식품 관련

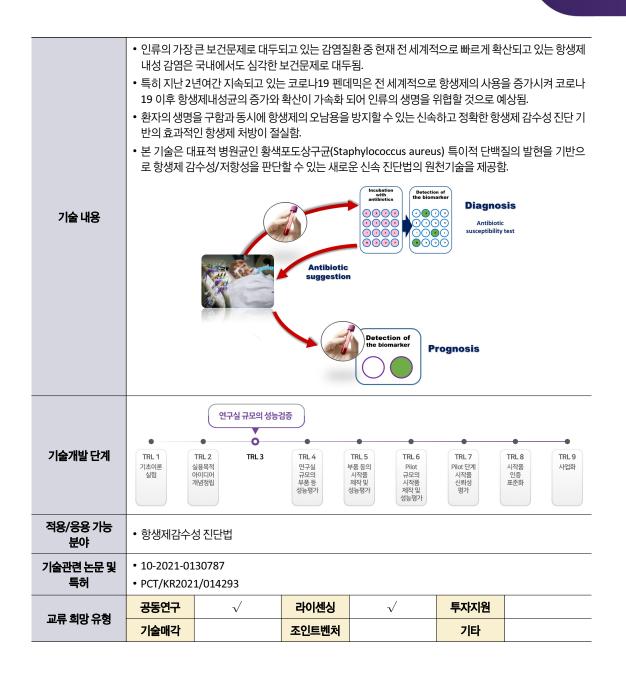
기술 내용	었음. 특히, 독 었으며, 국오 사율을 낮추 > 기술 내용 및 1. 건강한 힌 2. 분리된 유 섭취시켰 3. 유산균의 분석하였. 4. (유산균의 히였고, 설 고결과, (건 <u>화 효능이 있</u> 를 통한 신생	국내에서 발생하는 현  의 경우에도 생후 3~ 기 위해 설사병과 같  차별성 우신생 송아지로 부 산균주를 적정량으로 음. 투여유/무에 따른 산 고,  투여유/무에 따른) 실사증의 발생 유무어 강한 정상 한우 송아가 음을 직접적이고객	는우송아지설사 ·30일령송아지 은 소화기 질병을 터 유익 유산균 로 배양한 후에, 시 년생송아지의 장 설사증의 발생성 디따른 신생송아 디에서 분리된) 유 관적으로 확인하 료 및 관리에 주.	I Calf Diarrhea)은 표 병의 경우 포유기송( 의 발병이 심각하다고 에방하는 것이 매위 주를 직접 분리하였고 태로 태어난 한우신상 내 미생물총 변화를 r 상황 및 설사증의 주요 지의 생체정보 변화 유익 유산균제의 투여 였음. 따라서, 이번 인 요 지프로 활용될 수	가지의 97.6%가 2 알려짐. 따라서 <b>? 중요함</b> . 1, <b>! 송아지</b> 들에게 \$ nicrobiota anal <b>2 유발병원체에</b> 상을 비교 분석히 가 <u>신생 송아지 f</u> <sup>1</sup> 구의 결과는 <b>유</b> 있을 것으로 생격	감염된다고 보고되 네, <b>신생 송아지의 폐</b> 일정 기간 동안 <b>직접</b> lysis를 통하여 <b>비교</b> 대한 검출율을 비교 [였음. 설시증의 예방 및 완 익유산균제의 투여 각되며, 송아지의 건
기술개발 단계	TRL 1           기초이론           실험	생물총 형성에 도움	Pilot 규모의           TRL 4           연구실           규모의           부품 등	<b>제재의 개발</b> 방안에의 김 시작품 제작 및 성능평가 지자 등의 제작 및 성능평가	E 활용될 수 있을 Pilot 단계시작품	
적용/응용 가능 분야	<b>한우 신생 송</b> 내 <b>축산농가</b>	<b>아지 설사증의 치료</b> 들의 소득 증대를 가	<b>및 예방을 위한</b> 져올 수 있음. 또	<b>실러스 속 균주 기빈</b> <b>제제</b> (사료 첨가제 등 한, 한우 송아지 뿐만 5가들의 경제적 피해	)를 개발할 수 있 아니라 <b>다른 가</b>	L으며, 이를 통한 국 <b>축들 대부분에 적용</b>
기술관련 논문 및 특허	• 특허 출원 : 10-2022-0068961 • 국제특허기탁 : <i>L. amylovorus</i> 1394N20, KCCM12999P • 논문 : J Microbiology (2019) 57(2), 113~121. & JAST (2021) 63(5), 1207~1210.					
교류 희망 유형	공동연구           기술매각		라이센싱 조인트벤처	$\checkmark$	투자지원           기타	

## 박테리오파지를 이용한 **항생제**

연구자 김민수(주식회사 라이센텍) | 기술분야 바이오 의약

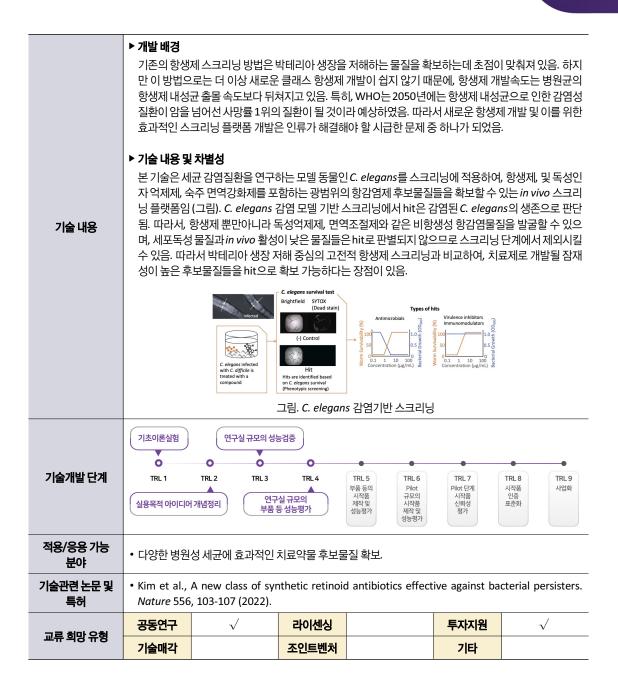
# 황색포도상구균(MRSA)의 **항생제 감수성 신속 진단**

연구자 장수진(한국파스퇴르연구소) | 기술분야 진단/평가/분석기술



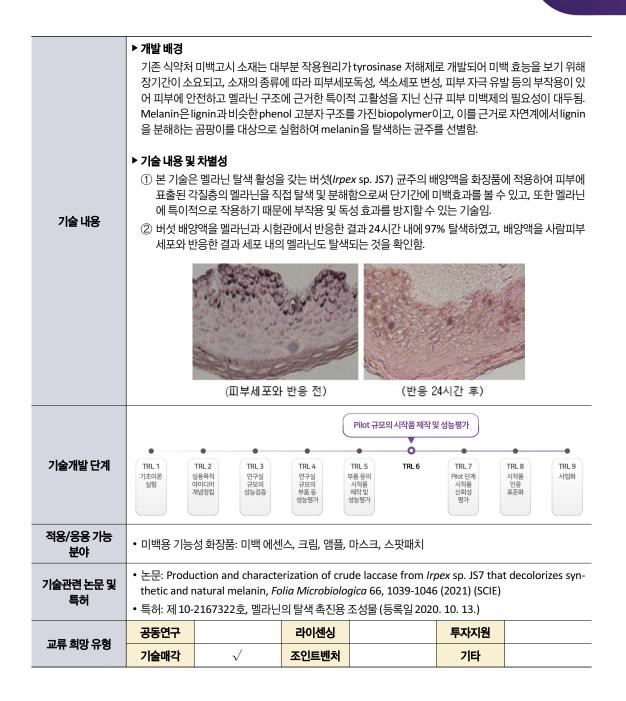
# 예쁜꼬마선충 감염모델 기반 <mark>광범위 세균감염치료물질 발굴법</mark>

연구자 김우성(이화여자대학교) | 기술분야 바이오 의약, 핵심/원천기술



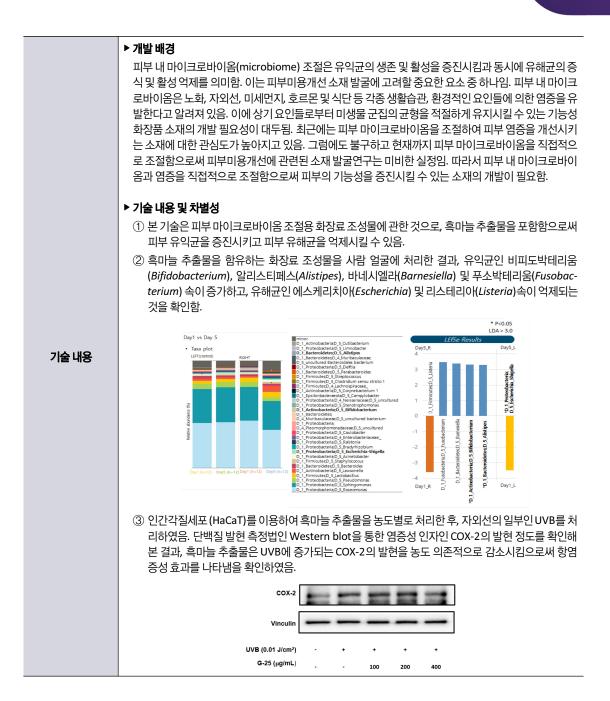
# 버섯 유래 멜라닌 탈색 효소를 **이용한 피부 미백 기술**

연구자 전숭종(동의대학교 의생명공학전공) | 기술분야 기능성 화장품 관련



# 피부미용개선 소재탐색 및 **피부마이크로바이옴 조절기술**

연구자 신학동, 임태규(세종대학교 탄수화물소재연구소) | 기술분야 기능성 화장품 관련



	부품 등의 시작품 제작 및 성능평가							
기술개발 단계	기초이론 · · · · · · · · · · · · · · · · · · ·	TRL 3         TRL 3           실용목적         연구실           아이디어         규모의           개념정립         성능검증	TRL 4           연구실           규모의           부품등           성능평가	TRL 5         TRL 6           Pilot         -           고모의         -           시작품         -           제작 및         -           성능평가         -	TRL 7 Pilot 단계 시작품 신뢰성 평가	TRL 8         TRL 9           시작품 인증 표준화         사업화		
적용/응용 가능 분야	• 피부 마이크로바이옴 조절 기능성 화장품: 외용연고, 크림, 클렌징 폼, 팩, 에센스, 로션, 비누, 스팟패치							
기술관련 논문 및 특허	• <b>특허</b> : 제 10-2021-0042595호, 피부 마이크로바이옴 조절용 화장료 조성물 (출원) • 논문: The effect of aged <i>Allium sativum</i> on human skin: skin inflammation suppression and skin microbiome modulation (In submission)							
그는 된다. 이정	공동연구	$\checkmark$	라이센싱		투자지원			
교류 희망 유형	기술매각	$\checkmark$	조인트벤처		기타			

## 건강기능성 및 질병 개선 **프로바이오틱스 소재화**

WiKim 세계김지연구 World Institute of Kim

연구자 최학종(세계김치연구소) | 기술분야 바이오 의약, 건강기능성 식품 관련

	▶ 개발 배경						
	<ul> <li>고령화 사회 진입과 함께 건강에 대한 의식수준이 높아지면서 전 세계적으로 건강기능식품 시장이 확대 되고 있으며, 건강기능식품은 일반 식품과 같은 보편화된 기능성 식품으로 기능성과 안전성을 동시에 섭 취할 수 있는 특성을 가지고 있음.</li> </ul>						
				· 우리 몸에게 유익한 분 수입으로 대체되고		것이 대중0	ㅔ게 널리
기술 내용		이옴 기반 질병과 연 새로운 대안이 될		미생물 구명 및 이를 Ξ	조절할 수 있는 4	소재 개발은	<u>-</u> 질병 예
	▶ <b>기술 내용 및</b> - 난치성 질환		선 프로바이오틱.	스 소재 발굴 및 지적;	재산권 획득		
				2틱스의 작용기작 및		음 조절 효고	┟규명
	- 소재의 유전	및 생화학적 안전	성 평가 및 물리적	. 화학적 안정성 평기	ł		
	연구실 규모의 부품 등성능평가 부품 등의 시작품 제작 및성능평가 Pilot 규모의 시작품						
기수개바 다게			TRL4	TRL 5 TRL 6			
기술개발 단계	기초이론 섬 실험 (	TRL 2         TRL 3           실용목적         연구실           바이디어         규모의           배념정립         성능검증	IKL 4	IKL5 IKL6	TRL 7 Pilot 단계 시작품 신뢰성 평가	TRL 8 시작품 인증 표준화	TRL 9 사업화
	• 과잉면역반응(아토피, 천식) 및 자가면역질환(류마티스관절염등) 개선 프로바이오틱스 소재개발 및 산						
적용/응용 가능 분야	업화 기초 연구 • 퇴행성 질환(퇴행성 관절염 및 근감소증) 개선 프로바이오틱스 소재 개발 및 산업화 기초 연구						
E -1	• 퇴행성 뇌질환(파킨슨병 및 치매) 개선 프로바이오틱스 소재 개발 및 산업화 기초 연구						
	<ul> <li>Lee J, Jang JY, Kwon MS, Lim SK, Kim N, Lee J, Park HK, Yun M, Shin MY, Jo HE, Oh YJ, Ryu BH, Ko MY, Joo W, Choi HJ. Mixture of Two Lactobacillus plantarum Strains Modulates the Gut Microbiota Structure and Regulatory T Cell</li> </ul>						
	Response in Diet-Induced Obese Mice. <i>Mol. Nutr. Food Res.</i> 62:1800329 (2018) • Kwon MS, Lim SK, Jang JY, Lee J, Park HK, Kim N, Yun M, Shin MY, Jo HE, Oh YJ, Roh SW, Choi HJ. <i>Lactobacillus sakei</i>						
	WIKIM30 Ameliorates Atopic Dermatitis-Like Skin Lesions by Inducing Regulatory T Cells and Altering Gut Microbiota						
기술관련 논문 및 특허	Structure in Mice. <i>Front. Immunol.</i> 9:1905 (2018) • Kwon MS, Shin MY, Lim SK, Lee J, Park HK, Kim N, Yun M, Jo HE, Oh YJ, Choi HJ. <i>Leuconostoc citreum</i> isolated from						
	kimchi suppresses the development of collagen-induced arthritis in DBA/1 mice. <i>J. Func. Foods</i> . 63:103579 (2019) • Kim N, Lee J, Song HS, Oh YJ, Kwon MS, Yun M, Lim SK, Park HK, Jang YS, Lee S, Choi SP, Roh SW, Choi HJ. Kimchi intake						
	alleviates obesity-induced neuroinflammation by modulating the gut-brain axis. Food Res Int. 158:111533 (2022)						
	• 락토코커스 락티스 아종 호르드니아에 균주 및 이의 퇴행성 뇌질환에 대한 예방, 개선 또는 치료 용도. 특허등록. 10-2404016. • 알레르기성 질환의 개선 활성을 갖는 락토바실러스 커베터스 WIKIM53 및 이를 포함하는 조성물. 특허등록. 10-1838281						
	공동연구		라이센싱		투자지원		
교류 희망 유형	-			1		l	

## 유전자변형미생물 LMO **안전성 평가 및 심사**

연구자 김성보(연세대학교) | 기술분야 진단/평가/분석기술

기술 내용	<ul> <li>기술 배경</li> <li>유전자변형미생물을 이용한 소재·효소는 글로벌 기업을 중심으로 산업적으로 매우 보편적으로 사용되고 있으나, 국내의 경우 그 산업화 사례가 제한적임.</li> <li>연구를 통하여 개발된 균주 및 재조합단백질은 그 용도에 따라 식품 혹은 산업용 LMO 안전성 평가 심사 및 생산공정이용 승인 등을 단계별로 거쳐야 하나, 아직 국내는 이에 대한 사례가 제한적이고 대부분 소수의 기업이 경험을 독점하고 있어 새로운 기술벤처 혹은 중·소기업에서 효과적인 기술 산업화에 어려움이 많음.</li> <li>인허가 심사는 신뢰도 확보를 위하여 해당 심의위원회에서 채택될 수 있는 올바른 시험 검증 프로토콜로 수행되어야 하고, 국내외 법규 현황이 서로 다른 부분이 많아 사업 추진 범위에 따라 그 적용 조건이나 범위의 설계가 잘 고려되어야 한다.</li> <li>LMO 심사 분야는 GLP 인증 전임상 독성평가 분야를 제외하면 시험평가 방법이 표준화/보편화되어 있지 않아, 시험평가 설계자에 따라 인허가 추진 전략과 소요되는 비용/일정의 수준이 서로 다를 수 있다.</li> <li>기술 내용 및 차별성</li> <li>유전자변형미생물의 식품 및 산업용 LMO 심사 승인 경험 다수 보유.</li> <li>최종제품(소재)의 국내·외 Novel Food Ingredient 심사 승인 경험 다수 보유.</li> <li>실질 심사 경험을 바탕으로 LMO 안전성 평가 및 심사에 대한 종합적인 인허가 추진 전략과 안전성 평가 시험법에 대한 자문 및 용역 과제 수행이 가능.</li> <li>(독점적 기술이전은 아니며, 노하우 기술이전 혹은 산업자문 형태로 기여 가능)</li> </ul>							
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적용/응용 가능 분야	<ul> <li>Lab 수준 연구개발 초기 단계에서 인허가 고려한 생산공정기술에 대한 자문</li> <li>개발 결과물(균주)에 대한 인허가 전략 및 안전성 평가 설계 자문</li> <li>안전성 평가에 대한 공동/위탁 등의 전부 혹은 일부 범위에 대한 연구</li> </ul>							
기술관련 논문 및 특허	[LMO 안전성 심사 경험 사례] - 식품용: <i>C. glutamicum</i> 균주 3건(FIS001, FIS002, FIS003) - 산업용: <i>C. glutamicum</i> 균주 1건(FIS002-1), <i>E.coli</i> 균주 1건(KCCM 80236)							
교류 희망 유형	공동연구 기술매각	$\checkmark$	라이센싱 조인트벤처		투자지원 기타			

MSK2022 International Meeting of the Microbiological Society of Korea

# Symposium [S16]

Sponsored by CHA University Laboratory of Bioantibacterials

### New Mechanisms of Anti-phage Defense

#### **Rotem Sorek**

Department of Molecular Genetics, Weizmann Institute of Science, Israel

The arms race between bacteria and phages led to the development of sophisticated anti-phage defense systems. Many of these defense systems have only recently been discovered, based on large scale genomic analyses. Our studies surprisingly reveal that multiple bacterial defense systems are ancient versions of central components of the cell-autonomous innate immune system in animals and plants. The talk will present progress in understanding the mechanisms of action of new bacterial immune systems, and will highlight cases in which bacterial defense from phage gave rise to key components in the eukaryotic innate immune system.

## How *Pseudomonas aeruginosa* Bacteriophages Evade CRISPR-mediated Demise

#### Joseph Bondy-Denomy

Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA 94403, USA

From humans to bacteria, the threat of viral attack is all too real. Bacteria protect themselves from viruses, called bacteriophages, with immune systems like restriction-modification, CRISPR-Cas, and a plethora of others that have been recently discovered. Our group focuses on the discovery and characterization of mechanisms that phages use to counter immune processes in *Pseudomonas aeruginosa*. Recent screening efforts have unveiled three advances in this area, which I will summarize in my lecture. First, the discovery of anti-CRISPR enzymes that covalently modify CRISPR-Cas proteins or degrade the Cas protein effectors. Second, the observation that some obligately lytic phages evade CRISPR-Cas and restriction enzymes in novel ways, including shielding their DNA with a "phage nucleus", as opposed to inhibiting CRISPR-Cas. Third, there has been recent expansion in the list of known bacterial anti-phage immune systems, some homologous to human innate immune processes. Many of these systems are found in the P. aeruginosa pangenome, which I will argue makes it the premier model organism for "bacterial immunology". We have developed new bioinformatic and genomic tools to discover anti-phage immune systems in this organism, identified genomic "defense hot spots" and launched experimental approaches to determine how they target phages and how phages fight back. Early progress on the CBASS anti-phage system (homologous to human cGAS-STING) will be discussed as an example. Together, we hope that studies of strategies deployed by lytic and temperate phages will provide a comprehensive understanding of phage-immune interactions in bacteria and fortify phage therapeutics.

### Quorum Sensing Anti-activation in the Phage-host Evolutionary Arms Race

#### **Karen Maxwell**

Department of Biochemistry, University of Toronto, Canada

Bacteria use a cell-cell communication system, known as quorum sensing, to collectively control group behaviours in a cell density dependent manner. These systems have been implicated in the upregulation of anti-phage systems, triggering their expression at high cell density when the risk of phage infection is greatest. For example, quorum sensing has been linked to upregulation of the CRISPR-Cas adaptive immune system, downregulation of phage receptors on the cell surface, and increased production of proteases that inactivate phage particles. We recently discovered a phage protein, Aqs1, expressed early in the infection cycle that binds to and inactivates LasR, the master regulator of quorum sensing in *Pseudomonas aeruginosa*. The 69-residue Aqs1 protein also inhibits PilB, the type IV assembly ATPase protein. This prevents assembly of the pilus on the surface of the cell and blocks superinfection by other phages. Since quorum sensing influences the expression of a variety of anti-phage defenses, Aqs1 provides a mechanism by which infecting phages might dampen multiple defenses simultaneously. Furthermore, as quorum-sensing systems are widely distributed among bacteria this phage counterdefense may play an important role in phage-host evolutionary dynamics.

# A Novel Bacterial Determinant for RNA Phage Entry in *Pseudomonas aeruginosa*

### Hee-Won Bae, Eun Sook Kim, and You-Hee Cho\*

Department of Pharmacy, College of Pharmacy and Institute of Pharmaceutical Sciences, CHA University

Bacteriophages (phages) that infect bacteria usually exhibit narrow host ranges, which means that they can infect only one or a few strains of the same bacterial species. Host range is determined by molecular interactions between phages and host strains throughout the various lifecycle stages during phage infection. We identified a novel determinant that affects the host range of PP7, a small RNA phage that infects the opportunistic Gram-negative pathogen, *Pseudomonas aeruginosa* strain PAO1 but not strain PA14. Based on the different susceptibilities observed for the two strains and by virtue of receptor engineering, we revealed that outer membrane (OM) integrity and fluidity might be a critical determinant for the RNA phage entry into *P. aeruginosa* strains. Topics discussed will include the presumable mechanism as well as the implications of our finding for phage application purposes, given that extreme diversity of OM structures is usually observed in various *P. aeruginosa* clinical isolates.

### In Silico Experiments for CRISPR-based Antimicrobials

### Ho-min Park<sup>1,2†</sup>, Yunseol Park<sup>1†</sup>, Urta Berani<sup>1</sup>, Eunkyu Bang<sup>1</sup>, Joris Vankerschaver<sup>1,3</sup>, Arnout Van Messem<sup>4</sup>, Wesley De Neve<sup>1,2</sup>, and Hyunjin Shim<sup>1\*</sup>

<sup>1</sup>Center for Biosystems and Biotech Data Science, Ghent University Global Campus, Incheon <sup>2</sup>Department of Electronics and Information Systems, Ghent University, Ghent, Belgium <sup>3</sup>Department of Applied Mathematics, Computer Science and Statistics, Ghent University, Ghent, Belgium <sup>4</sup>Department of Mathematics, University of Liège, Liège, Belgium <sup>†</sup>These authors have contributed equally to this work

Conducting in silico experiments that accurately predict the results of laboratory experiments is a long-standing goal of many computational biologists. The recent advances in computational methods such as machine learning in structural biology and in genomics bring the possibility of achieving such challenging tasks closer to reality. We introduce two studies to demonstrate the utility of in silico experiments as preliminary steps in designing CRISPR-based antimicrobials. Antimicrobial resistance is one of the most urgent public health issues that causes 700,000 deaths each year, but few antibiotics are in clinical development to meet current and future needs. Given the programmability and specificity of CRISPR-Cas systems, CRISPR-based antimicrobials have the potential to be repurposed as new types of antibiotics. Unlike traditional antibiotics, CRISPR-based antimicrobials can be designed to target specific bacteria and minimize negative effects on the human microbiome. Our first study explores the potential of anti-CRISPR proteins, which are small proteins from bacteriophages that counter-defend against the prokaryotic adaptive immunity of CRISPR-Cas systems. Through accurate structural prediction using deep-learning-based AlphaFold, we show that these phage-derived proteins are extremely distinct in structure, some of which have no homologues in the current protein structure domain. Furthermore, we find a novel family of anti-CRISPR proteins which are structurally similar to the recently discovered mechanism of manipulating host proteins through enzymatic activity, rather than through direct inference. Our second study explores the potential of CRISPR-Cas13-based antimicrobials, which degrade specific foreign RNAs, leading to non-specific RNase activities and cell cycle arrest. Here, we investigate the RNA-protein interactions of the Cas13-based systems, as high proportion of the Cas13 systems have no colocalized CRISPR arrays. From the in silico docking of CRISPR RNAs with the Cas13 proteins, we find a number of candidate CRISPR RNAs that have comparable or better in silico docking with the Cas13 proteins of the current tools.

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# Symposium [S17]

Co-organized by Global Research Collaboration for Infectious Disease Preparedness KOREA / KRIBB

## R&D Policy of Ministry of Science and ICT to Strengthen Infectious Disease Response Capabilities

#### Tae-ho Lee

Office of R&D Policy Bioscience Technology Division, Ministry of Science and ICT

The Ministry of Science and ICT is promoting a variety of policies to strengthen fundamental scientific and technological capabilities against new strains of infectious diseases.

First, basic research in the virus field is being strengthened, and the source technology research of next-generation vaccines and therapeutics is also being promoted.

Second, the establishment of infrastructure to support infectious disease research is being promoted. Research facilities that can be used by the private sector are being expanded, and training of specialized research personnel is being promoted.

In addition, research cooperation to establish an international cooperation system in the field of infectious diseases will be strengthened.

## Asia-Pacific Infectious Disease Shield: A GloPID-R Regional Hub Project

### Jieun Lee, Jiwon Yang, and Choong-Min Ryu\*

Infection Disease Research Center, Korea Research Institute of Bioscience and Biotechnology

The Asia - Pacific region offers ideal climatic, geographic, demographic, and cultural conditions for novel pathogens to enter the human population. In combination with the high population density across many of the countries, these factors are conducive to the development of local epidemics. But more worryingly, the pathogen can rapidly spread via the main flight routes from the major hub airports. This can potentially result in a global pandemic, as we have seen during the current COVID-19 crisis. The emergence of COVID-19 has exposed the lack of any regional (clinical) research networks and governance structures for coordinated cross-border control measures to contain, if not prevent, the spread of the virus more efficiently. With the exception of nations like Japan, South Korea, Singapore, New Zealand, and Australia, the majority of countries in this region are classified as low-middle income countries (LMICs). To solve the fundamental problem in Asia LMICs, continental level networking in responding infectious diseases is critical. Therefore, we proposed "Asia-Pacific Infectious Disease Shield (APIS)" as a regional hub project of GloPID-R, which is an international organization to invest in research capacity and capabilities to support the rapid initiation of scientific research in case of an outbreak. Our short-term goal is to establish sustainable networking in between Asia countries for helping LMICs in their prevention and control against infectious diseases. The long-term goal of APIS is to prevent and manage outbreaks of emerging and re-remerging infectious diseases in Asia-Pacific LMICs with cooperation between countries in Asia-Pacific region. In this year, we are conducting mapping-survey activity for identifying target project. At the end of this year, we will complete to understand the landscape of infectious diseaserelated projects and activity across Asia-Pacific region. To best our knowledge, APIS is a first Koreainitiated international collaboration program for sustainable Asia-Pacific infectious disease network for rapid response to combat infectious diseases in the emergency and peace time.

## International Collaboration of TIDCL (Tropical Infectious Diseases Cooperation Laboratory)

#### **Ho-Joon Shin**

Director and Principal Researcher in TIDCL Department of Microbiology, Ajou University School of Medicine

Global climate warming and frequent human trafficking are causing an increase in tropical infectious diseases, alike currently COVID-19. And then, we are living in a situation where new tropical infectious diseases such as malaria, dengue and other infections are constantly becoming a social problem in Korea. Tropical Infectious Diseases Cooperation Laboratory (TIDCL) was constructed in Ajou University School of Medicine, for achieving the research title "Global joint research programs for the defense on high-risk importable infectious diseases" (June 2018 ~ May 2022: funded by KRF/MSIT). TIDCL consists of four "Domestic Cooperation Organizations" - Ajou University, Gyeongsang University, Incheon University and Chungnam University (KOICID), and three "Oversea Cooperation Organizations" - DMRPOLB (Department of Medical Research Pyin Oo Lwin Branch, Myanmar), ICMR-NIMR (Indian Council of Medical Research -National Institute of Malaria Research, India) and TIDREC (Tropical Infectious Diseases for Research and Education Center, Malaysia). The TIDCL collaborative activities are in below: 1. Construction of joint research center or laboratory for tropical diseases. 2. Training program operation for human resource development at home and aboard. 3. Sharing data, documents, research materials including clinical samples (operating Biobank). 4. Execution of joint research projects for malaria, dengue, insect-borne diseases and COVID-19. TIDCL with DMRPOLB, TIDREC, ICMR/NIMR, and IMPE is actively conducting the human resource development, international researches, and Biobank cooperation for topical infections.

## Facilitating R&D Based International Network to Tackle Antibiotic Resistance

### Robin Guevarra<sup>1</sup>, Juchan Hwang<sup>1</sup>, Hein Min Tun<sup>2</sup>, Phong Quoc Le<sup>3</sup>, Soojin Jang<sup>1\*</sup>, MetaSUB consortium, and RAPID

<sup>1</sup>Antibiotic Resistance Laboratory, Institut Pasteur Korea <sup>2</sup>Division of Public Health Laboratory Sciences, LKS Faculty of Medicine the University of Hong Kong <sup>3</sup>Microbiology Laboratory, Center for Food Safety, Pasteur Institute in Nha Trang

The COVID-19 pandemic has lasted over 2 years giving us an important lesson how constant preparedness through international collaboration is crucial to prevent and respond to infectious diseases. Antibiotic resistant bacterial infections are the urgent global health threat responsible for 4.95 million deaths in 2019 alone. It is necessity to comprehend the current status of antibiotic resistance not only human sector but also animal and environmental sectors in order to build an effective strategy for the battle against it. Approximately 10<sup>6</sup> of particles that likely contain microbes are hourly shed from human body becoming seeds of the microbiome surrounding environments of humans, which in turn greatly affects human health. Environment microbiome has, therefore, become an important subject especially for the built environments, the human-made place where people live and work on a daily basis. We recently established an international R&D alliance to monitor potential pathogens by metagenomic analysis of environmental samples collected from several Asian countries and create common database to share all generated data within the alliance. This presentation discusses advantages of investigation of the built environment microbiome as a way to survey pathogens and antibiotic resistance in community showing how international collaborations can facilitate the work.

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# Symposium [S18]

Co-organized by Korean Collection for Type Cultures

### New Paradigm on Microbial Resources: Plant Microbiome

### Hyun Kim and Yong-Hwan Lee\*

Department of Agricultural Biotechnology and Center for Plant Microbiome Resources, Seoul National University

The Center for Fungal Genetic Resources (CFGR) was established in 2005 at Seoul National University to collect, maintain and distribute genetic resources mainly from fungi. Fungi are eukaryotic organisms of ecological, industrial, and agricultural significance. CFGR has also developed user-friendly databases to maintain genetic information of fungal stocks and help to solve questions on fungal pathogenicity, population genetics, development, and evolution. These efforts have been important for both educational and research purposes. During last 10 years, new paradigm on microbial resources has been emerged: shift from individuals to community level, Microbiome. To meet the needs of new paradigm, CFGR was reestablished as The Center for Plant Microbiome Resources (CPMR) in 2021 to add plant microbiome resources from rice and several medicinal plants. In this talk, we will introduce overall activities of CPMR and rice microbiome as a model for crop microbiome research.

## Exploring Fungal Diversity and Their Potential Applications in Bioindustries

#### **Hyang Burm Lee**

Department of Agricultural Biological Chemistry, Chonnam National University

Fungi are primarily heterotrophic, nutrition-absorptive (osmotrophic) eukaryotes that exist in every ecological niche, playing important roles in all the biogeochemical cycles in both terrestrial and aquatic ecosystems. An updated estimate of global fungal diversity showed that the fungal species ranged from 2.2 to 3.8 million worldwide. However, only 150,000 fungal species have been described to date and more species are still likely hidden in various niches, waiting to be discovered. The Kingdom Fungi contains more than two hundred orders into twenty phyla. Fungi represent invaluable sources of natural products, including secondary metabolites. Many secondary metabolites derived from fungi have been developed into important pharmaceutical products as antibiotics, tumor inhibitors and biofunctional sources. Although numerous natural products are being identified every day, there are still so much that remains unknown. Therefore, exploring undiscovered fungal taxa may be one of the promising ways for finding novel drug candidates and antimicrobials. The objectives of the project were to explore more novel fungal diversity, to screen and extract novel bioactive compounds with their potential applications in the functional food, functional cosmetics, and pharmaceutical bioindustries.

# Korean Culture Collection of Microalgae, and Toxin-producing Dinoflagellates

### Hyeon Ho Shin<sup>1\*</sup>, Joo Yeon Yoon<sup>1</sup>, Kyeong Yoon Kwak<sup>1</sup>, and Zhun Li<sup>2</sup>

<sup>1</sup>Library of Marine Samples, Korea Institute of Ocean Science & Technology <sup>2</sup>Biological Resource Center/Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology

The Korean culture collection of microalgae at the Korea Institute of Ocean Science and Technology (KIOST) was established in 2015. The cultures are used for research, teaching, biotechnology development, food for aquatic animals and a variety of other purpose. The principal function is the maintenance of diverse stock of living microalgae in order to make cultures available for user community worldwide. Over 1,900 different strains of microalgae, representing approximately 500 different genera, have been maintained at 2 culture rooms of KIOST. During the past five years, 800 strains were provided to user community, leading to published 100 scientific papers. Recently, toxin-producing dinoflagellates responsible for paralytic shellfish poisoning (PSP) and ciguatera fish poisoning, such as *Alexandrium* species, *Gymnodinium catenatum, Centrodinium punctatum* and *Fukuyoa* species, were collected from Korean coastal area. The established strains and mass cultures of these species will be utilized to produce the certified calibration solutions of PSP and ciguatoxin.

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## Establishment of a Public Probiotics Bank and Case Studies for Supporting Industrialization

#### **Doo-Sang Park**

KCTC/Biological Resources Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB)

Probiotics market is estimated to be valued at \$2.3 Billion in 2020, and is projected to reach a value of \$4.15 Billion by 2027, at a CAGR of 8.7%. On the basis of source, the probiotics market has been segmented into bacteria and yeast. The bacterial segment (*Lactobacilli, Bifidobacteria, Streptococcus thermophilus*) accounted for the largest share in this market. There are a large quantity of probiotic bacterial strains have been isolated by several company and research teams, however, a public probiotic bank is not available for a researcher and medium and small sized enterprises in Korea. Lately, KCTC/BRC have developed a probiotic bank and it retain over 3,000 lactic acid bacteria represent 98 species that belong to *Lactobacilli, Bifidobacteria, Enterococcus, Leuconostoc* and *Weissella* etc. All the strains were isolated from human, animals and traditional fermented foods and were characterized for their short chain fatty acid production, antibiotics resistance, pH and bile acid resistance etc. In this presentation, I will briefly introduce the probiotics strain bank established in KCTC/BRC, and present cases such as the development of several probiotics, as well as the role of probiotics industrialization supporting center which supported by the Ministry of Science and ICT.

### Function of Novel Korean Gut Bacteria in Human Disease

<u>Ji-Sun Kim</u><sup>1</sup>, Ju Huck Lee<sup>1</sup>, Seung-Hwan Park<sup>1</sup>, Se Won Kang<sup>1</sup>, Mi Kyung Eom<sup>1</sup>, Min Kuk Suh<sup>1</sup>, Han Sol Kim<sup>1</sup>, Byeong Seob Oh<sup>1</sup>, Jam-Eon Park<sup>1</sup>, Seung Yeob Yu<sup>1</sup>, Seoung Woo Ryu<sup>1</sup>, Seung-Hyeon Choi<sup>1</sup>, Ji Young Choi<sup>1</sup>, Dong-Ho Lee<sup>2</sup>, Hyuk Yoon<sup>2</sup>, Je Hee Lee<sup>3</sup>, Insu Jang<sup>4</sup>, and Jung-Sook Lee<sup>1,5\*</sup>

<sup>1</sup>Korean Collection for Type Cultures, Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology <sup>2</sup>Seoul National University Bundang Hospital <sup>3</sup>CI Bioscience, Inc. <sup>4</sup>Korean Bioinformation Center, Korea Research Institute of Bioscience and Biotechnology <sup>5</sup>University of Science and Technology

The human gut is colonized with complex and diverse communities known as billions of gut microbiota. Metagenome-based diversity analyses to date have shown the potential that the gut microbiome may mediate or modulate human health and disease risk. Therefore, in order to study the direct relationship between human disease and the gut microbiome, it is very important to conduct physiological and multiomics studies using gut microbiota isolated from human. However, many researchers have encountered difficulties in follow-up studies using disease-related gut microbiota due to the difficulty of their isolation/ cultivation and the different characteristics of strains. To solve these problems, we isolated 13,066 strains belong to 457 species from 835 healthy Koreans through Korean Gut Microbiome Bank (KGMB) project. Based on multi-omics analyses of these isolates, the therapeutic effect of these gut bacteria on various human diseases such as cancer, obesity, and inflammatory bowel disease was screened. As a result, it was discovered that novel gut microbes have significant therapeutic and preventive effects on these diseases. [This work was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (NRF-2016M3A9F3947962) and Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program.]

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# Symposium [S19]

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### Systems and Synthetic Biology: Constructing Smart and Programmable Microbes to Address Global Problems

#### **Tae Seok Moon**

Department of Energy, Environmental and Chemical Engineering, Division of Biology and Biomedical Sciences, Washington University in St. Louis, USA

The past decade has witnessed the tremendous power of systems and synthetic biology in the creation of genetic parts, devices, and systems, which helps understand complex biological systems. However, its potential for real-world applications has not been fully exploited. One of its promising applications is the construction of programmable cells that are able to integrate multiple environmental signals and to implement synthetic control over biological processes. My research interests are focused on developing microbes that are able to process multiple input signals and to generate user-defined outputs. Specifically, I aim to build genetic programs in order to control various bacterial processes such as gene expression, chemical reactions, and evolution. I will present unpublished results of my research projects by discussing the potential and challenges of systems and synthetic biology to address global problems, including plastic and agricultural waste issues, non-invasive diagnostics and disease treatment using smart probiotics, sustainable bioproduction, and biocontainment of engineered organisms.

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# CRISPR-Mediated Microbial Genome Editing and New Target Finding

### Ho Joung Lee, Song Hee Jung, Hyun Ju Kim, and Sang Jun Lee $^{*}$

Department of Systems Biotechnology, Institute of Microbiomics, Chung-Ang University

CRISPR-Cas was discovered as an adaptive immune system in prokaryotes and emerged as a genome editing tool a decade ago. Since the CRISPR-Cas system is modularly composed of proteinaceous Cas nuclease and target recognition RNA, it is more convenient to design for treatment of genetic diseases and advancement of genetically modified organisms such as crops, and livestock. However, there are still intrinsic problems with PAM restriction and mismatch tolerance that affect target selection and correct genome editing, respectively. Here, we present how to precisely edit the desired genomic sequence with current CRISPR technology. We will also discuss CRISPR screening and sensor technologies for target identification *in vivo* and *in vitro*.

### **CRISPR-based DNA Recording System for Gut Inflammation**

#### Dae-Hee Lee

<sup>1</sup>Synthetic Biology Research Center, Korea Research Institute of Bioscience and Biotechnology <sup>2</sup>KRIBB School of Biotechnology, University of Science and Technology

Microbes living and interacting with host cells in the human gut are essential to human health. Changes in the composition of this gut microbiome cause disease-associated symptoms, which motivates the engineering of microbes to diagnose, treat, memorize, or prevent disease. Microbes reprogrammed using advanced DNA-based recording systems are envisaged as emerging living diagnostics and therapeutics for a wide range of diseases and play key roles in regulating gut microbiota to treat disease in a noninvasive manner. Here, we present a CRISPR-aided genetic switch that uses various Cas effectors to convert the mRNA information into RNA-guided transcriptional regulation or base editing. We combined the transcription factor-based genetic circuit with the CRISPR system and optimized the genetic switch performance by tuning and processing the gRNA. We then developed a DNA-based recording system for sensing and recording multiplex inflammation markers. These strategies developed here present a simple and robust approach to convert many biological signals into a DNA-based recording system, expanding the toolkit for biotechnological applications.

### Efficient CRISPR Editing with a Hypercompact Cas12f1 and Engineered Guide RNAs Delivered by Adeno-associated Virus

<u>Jeong Mi Lee</u><sup>1†</sup>, Do Yon Kim<sup>2†</sup>, Su Bin Moon<sup>1</sup>, Hyun Jung Chin<sup>1</sup>, Seyeon Park<sup>2</sup>, Youjung Lim<sup>2</sup>, Daesik Kim<sup>3</sup>, Taeyoung Koo<sup>4</sup>, Jeong-Heon Ko<sup>1</sup>, and Yong-Sam Kim<sup>1,2\*</sup>

<sup>1</sup>Genome Editing Research Center, Korea Research Institute of Bioscience & Biotechnology <sup>2</sup>GenKOre

<sup>3</sup>Department of Development and Regenerative Medicine, Sungkyunkwan University <sup>4</sup>Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University <sup>†</sup>These authors contributed equally

Gene therapy would benefit from a miniature CRISPR system that fits into the small adeno-associated virus (AAV) genome and has high cleavage activity and specificity in eukaryotic cells. One of the most compact CRISPR-associated nucleases yet discovered is the archaeal Un1Cas12f1. However, Un1Cas12f1 and its variants have very low activity in eukaryotic cells. In the present study, we redesigned the natural guide RNA of Un1Cas12f1 at five sites: the 5' terminus of the trans-activating CRISPR RNA (tracrRNA), the tracrRNA-crRNA complementary region, a penta (uridinylate) sequence, the 3' terminus of the crRNA and a disordered stem 2 region in the tracrRNA. These optimizations synergistically increased the average indel frequency by 867-fold. The optimized Un1Cas12f1 system enabled efficient, specific genome editing in human cells when delivered by plasmid vectors, PCR amplicons and AAV. As Un1Cas12f1 cleaves outside the protospacer, it can be used to create large deletions efficiently. The engineered Un1Cas12f1 system showed efficiency comparable to that of SpCas9 and specificity similar to that of AsCas12a.

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# Symposium [S20]

### Modulation of Pathogenicity by Endoribonucleases and Their Regulators in Pathogenic Bacteria

### Jaejin Lee<sup>1</sup>, Minkyung Ryu<sup>1</sup>, Young-Jin Seo<sup>1</sup>, Seungmok Han<sup>3</sup>, Yong-Hak Kim<sup>2\*</sup>, Kangseok Lee<sup>1\*</sup>, and Minho Lee<sup>3,4\*</sup>

<sup>1</sup>Department of Life Science, Chung-Ang University <sup>2</sup>Department of Microbiology, Daegu Catholic University School of Medicine <sup>3</sup>Department of Microbiology, College of Medicine, Hallym University <sup>4</sup>Institute of Medical Science, College of Medicine, Hallym University

Gene expression in bacteria is regulated at many levels, including transcription initiation, RNA processing and decay, RNA/RNA interactions, and post-transcriptional regulation involving enzymes that alter translational efficiency. Moreover, pathogenic bacteria utilize gene expression via RNA metabolism to rapidly adapt to environmental changes during host infection. However, the molecular mechanisms underlying RNA metabolism-associated pathogenesis have not yet been characterized.

In this study, we discuss the endoribonucleases and their regulators that control pathogenicity through regulation of transcription, post-transcription, and RNA stability by RNA processing and degradation. RNA-sequencing, qRT-PCR, and proteomic analyses indicated the effects of endoribonucleases and their regulators levels on the abundance of mRNA species. Primer extension and *in vitro* cleavage analyses further identified endoribonuclease cleavage in the 5' untranslated region (UTR) of the target mRNA. In addition, endoribonuclease- and their regulators-deleted cells showed decreased invasion ability and cytotoxicity in infection of human cell lines and reduced mortality in a mouse infection model compared to wild-type cells.

This study provides strong experimental evidence that the coordinated modulation of pathogenicity by endoribonucleases and their regulators constitutes an additional regulatory layer upstream of a complex feed-forward loop controlling global regulatory systems in pathogenic bacteria. Furthermore, we suggest that these regulatory pathways play key roles in the virulence of pathogenic bacteria in the host.

This study improves the understanding of the molecular mechanisms through which pathogenic bacteria sense the host environment and respond precisely by expressing gene products required for adaptation to that particular niche.

### **Bacterial Toxins during Host Infection**

### Soomin Choi, Seungwoo Baek, and Eun-Jin Lee\*

Department of Life Sciences, Korea University

Many bacteria are often resistant to antibiotic treatment and drugs because, even if these drugs are effective, bacteria can slow down their growth rate and thus attenuate the effectiveness of the drug. A similar growth-rate control is detected in pathogenic bacteria that infect and persist inside their hosts. The bacterial growth rate within host cells can be regulated by multiple signaling pathways, most of which are still unknown. A toxin-antitoxin (TA) system is one of the candidates for controlling bacterial growth because the TA system could slow down growth by expressing a toxin component. The toxin protein can be neutralized by the antitoxin component, serving as a non-heritable phenotypic switch for growth rate. In this study, we investigated a type I toxin-antitoxin system from the intracellular bacterial pathogen *Salmonella enterica* serovar Typhimurium. We characterized residues required for toxin's activity and a potential mechanism of the toxin by searching for its target via bacterial two-hybrid screening. Understanding the underlying mechanism of toxin-mediated persister formation and growth rate control within host cells will provide a new alternative to treat antibiotic resistant bacteria or intracellular bacteria surviving within host cells.

# A Bacterial Strain Saves Microbial Community: Colistin-degrading Proteases Confer Collective Resistance in Polymicrobial Infection Communities

Do-Hoon Lee<sup>1</sup>, Ju-Hee Cha<sup>1</sup>, Dae-Wi Kim<sup>1,2</sup>, Kihyun Lee<sup>1</sup>, Yong-Seok Kim<sup>1</sup>, Hyo-Young Oh<sup>3</sup>, You-Hee Cho<sup>3</sup>, and Chang-Jun Cha<sup>1\*</sup>

<sup>1</sup>Department of Systems Biotechnology and Center for Antibiotic Resistome, Chung-Ang University <sup>2</sup>Division of Life Sciences, Jeonbuk National University Department of Dharmacy, Collage of Pharmacy and Institute of Pharmacoutical Sciences, CHA Universit

<sup>3</sup>Department of Pharmacy, College of Pharmacy and Institute of Pharmaceutical Sciences, CHA University

The increasing prevalence of resistance against the last-resort antibiotic colistin is a significant threat to global public health. Here, we discovered a novel colistin resistance mechanism via enzymatic inactivation of the drug. A bacterial strain of the Gram-negative opportunistic pathogen *Stenotrophomonas maltophilia* capable of degrading colistin and exhibiting a high-level colistin resistance was isolated. A colistin-degrading protease (Cdp) was identified in this strain and its contribution to colistin resistance was demonstrated. Coculture and coinfection experiments revealed that *S. maltophilia* carrying *cdp* gene could inactivate colistin and protect otherwise susceptible *Pseudomonas aeruginosa*, which may seriously affect the clinical efficacy of the drug for the treatment of cystic fibrosis patients with polymicrobial infection. Our study suggests that Cdp should be recognized as an emerging colistin resistance determinant conferring collective resistance in microbial communities during polymicrobial infections. We propose a hitherto unrecognized colistin resistance mechanism that should be under surveillance in clinical settings.

## Clostridial Toxin-medicated Gut Inflammation and the Association with Gut Microbiota

#### **Eun-Jeong Yoon**

Division of Antimicrobial Resistance Research, National Institute of Health, Korea Disease Control and Prevention Agency

*Clostridioides difficile* is a Gram-positive, spore-forming, and toxin-producing anaerobic bacterial pathogen, which causes intestinal infections. Since the bacteria is intrinsically resistant to many classes of antimicrobials and the ability to be persistent owing to the endospores, *C. difficile* is one of the most common cause of nosocomial antimicrobial-associated diarrhea. According to the given characteristics of patients, *C. difficile* infections present a wide range of clinical features, asymptomatic colonization, diarrhea, varied types of colitis including fulminant colitis and pseudomembranous colitis, and attributable death. The colitis is mainly mediated by two large clostridial toxins, *i.e.*, toxin A and toxin B, triggering a complex cascade of immune responses of host cells resulting in inflammation and tissue necrosis. The intestinal environment, which is generated by the gut microbiota, is critical for the spore germination and outgrowth of the bacterial strain in the gut. In this talk, with a recent research work for the *C. difficile* infection *in vivo* model using mice having varied microbiota, differed outcomes by clostridial toxins will be presented together with the association between the gut microbiota and effects of the toxins. [This work was supported by funding from the Korea Disease Control and Prevention Agency (grant number 2022NI01800).]

### Saccharomyces cerevisiae: A Sexy Yeast with A Prion Problem

#### **Moonil Son**

Department of Microbiology, Pusan National University

Prions are infectious proteins, mostly having a self-propagating amyloid (filamentous protein polymer) structure consisting of an abnormal form of a normally soluble protein. These prions arise spontaneously in the cell without known reason, and their effects were generally considered as fatal based on prion diseases of human or mammals. However, the wide array of prion studies in yeast, Saccharomyces cerevisiae, including filamentous fungi revealed that their effects can range widely, from lethal to very mild (even cryptic) or functional, depending on the nature of the prion protein, and the specific prion variant (or strain) made by the same prion protein but with a different conformation. This prion biology is affected by an array of molecular chaperone systems, such as Hsp40, Hsp70, Hsp104, or their combination. In parallel with the systems required for prion propagation, yeast has multiple anti-prion systems, constantly working in the normal cell without overproduction or deficiency of any protein, which have negative effects on prions by blocking their formation, curing of many prions after arising, preventing prion infections and reducing the cytotoxicity produced by prions. From the protectors of nascent polypeptides (Ssb1/2p, Zuo1p and Ssz1p), to the protein sequester (Btn2p), the disaggregator (Hsp104), and the mysterious Cur1p normal levels of each can cure prion variants arising in its absence. The controllers of mRNA quality, nonsense-mediated mRNA decay proteins (Upf1, 2, 3), can cure newly formed prion variants by association with a prion forming protein. The regulator of inositol pyrophosphate metabolic pathway (Siw14p) cures certain prion variants by lowering the levels of certain organic compounds. Some of these proteins have other cellular functions (e.g., Btn2), while others produce an anti-prion effect through their primary role in the normal cell (e.g., ribosomal chaperones). Thus, these anti-prion actions are the innate defense strategy against prions. Here, we outline the anti-prion systems in yeast that produce innate immunity to prions by a multi-layered operation targeting each step of prion development.

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# Symposium [S21]

## Emulating Host-Microbiome Crosstalk in a Microphysiological Human Gut-on-a-chip

#### Hyun Jung Kim

Department of Inflammation and Immunity, Lerner Research Institute, Cleveland Clinic, USA

The human gut microbiome substantially orchestrates intestinal homeostasis, disease development, and metabolic functions of the human body. Indeed, dysbiosis of human intestinal microbiota develops diverse intestinal disorders that can potentially lead to inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, colorectal cancer (CRC), infectious diseases, or irritable bowel syndrome (IBS) that manifests a dysfunction in the gut-brain axis. However, a lack of robust models of the living human intestine that demonstrates host-microbiome crosstalk remains a critical unmet need. Human intestinal organoids have emerged as an advanced tissue-engineered model, but an enclosed lumen cultured in a static condition considerably hampers to recapitulate host-microbiome crosstalk in vitro. My group has spearheaded to offer a human Gut-on-a-chip microphysiological model that demonstrates intestinal physiology, three-dimensional (3D) morphogenesis, accurate oxygen control, mechanodynamic bowel movement, and longitudinal host-microbiome co-cultures. The accessibility and modularity of a microengineered Gut-on-a-chip can identify a specific disease trigger by recoupling the uncoupled complex factors germane to disease development in a spatiotemporal manner. In a pathomimetic human intestinal inflammation-on-a-chip model, we discovered that the intact epithelial barrier is necessary and sufficient to maintain the homeostatic tolerance of the gut under complex host-microbiome crosstalks. Furthermore, an integrative culture of patient-derived organoid epithelium in a Gut-on-a-chip enables to simulate patient-specific host responses under various microbial stimulations in an IBD Chip and a CRC Chip. Finally, we highlight the breakthrough of our new disease models and discuss the future impact of investigating the etiology and therapeutic targets in multifactorial human gastrointestinal diseases.

## Development of High-throughput Screening Technique for Microbiome Using MALDI-TOF Mass Spectrometry

#### Kun Cho

Bio-Chemical Analysis Team, Korea Basic Science Institute

A rapid and reliable approach to the identification of microorganisms is a critical requirement for large-scale culturomics analysis. MALDI-TOF MS is a suitable technique that can be a better alternative to conventional biochemical and gene sequencing methods as it is economical both in terms of cost and labor. The applications of MALDI-TOF MS for the comprehensive identification of microorganisms for culturomics-based approaches have been widely explored. Although in the preliminary stage, MALDI-TOF MS-based sub-species and strain typing, detection of drug-resistant strains, identification of pathogens in blood cultures and generation of MS profile for microbial metabolites for the assessment of functional traits in microorganisms have been studied which requires further standardization for better reliability. However, the restriction of this technique is attributed to insufficient coverage of the mass spectral database. To improve the applications of this technique for the identification of novel isolates, the spectral database should be updated with the peptide mass fingerprint (PMF) of type strains with not only microbes with clinical relevance but also from various environmental sources. Further, the development of enhanced sample processing methods and new algorithms for automation and de-replication of isolates will increase its application in microbial ecology studies.

# Direct and Rapid Antimicrobial Susceptibility Testing (dRAST) Using Artificial Intelligence to Accelerate Therapeutic Decisions for Blood Stream Infection

### Sunghoon Kwon<sup>1,2</sup>

<sup>1</sup>Department of Electrical and Computer Engineering, Seoul National University <sup>2</sup>QuantaMatrix Inc.

Bloodstream infection (BSI) has become a serious threat to human healthcare, globally. If not recognized early, the disease can further develop into sepsis which is one of the leading causes of death and hospital readmission. Without prompt prescription and administration of optimal antibiotics, the mortality rate can steadily increase by 7-9% every hour, critically suggesting the need of fast and accurate antimicrobial susceptibility testing (AST). However, conventional AST is extremely time consuming which typically takes up to 3 days or more based on the infecting species. During this time, patients are empirically treated with broad-spectrum antibiotics. Although these broad-spectrum antibiotics are important asset for humanity, they have been occasionally misused or overused, accelerating the undesirable proliferation of antibiotic resistance unexpectedly. High antibiotic resistance for these broad-spectrum antibiotics results in ineffective empirical treatment as high as 40%. Optimal prescription of antibiotics from expedited AST is needed to increase survival rate of BSI and to reduce unnecessary broad-spectrum treatment. In this talk, I will discuss a direct and rapid AST (dRAST) platform that can reduce the sample-to-answer time by up to 2 days compared to conventional AST methods. In our system, a 96 micro-structured, plastic cartridge is inoculated with bacterial suspension directly from positive blood culture to evaluate the morphological changes of bacteria using time-lapse microscopic imaging and artificial intelligence. I will also introduce recent clinical studies that demonstrate the clinical applicability of our platform to accelerate therapeutic decisions for earlier and adequate antibiotic treatment. Finally, I will introduce how QuantaMatrix Inc. successfully translated innovative academic research into life-saving clinical diagnostic products.

## SARS-CoV-2 Aberrantly Elevates Mitochondrial Bioenergetics to Induce Robust Virus Propagation

Hye Jin Shin<sup>1</sup>, Keun Bon Ku<sup>1</sup>, Gun Young Yun<sup>1</sup>, Hyun-Woo Moon<sup>1</sup>, Chonsaeng Kim<sup>1</sup>, Mi-Hwa Kim<sup>1,2</sup>, Bum-Tae Kim<sup>1</sup>, Jong-Won Oh<sup>3</sup>, Aleem Siddiqui<sup>4</sup>, and Seong-Jun Kim<sup>1\*</sup>

<sup>1</sup>Department of Convergent Research of Emerging Virus Infection, Korea Research Institute of Chemical Technology <sup>2</sup>Bioenvironmental Science and Toxicology Division, Gyeongnam Branch Institute, Korea Institute of Toxicology <sup>3</sup>Department of Biotechnology, Yonsei University

<sup>4</sup>Division of Infectious Diseases, School of Medicine, University of California, San Diego, La Jolla, CA 92093, USA

SARS-CoV-2 is a respiratory pathogen that can cause serious multi-organ damage. However, knowledge on SARS-CoV-2-induced cellular alterations is limited. In this study, we report that SARS-CoV-2 aberrantly elevates mitochondrial bioenergetics and activates EGFR-mediated cell survival signal cascade to sustain SARS-CoV-2 persistence. SARS-CoV-2 causes an increase in mitochondrial transmembrane potential via the SARS-CoV-2 RNA-nucleocapsid cluster, thereby abnormally promoting mitochondrial biogenesis and OXPHOS process followed by enhancing ATP production. SARS-CoV-2 activates the EGFR signal cascade and mitochondrial EGFR accumulation to maintain abnormal OXPHOS and viral propagation. Approved EGFR inhibitors remarkably reduced SARS-CoV-2 propagation, among which vandetanib showed the highest efficacy and potent activity against various SARS-CoV-2 variants of concern, including alpha, beta, delta, and omicron, in both *in vitro* cell culture and *in vivo* animal experiments, suggesting that EGFR is an attractive host target for combatting COVID-19. Our results suggest that SARS-CoV-2 induces aberrant mitochondrial dynamics and bioenergetics, significantly contributing to robust SARS-CoV-2 propagation.

### Self-Amplifying mRNA (SAM): Next Generation Expression Platform and Its Application

Tan Lu<sup>1</sup>, Seong-Kug Eo<sup>2</sup>, Sang-hwa Lee<sup>3</sup>, Joong-Chul Lee<sup>3</sup>, and Dal Young Kim<sup>1,3\*</sup>

<sup>1</sup>Department of Infectious Diseases and Public Health, CityUniversity of HongKong <sup>2</sup>College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University <sup>3</sup>Winastabio Inc.

Since the development of infectious cDNA clones (reverse genetics system) of viral RNA genomes and the methods of delivery of the in vitro-synthesized RNA into susceptible cells, alphaviruses have become an efficient system for the expression of heterologous genetic material. Alphaviruses replicate exclusively in the cytoplasm, and their genetic material cannot interfere with cellular DNA. Alphavirus genome-based, self-replicating RNAs (replicons) are widely used vectors for the expression of heterologous proteins. Their current design relies on the replacement of structural genes, encoded by subgenomic RNAs (SG RNA), with a heterologous gene of interest. During the COVID19 pandemic era, the unprecedented fasttracked introduction of vaccines based on the mRNA platform by Pfizer-BioNTech and Moderna shortened the production time. It demonstrated a fascinating protective efficacy in the human population. Traditionally, a well-characterized and deeply studied one of the alphaviruses oriented vectors, SAM (selfamplifying mRNA) is capable of being the next generation mRNA platform with a series of unrivaled factors. (i) With the equipment of replication machinery, SAM can extend the duration of expression *in vivo* (ii) Relatively, SAM is known to be inducing a high-level expression of heterologous genetic information (iii) In an exclusive cytoplasm replication, SAM does not recombine with host chromosome (iv) The prolonged expression of the immunogenic or therapeutic protein can trigger a long-lasting humoral and cell-mediated immune response (v) Comparable to the conventional mRNA (cmRNA), with a diminished amount of dose volume, SAM can provoke a similar efficient immune response as cmRNA. Additionally, we can inject SAMoriented vaccine to the host without LNP (lipid-nanoparticle) formulation or other protective coating materials essential to encapsulate conventional mRNA. With two components (replicon genome and helper genome) packaging system, we can generate high-titered non-infectious VRP (virus replicon particle), the ambivalent approach can be readily applied according to the field situation. We developed diverse COVID19 vaccine libraries and tested them on the susceptible rodent model such as Syrian hamster and hACEs transgenic mice. In vivo study, we demonstrated a spike protein-specific high-level neutralizing antibody titers against a variant of concerns and measured the degree of viral RNA in the different internal organs and its protective effects in a challenge study. The SARS-CoV-2 SAM (VRP) vaccine candidates have a promising safety profile and elicit potent protective immune responses against multiple SARS-CoV-2 variants.

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# Symposium [S22]

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#### S22-1

### Laws and Regulations for Safety Management of LMOs for R&D

#### Yong-Ik Kwon, Sun-Hwa Lee, and Young-Hee Roh\*

Korea Research Institute of Bioscience & Biotechnology, National Research Safety Headquarters

Genetically modified organism (GMO) refers to an organism containing genetic material newly combined using modern biotechnology. In biological laboratories, the word 'Living Modified Organisms' (LMO) is used instead of GMO. The reason is the term that emphasizes the possibility of live reproduction/ reproduction and it was first used in the biosafety protocol. LMO safety management was beginning with the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, which was adopted in January 2000, and entered into force in September 2003. In Korea, the 'Act on Transboundary Movement of Genetically Modified Organisms' (LMO Act) was enacted for implementation of the protocol and it took enforced since 2008. When using or making LMO for R&D, safety management must be performed in accordance with the LMO Act. The Ministry of Science and ICT is in charge of safety management of LMO for R&D. In addition, NRSH carries out biosafety management tasks such as R&D LMO import reporting, export notification and so on. This presentation introduces Korean legal system applied to R&D LMO, such as the research facility reporting system and the LMO import declaration system based on the LMO Act. S22-2

#### The Method of R&D LMO Safety Management

#### Seung-chul Shin, In-Ja Song, and Young-Hee Roh\*

Korea Research Institute of Bioscience & Biotechnology, National Research Safety Headquarters

In order to establish a LMO safety management system, Korea has been implementing the <sup>「</sup>TRANS-BOUNDARY MOVEMENT, ETC. OF LIVING MODIFIED ORGANISMS ACT」 (hereinafter, <sup>「</sup>LMO Act」) from January 1, 2008. In particular, the <sup>「</sup>LMO Act」 stipulates matters that researchers must comply with, when using LMOs for Research and development (R&D). In this presentation, we would like to introduce the export/import report method according to the <sup>「</sup>LMO Act」 and detailed safety management standards for LMO research facilities. In addition, detailed method of compliance suitable for the characteristics of the research site will be introduced. The content is currently under the jurisdiction of the Ministry of Science and ICT, and the task is entrusted to and managed by the National Research Safety Management Headquarters (NRSH). Additional information about the announcement can also be found in the 'LMO Information System (https://www.Imosafety.or.kr)'.

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## **Graduate Students' Forum**

### Enhancement of the Solubility of Recombinant Proteins by Fusion with a Short-disordered Peptide

#### Jun Ren<sup>†</sup>, <u>Suhee Hwang</u><sup>†</sup>, Junhao Shen, Hyeongwoo Kim, Hyunjoo Kim, Jieun Kim, Soyoung Ahn, Min-gyun Kim, Seung Ho Lee, and Dokyun Na<sup>\*</sup>

Department of Biomedical Engineering, Chung-Ang University

Bacterial cells, especially *Escherichia coli*, are widely used as a host to produce recombinant proteins. However, heterologous proteins often become insoluble and thus lose their activity. There have been developed many fusion tags to transform insoluble heterologous into soluble ones: maltose-binding proteins (MBP), glutathione-S-transferase, TrxA, SUMO, etc. Though they are effective in solubilizing recombinant proteins, due to their large size they also pose a burden to the host cell, which may reduce production yield. In this study, we developed a short fusion tag, 7 kDa, that is as effective as conventional fusion tags. We hypothesized that fully disordered peptides enhanced the solubility of recombinant protein. From a database of fully disordered proteins (DisProt), we collected 10 short-disordered peptides and evaluated their solubilization ability using GFP-GFIL4, an engineered aggregation-prone GFP, highly insoluble *E. coli* proteins, YagA and YdiU. Consequently, we found two short-disordered peptides (7 kDa) were able to solubilize recombinant proteins up to 90%, and were as effective as a known highlysolubilizing tag, MBP. To confirm their effect on the activity of recombinant proteins, the two disordered peptides were then evaluated with I-Scel, which is a restriction enzyme and has a folding issue when expressed. The disordered peptides not only enhanced the solubility of I-Scel but also did not disturb the activity I-Scel. On the other hand, I-Scel fused with conventional fusion tags, MBP and TrxA, had no activity. The discovered two short-disordered fusion tags should have a practical application in industrial protein production.

### Genomics and Transcriptomics of Laboratory-evolved Multidrug-resistant *Acinetobacter baumannii* under Nutritional Stresses

#### Sohyeon Yun and Woojun Park\*

Laboratory of Molecular Environmental Microbiology, Department of Environmental Science and Ecological Engineering, Korea University

Bacterial evolution offers a wide range of genomic mutations and new adaptive pathways, and decreasing fitness costs for new environments is a beneficial product of evolution. We induced the laboratory evolution of multidrug-resistant *Acinetobacter baumannii* NCCP 16007 (WT) isolated from patient urine under nutritional stress to obtain two new cell types, Evolved *A. baumannii* 1 (EAB1) and Evolved *A. baumannii* 2 (EAB2). Demonstrated increased fitness through high-dimensional evolution in EAB1 - oligotrophic and EAB2 - eutrophic environments. Genomic analysis shows extensive gene loss, gain, and, rearrangement in evolutionary strains. Gain of genes was caused by insertion sequences, which is a result supported by transcript analysis to interrupt gene coding or increase gene expression level. The *ata* loss of EAB1 and the *bap* overexpression of EAB2 caused differences in bacterial adhesion, demonstrating a decrease in EAB1 and an increased biofilm-forming ability of EAB2. Evolutionary strain maintain all of the antibiotic resistance genes shown in the WT, however, the antibiotic resistance of the polymyxin B and clindamycin has been reduced. We predicted that there is a novel mechanism by which mutations are synergistic to make them sensitive to antibiotics. These data provide the remarkable genetic plasticity of *A. baumannii* and the ability to develop strong adaptive functions to specific environmental niches.

### A Novel Spore-specific Transcription Factor is Essential for Conidial Maturation and Dormancy in *Aspergillus* Species

#### Ye-Eun Son<sup>1</sup> and Hee-Soo Park<sup>1,2\*</sup>

<sup>1</sup>School of Food Science and Biotechnology, Kyungpook National University <sup>2</sup>Department of Integrative Biology, Kyungpook National University

Aspergillus, a filamentous fungus that makes up the majority of airborne fungi, mainly propagates by forming asexual spores called conidia. Conidia formation is regulated by various transcription factors (TFs). Although previous studies have shown that some TFs, such as VosA, VelB, and WetA, involve in conidia formation and maturation, there are still unknown TFs for conidiogenesis. Therefore, we studied putative spore-specific TFs in three representatives Aspergillus species based on transcriptomic analysis of conidia and hyphae. As a result of the analysis, twenty-two spore-specific TFs were identified, and each deletion mutant was phenotypically analyzed in A. nidulans. Among them, we characterized one of the spore-specific- $C_2H_2$  zinc finger A SscA in A. nidulans. The  $\Delta$ sscA mutant showed defective conidiation, conidial viability, and reduced stress tolerance in A. nidulans. The amount of trehalose in the  $\Delta$ sscA mutant was decreased compared to that of the WT and deletion of sscA caused induced germ tube formation with or without glucose. Furthermore, transcriptome data suggested that SscA affected the mRNA expression of various genes in A. nidulans conidia. Interestingly, deletion of sscA resulted in gene expression alterations involved in the response of conidia to stimuli and stress. The mRNA levels of the eta-glucan biosynthesis gene and the sterigmatocystin gene cluster were upregulated in sscA mutant conidia. These results were validated by the phenotypic analyses. In addition, we confirmed that the roles of SscA in conidia were conserved in A. flavus and A. fumgiatus. Taken together, these results suggest that SscA is a novel spore-specific transcription factor, essential for proper conidia formation, conidia maturation, conidia dormancy and secondary metabolites in A. nidulans. And the functions of SscA in conidia are conserved in three representative Aspergillus spp.

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### Study of Microbial Carotenoid: A Focus on the Optimization of Bacterioruberin Production and Its Antioxidant Properties

Chi Young Hwang<sup>1</sup>, Eui-Sang Cho<sup>1</sup>, and Myung-Ji Seo<sup>1,2,3\*</sup>

<sup>1</sup>Department of Bioengineering and Nano-Bioengineering, Graduate School of Incheon National University <sup>2</sup>Division of Bioengineering, Incheon National University <sup>3</sup>Research Center for Bio Materials & Process Development, Incheon National University

Carotenoids are natural pigments synthesized by plants, fungi, algae, bacteria, and archaea. Carotenoids have drawn a lot of attention due to their potential health benefits for people. At present, the natural carotenoids by microbial production and their application have been increased. The diversity of carotenoid structures that can be produced by microbes has great potential. In this study, we isolated the novel carotenoid producers and profiled the pigments extracted from microbial sources. Carotenoid profiling was performed by high-performance liquid chromatography photodiode array (HPLC-PDA) analysis.  $C_{30}$  carotenoids (diapophytoene and 1-glycosyl-apo-8'-lycopene) were produced by the family *Bacillaceae*. In the case of  $C_{40}$  carotenoids ( $\beta$ -carotene, lutein, and zeaxanthin) were detected in the family Flavobacteriaceae. Bacterioruberin and its intermediates including monoanhydrobacterioruberin and bisanhydrobacteiroruberin were confirmed in the family Halorubraceae and Haloferaceae. As focusing on bacterioruberin as C<sub>50</sub> carotenoid, one novel candidate species of the genus Halorubrum designated MBLA0099 was selected. Based on the culture optimization in the flask level, the bacterioruberin production increased 2.48 times compared to initial conditions. In 7 L lab-scale fermentation, the optimized conditions achieved a 6.05-fold increase. The antioxidant activity was also evaluated both in vitro and in vivo levels using the carotenoid extracts (majorly including bacterioruberin) from strain MBLA0099. In vitro antioxidant assessment of bacterioruberin extracts showed the highest antioxidant activity compared to other C<sub>40</sub> carotenoids (lycopene,  $\beta$ -carotene, and astaxanthin). In vivo evaluation by investigating the survival rate of *Caenorhabditis elegans* revealed the highest level in  $H_2O_2$  treatment when fed with bacterioruberin. In conclusion, the current results suggest that the strain MBLA0099 could be considered to be a potential bacterioruberin-producer that could be applied to the development of functional materials in bio-industrial fields.

### Persistence of Antibiotic Resistance from Agricultural Effluents to Surface Water Revealed by Genome-centric Metagenomics

#### Jin Ju Kim<sup>1†</sup>, Hoon Je Seong<sup>1†</sup>, Chang-Jun Cha<sup>1</sup>, Timothy A. Johnson<sup>2</sup>, Woo Jun Sul<sup>1\*</sup>, and Jong-Chan Chae<sup>3\*</sup>

<sup>1</sup>Department of Systems Biotechnology, Chung-Ang University <sup>2</sup>Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA <sup>3</sup>Division of Biotechnology, Jeonbuk National University

Concerns about antibiotic resistance genes (ARGs) released from wastewaters of livestock or fish farming into the natural environment are increasing, but studies on unculturable bacteria related to the dissemination of antibiotic resistance are limited. Here, we reconstructed 1,100 metagenome-assembled genomes (MAGs) by our presented genome binning refiner method, ACR, to assess the impact of microbial antibiotic resistome and mobilome in wastewaters from Korean rivers. Our results indicate that ARGs harbored in the MAGs were disseminated from wastewater effluents into downstream rivers. Moreover, it was found that ARGs are more commonly co-localized with mobile genetic elements (MGEs) in agricultural wastewater than in river water. Among the effluent-derived phyla, superphylum Patescibacteria possessed a high number of MGEs, along with co-localized ARGs. Our findings suggest that members of the Patesibacteria are a potential vector for propagating ARGs into the environmental community. Therefore, we propose that the dissemination of ARGs by uncultured bacteria should be further investigated in multiple environments.

### Functional Characterization of 4-α-Glucanotransferase and α-Amylolytic Enzyme from Hyperthermophilic *Fervidobacterium islandicum* AW-1

Sondor Ganbat<sup>1</sup>, Jae-Hong Park<sup>1</sup>, Bo Gyoung Choi<sup>1</sup>, Dariimaa Ganbat<sup>1</sup>, Dong-Woo Lee<sup>2</sup>, Seong-Bo Kim<sup>3</sup>, Han-Seung Lee<sup>1</sup>, and Sang-Jae Lee<sup>1\*</sup>

> <sup>1</sup>Department of Bioscience, Silla University <sup>2</sup>Department of Biotechnology, Yonsei University <sup>3</sup>Bio-Living Engineering Major, Global Leaders College, Yonsei University

Starch is one of the most used polysaccharides in both food and non-food applications because of its broad functionality. The combination of starch-active enzymes in the glucanohydrolase and glucanotransferase families has been widely studied to identify enzymes that successfully combined to produce novel and functional isomaltooligosaccharides. In this research, functional characterization of  $\alpha$ -amylase and 4- $\alpha$ -glucanotransferase from hyperthermophilic *Fervidobacterium islandicum* AW-1, which can be key enzymes for innovative glucan structures was conducted. As a result of the CAZy (Carbohydrate-Active enZYmes) analysis, three genes annotated as  $\alpha$ -amylase in *F. islandicum* AW-1 genome. Among them, we selected two genes (NA23\_09645 and NA23\_09780) without signal peptide. The genes were cloned and expressed in *E. coli* BL21 using the pET system. The recombinant enzyme NA23\_09645 (49 kDa) could not hydrolyze starch and amylose; however, it reacted with hydrolyzed small maltooligosaccharides such as maltotriose to form various maltooligosaccharides. It showed the enzyme was not  $\alpha$ -amylase but 4- $\alpha$ -glucanotransferase (FIGTase). The enzyme NA23\_09780 (81 kDa) showed high hydrolysis activity not only with maltooligosaccharides or maltodextrin but also cyclodextrins, which indicates that the enzyme was  $\alpha$ -amylase (FIAmyA). The recombinant FIGTase and FIAmyA enzymes exhibited maximal activity at 80°C and 65°C, respectively, and pH 6.0.

### Enhancement of Anti-inflammatory and Anti-osteoporosis Effects of Fermented *Abelmoschus Manihot* L. by *Bacillus licheniformis* CP6

Min Kyeong Kim<sup>1</sup>, Yu Jeong Yeom<sup>1</sup>, Yong-Jik Lee<sup>2</sup>, Sang-Jae Lee<sup>1</sup>, and Mi-Hwa Park<sup>3\*</sup>

<sup>1</sup>Major in Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University <sup>2</sup>Department of Bio-cosmetics, Seowon University <sup>3</sup>Department of Food and Nutrition, Silla University

A *Abelmoschus Manihot* L. (AM) was fermented by *Bacillus licheniformis* CP6 at 37°C for 1 days (CP6-1D). And we investigated the anti-inflammatory effects on LPS - stimulated RAW 264.7 macrophages and the effects on osteoblast differentiation in MC3T3-E1 cells. According to our findings, the CP6-1D suppressed ROS, and the expression of iNOS and COX-2. Also, CP6-1D showed inhibitory effect on the production of pro-inflammatory cytokines such as IL-6, IL-1 $\beta$  and TNF- $\alpha$ . Moreover, nuclear translocation of NF- $\kappa$ B and phosphorylation of MAP kinase were strongly suppressed by CP6-1D in LPS-stimulated RAW 264.7 cells. Furthermore, CP6-1D increased cell proliferation in MC3T3-E1 osteoblasts. Compared to incubated AM treatment, CP6-1D markedly promoted alkaline phosphatase activity and mineralization. Alizarin Red S staining demonstrated that CP6-1D treatment tended to increase extracellular matrix calcium accumulation. Taken together, our data suggests that fermentation may be a useful strategy for improving the biological properties of raw materials including their anti-inflammatory and anti-osteoporosis properties. Thus, CP6-1D is suggested to be a preventive medicinal food against inflammatory bone disorders.

### Antimicrobial Spectrum and Characterization of Purified Recombinant Micro Halocin HB384 Derived from Halophiles

#### <u>Bo Hyeon Park</u><sup>1</sup>, Ga Eul Jeong<sup>1</sup>, Dariimaa Ganbat<sup>1</sup>, Ga Yeon Nam<sup>1</sup>, Kwang Soo Jung<sup>1</sup>, Dong-Woo Lee<sup>2</sup>, Seong-Bo Kim<sup>3</sup>, Yong-Jik Lee<sup>4</sup>, and Sang-Jae Lee<sup>1\*</sup>

<sup>1</sup>Major in Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University <sup>2</sup>Department of Biotechnology, Yonsei University <sup>3</sup>Bio-Living Engineering Major, Global Leaders College, Yonsei University <sup>4</sup>Department of Bio-Cosmetics, Seowon University

Excessive misuse of conventional antibiotics leads to antibiotic resistance in bacteria, which causes super bacteria, multi-drug resistant organisms. To solve this problem, several alternative strategies have been proposed, among which antimicrobial peptides (AMPs) are the most promising due to they do not reveal the resistance problem. In this study, AMPs derived from halophiles were obtained based on the gene information of halocin peptides, which are bacteriocin-like substances produced from halophiles. It was named HB384, and the gene encoding HB384 was cloned into pGST vector and the recombinant HB384 was expressed in *E. coli* BL21. The recombinant protein was purified by GST affinity chromatography, and the molecular weight of HB384 was 3.14 kDa. Disk diffusion assays were performed to evaluate antimicrobial activity. HB384 showed antimicrobial activity against Gram-positive bacteria *S. aureus, B. subtilis*, and Gram-negative bacteria *S. typhimurium, E. coli*. Moreover, HB384 was stable at 99°C for 8 h, and antimicrobial activity against pathogen increased with the increase in the concentration used. As a result, purified HB384 is expected to be used as a substitute for antibiotics. In future research, we plan to conduct a study to analyze the antiviral potential of HB384.

### Identification of Imidazole Propionate Producing Lactobacillus Bacteria in the Human Gut

#### Mahrukh Butt, Kyung Hyun Kim, and Che Ok Jeon\*

Department of Life Science, Chung-Ang University

Trillions of microbes inhabit in human gut and interact with host by producing bioactive metabolites. These bioactive metabolites can be beneficial or harmful to human host. Gut microbes produce amino acid-derived metabolites, such as imidazole propionate which is causative molecule to induce type 2 diabetes by impairing insulin signaling at the level of insulin receptor substrate (IRS). This study identified one of the key-players Lactobacillus genus involved in producing imidazole propionate in the gut. From sequence similarity network analysis using proteins sequences from Uniprot and HMP database, 28 putative urocanate reductase (UrdA) clusters were identified and, among all clusters, abundance analyses of gut metagenomes identified a cluster of Lactobacillus bacteria (cluster 4) harboring putative UrdA genes showed significantly high abundance in case of T2D. Lactobacillus gasseri cells produce imidazole propionate from urocante in vitro, which was confirmed by LC-ToF/MS analysis. In addition, through cloning, heterologous protein expression, and enzyme activity assay of putative UrdA of Lactobacillus gasseri, it was confirmed that Lactobacillus gasseri harboring the putative UrdA could catalysis the irreversible reaction of two-electron reduction of urocanic acid to imidazole propionate by using NADPH as electron donor. These results indicated that Lactobacillus gasseri, one of famous probiotics, can produce imidazole propionate and it may be causative factor to induction of T2D in the human. In the future, in vivo test is needed to examine the effect of Lactobacillus gasseri in the development of T2D in host.

### Characteristics and Biological Activity of Exopolysaccharide Produced by Lysobacter sp. MMG2 Isolated from the Roots of Tagetes patula

Inhyup Kim, Geeta Chhetri, Yoonseop So, Jiyoun Kim, and Taegun Seo\*

Department of Life Science, Dongguk University-Seoul

In the present study, exopolysaccharide (EPS) produced by *Lysobacter* sp. MMG2 (IyEPS) was characterized and purified. The IyEPS-producing strain *Lysobacter* sp. MMG2 was isolated from the roots of *Tagetes patula*. When IyEPS was produced in tryptic soy broth with 1% glucose and the Iyophilized powder was measured, the yield was found to be 0.67 g/L. The molecular weight (Mw) of IyEPS was 1.01 ×  $10^5$  Da. Its monosaccharide composition includes 84.24% mannose, 9.73% glucose, 2.55% galactose, 2.77% arabinose, 0.32% xylose, and 0.03% rhamnose. Scanning electron microscopy (SEM) revealed that IyEPS has various round and rough surfaces. Fourier-transform infrared (FTIR) analysis identified its carbohydrate polymer functional groups. Moreover, thermogravimetric analysis of IyEPS revealed two events of mass loss: the first was water loss, which resulted in 3.97% mass loss and the second event occurred at approximately 212°C. IyEPS could inhibit biofilm-producing pathogenic bacteria without any antimicrobial activity. Furthermore, IyEPS at a concentration of 4 mg/ml could exhibit potent 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity (89.25%). These results indicate that IyEPS could be a promising candidate for industrial development if its biological activity is further explored.

### The Effect of Plant-derived Biological Nitrification Inhibitors (BNIs) on the Nitrification in Co-culture of Three Different Ammonia-oxidizing Microorganisms

Seongwook Kim<sup>1</sup> and Man-Young Jung<sup>1,2\*</sup>

<sup>1</sup>Interdisciplinary Graduate Program in Advance Convergence Technology and Science, Jeju National University <sup>2</sup>Department of Biology Education, Jeju National University

In the global nitrogen (N) cycle, the nitrification, the conversion of ammonia (NH<sub>3</sub>) to nitrate (NO<sub>3</sub><sup>-</sup>) via nitrite ( $NO_2$ ) by soil ammonia-oxidizing microorganisms [AOMs; ammonia-oxidizing archaea (AOA), bacteria (AOB), and complete ammonia oxidizers (Comammox)] and nitrite-oxidizing bacteria, is the major N transformation process in soil. Many studies have indicated that nitrification plays a significant role in environmental pollution, i.e., nitrous oxide ( $N_2O$ ) emissions,  $NO_3^-$  leaching into groundwater, and N loss in agricultural systems. Using plant-derived biological nitrification inhibitors (BNIs), which block two key enzymes (AMO and HAO) of ammonia oxidation to suppress soil ammonia oxidation, is a competent strategy to mitigate these problems by disrupting the activity of AOMs. Previously published research on BNI focus mainly on determining the inhibitory effect on ammonia oxidation in the pure culture of nitrifiers individually; however, the environmental system is much more complex with the co-existence of a different group of nitrifiers. Therefore, the main purpose of this study is to investigate the inhibitory effects of different BNIs [methyl 3-(4-hydroxyphenyl) propionate, sakuranetin, linoleic acid, linolenic acid, methyl linoleate] capacity on various ratios of three different groups of AOM (AOA: Nitrosocosmicus oleophilus, Nitrosoarchaeum koreensis, and Nitrososphaera viennensis, AOB: Nitrosomonas europaea, Comammox: Nitrospira inopinata) in a co-culture by measuring gross nitrification rate and cell growth. In addition, these results are applied to an *in situ* or microcosm experiment in a pot similar to a real soil environment, in which these three nitrifiers coexist. Taken together, our findings provide better insights into how we can apply the BNIs to regulate nitrification with specific AOM in the terrestrial soil to reduce the amount of N fertilizer used, and N<sub>2</sub>O emissions contribute to the "Carbon Net-zero" by 2030.

### Transmembrane Helix Stability of Lysis Protein in RNA Phage Lifecycle

#### So-Yeon Kim, Hee-Won Bae, Eun Sook Kim, and You-Hee Cho\*

Department of Pharmacy, College of Pharmacy and Institute of Pharmaceutical Sciences, CHA University

PP7 is an RNA phage that infects *Pseudomonas aeruginosa*. Lysis protein (LP), one of the four phage proteins, is critical for the proper release of the phage progenies, but the actual lysis mechanism of LP remains elusive. As an initial attempt to elucidate the LP-mediated lysis mechanism, we divided the 55-aa LP into 3 regions, based on the sequence characteristics: the N-terminal hydrophilic region (1-20 aa), the transmembrane (TM) domain (21-43 aa), and the C-terminal hydrophobic region (44-55 aa). Considering the critical roles of the TM domain or the TM helix stability, in the lysis function, we analyzed the helical propensity of the individual amino acids in the TM domain and introduced the mutations that change the helical propensity of the amino acids. Topics discussed will include our results on the characterization of the LP muteins with altered TM helix stability in regards to membrane association and lysis function as well as phage production.

### Characterizations of Zinc-ion Dependent Phosphatase (YktC1) and Alcohol Dehydrogenase (GutB1) for 1-Deoxynojirimycin Biosynthesis in *Bacillus amyloliquefaciens* MBLB0692

Hyo Jung Lim<sup>1</sup>, Jeonghye Chun<sup>2</sup>, Hyunjin Lee<sup>1,3</sup>, Sungho Jang<sup>1,3,4</sup>, and Myung-Ji Seo<sup>1,3,4\*</sup>

<sup>1</sup>Department of Bioengineering and Nano-Bioengineering, Incheon National University <sup>2</sup>Biofood & Living Engineering Laboratory, Global Leaders College, Yonsei University <sup>3</sup>Research Center for Bio Materials & Process Development, Incheon National University <sup>4</sup>Division of Bioengineering, Incheon National University

1-Deoxynojirimycin (1-DNJ), a poly-hydroxy derivative of piperidine ((CH<sub>2</sub>)<sub>5</sub>NH), is a potent  $\alpha$ -glucosidase inhibitor since their structural resemblance to D-glucose. The food-grade 1-DNJ production can be accomplished by some Bacillus spp. isolated from fermented food sources. However, there have been few studies about the functional roles of core 1-DNJ biosynthesis genes in Bacillus spp. Here, we characterized two genes (yktC1 and gutB1) of the core 1-DNJ biosynthetic genes from Bacillus amyloliquefaciens MBLB 0692, which was isolated from cucumber kimchi. We successfully expressed recombinant YktC1 (rYktC1) and GutB1 (rGutB1) in *Escherichia coli* BL21(DE3) as a His<sub>6</sub>-tagged protein. First, the zinc-ion dependent phosphatase (yktC1), which converts 2-amino-2-deoxy-p-mannitol-6-phosphate (ADMP) to 2-amino-2deoxy-p-mannitol (ADM), was investigated using alternative substrates, p-nitrophenyl phosphate (pNPP). The purified rYktC1 had optimal pH and temperature at pH 5.5 and 65°C for pNPP. The substrate specificity for pNPP of rYktC1 was narrower at optimal pH 5.5 than pH 8.0. The activity of rYktC1 for pNPP as substrate was largely dependent on Zn ion. The  $K_m$ ,  $V_{max}$  and  $k_{cat}$  values of the rYktC1 for pNPP were 7.6 mM, 0.0011 mM/sec, and 0.0015 sec<sup>-1</sup>, respectively. Second, the alcohol dehydrogenase (gutB1), which converts ADM to mannojirimycin, was investigated using ADM. The purified rGutB1 showed maximum activity at 40°C and pH 10.0. The most preferred substrate of the rGutB1 was ADM, though accepted other substrates such as sorbitol, mannitol, galactitol, mannosamine, glucosamine, and galactosamine at reduced activity (21.8-34.5%). The addition of MnSO<sub>4</sub> and CaCl<sub>2</sub> increased the rGutB1 activity, while CuCl<sub>2</sub>, AgNO<sub>3</sub> and ZnCl<sub>2</sub> inhibited the activity. The  $K_m$ ,  $V_{max}$  and  $k_{cat}$  values of the rGutB1 were 1.2 mM, 0.0018 mM/min, and 0.060 min<sup>-1</sup> for ADM and 0.82 mM, 0.0037 mM/min, and 0.12 min<sup>-1</sup> for NAD<sup>+</sup>. Finally, this study will provide practical basis for application of YktC1 and GutB1 to industrial 1-DNJ production.

# Characterization of Integral Membrane Protein, *CpImp1*, of the Chestnut Blight Fungus, *Cryphonectria parasitica*

#### Jaehyeon Lee<sup>1</sup>, Jeesun Chun<sup>2</sup>, Yo-han Ko<sup>2</sup>, and Dae-hyuk Kim<sup>1,2</sup>

<sup>1</sup>Department of Bioactive Material Sciences, Jeonbuk National University <sup>2</sup>Institute for Molecular Biology and Genetics, Jeonbuk National University

The chestnut blight fungus, *Cryphonectria parasitica*, and its interaction with hypovirus, Cryphonectria Hypovirus 1 (CHV1), is known to be a useful model to study the mechanism of fungus-virus interaction. In our previous study, the sectorization was observed in two gene (*CpBckl, CpSlt2*) mutants associated with cell wall integrity pathways. In addition, 73 genes were found to be common differentially expressed genes (DEGs) between the sectored progenies and the corresponding parental mutant strains (*CpBck1*-sectored progeny vs *CpBck1*-null mutant and the *CpSlt2*-sectored progeny vs *CpSlt2*-null mutant) via RNA sequencing analysis. Among common 73 DEGs, 22 DEGs were found to be affected by CHV1 infection.

In this study, a gene encoding integral membrane protein (*CpImp1*) was selected among those genes affected by both CHV1 and sectorization. BLAST search revealed that the cloned gene showed high homology with one of membrane protein family of *Coniella lustricola*. To analyze the biological function of the putative membrane protein of *Cryphonectria parasitica*, we constructed *CpImp1*-null mutant via transformation based on homologous recombination. *CpImp1*-null mutant was confirmed by using PCR screening among the 212 transformants. The phenotype of *CpImp1*-null mutant was similar to the wild-type strain, EP155/2, on PDAmb and no differences in responsiveness was observed to various stressors such as ROS, and high osmotic pressure. To confirm the pathogenicity, Bavendamm assay was conducted for measuring phenoloxidase activity. The colony size of the *CpImp1*-null mutant was not different from that of EP155/2, but the *CpImp1*-null mutant's level of brown coloration was higher than EP155/2. And the virulence assay performed on the excised chestnut tree bark revealed that the *CpImp1-null* mutants produced similar size of necrotic areas compared with the wild type EP155/2. Thus, our study strongly suggests that the protein product of *CpImp1* has an important role in secretion of phenoloxidase but its enhanced secretion does not affect the fungal virulence.

### Effects of Human Activities and Environmental Factors on Soil Microbiome of Urban Green Space

#### Yerang Yang, Jaeyoung Yoo, Jeongeun Yun, Jiwon Choi, Jaehyun Lee, and Hojeong Kang\*

School of Civil & Environmental Engineering, Yonsei University

Urban green space (UGS) plays an important role in maintaining urban ecosystem functions such as pollution remediation, water supply, carbon storage, and nutrient cycling. Although such functions are mainly mediated by soil microorganisms, we have a limited understanding on the structure and functions of the soil microbiome and key environmental factors influencing it. This understanding is essential to managing soils of UGS and to constructing a sustainable city. Here, we selected and collected soil samples from 32 UGS in Seoul, Korea with different characteristics including the size of the area, construction year, location, population, and the number of visitors. Soil and microbial properties such as community composition, microbial diversity, and metabolic activity were analyzed. To compare how these properties of USG differ from those of the natural ecosystems, soil samples were also collected from nearby natural forests and the same analysis was conducted. The contents of soil moisture, soil organic matter (SOM), and dissolved organic carbon were higher in the natural ecosystems compared with UGS and soil pH was higher in UGS than that of natural ecosystems. Soil microbial diversity was higher in natural ecosystems than that of UGS. However, there was no significant difference in soil metabolic diversity between UGS and the natural ecosystems. Soil factors such as soil pH and SOM content were fundamental drivers of soil microbial communities on UGS. Our study provides a more comprehensive understanding of soil microbiome of UGS essential for sustainable unban management.

### Identification of Multiple dsRNA Mycoviruses of *Trichoderma polysporum* NFCF205 and Their Effects on Antifungal Activities of *T. polysporum*

Hae-ryeong Yoon<sup>1</sup>, Jeesun Chun<sup>2</sup>, and Dae-Hyuk Kim<sup>1,2,3\*</sup>

<sup>1</sup>Department of Bioactive Material Science, Jeonbuk National University <sup>2</sup>Institute for Molecular Biology and Genetics, Jeonbuk National University <sup>3</sup>Department of Molecular Biology, Jeonbuk National University

We reported the 15 agarose gel band patterns of double-stranded RNA (dsRNA) from *Trichoderma* spp.. We revealed that band pattern XIII in *Trichoderma polysporum* NFCF205 was co-infected with two mycoviruses related to the members of families "Fusagraviridae" and Partitiviridae. Based on the evaluation of gene organization and sequence similarity, we have named Trichoderma polysporum fusagravirus 1 (TpFV1) and Trichoderma polysporum partitivirus 1 (TpPV1), respectively. Using successive single-spore isolation, we were able to obtain mycovirus-free and single-infected strains with either TpFV1 or TpPV1. We observed growth rate of four strains mentioned above. And we also checked growth inhibition rate of all four strains through dual culture method, water soluble assay, and VOCs profiles exposed to *Rhyzoctonia solani* and *Pleurotus ostreatus*. In this study, we report the presence of novel dsRNA mycoviruses and investigate their possible roles in antifungal activities of *T. polysporum*.

### Predatory Bacteria and Violacein as Alternative Antibiotics under Microgravity

Hyochan Jang<sup>1</sup>, Seong Yeol Choi<sup>1</sup>, Wonsik Mun<sup>1</sup>, Seok Hoon Jeong<sup>2</sup>, and Robert J. Mitchell<sup>1\*</sup>

<sup>1</sup>School of Life Sciences, Ulsan National Institute of Science and Technology (UNIST) <sup>2</sup>Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine

As mankind moves toward permanently inhabiting outer space and other planetary bodies, alternatives to antibiotic that can effectively control drug-resistant pathogens are needed. The activity of one such alternative, *Bdellovibrio bacteriovorus* HD100, was explored here, and was found to be as active or better in simulated microgravity (SMG) conditions as in flask and normal gravity (NG) cultures, with the prey viabilities decreasing by 3- to 7-log CFU/ml in 24 h. The activity of *B. bacteriovorus* HD100 under SMG was also appraised with three different antibiotic resistant pathogens. In addition to being more efficient at killing two of these pathogens under SMG conditions (with losses of 5- to 6-log CFU/ml), we also explored the ability of *B. bacteriovorus* HD100 to hydrolyze the antibiotic resistant gene pools, i.e., mcr-1,  $bla_{kPC-2}$  and  $bla_{0XA-51}$ , present in pathogens. We found removal efficiencies of 97.4±0.9%, 97.8±0.4% and 99.3± 0.1%, respectively, in SMG cultures, while similar reductions were also seen in the flask and NG cultures. However, predatory bacteria cannot attack Gram-positive bacteria. Violacein is a bisindole antibiotic which is effective to several Gram-positive bacteria. Under SMG, antimicrobial activity of violacein increased against *S. aureus* compared to NG. These results illustrate the potential applicability of *B. bacteriovorus* HD100 and violacein as an antibiotic to combat the ever-growing threat of multidrug-resistant (MDR) pathogens during spaceflight.

### BAI22-derived Extracellular Vesicle as a New Synergistic Antibacterial Platform in the Control of Gram-negative Bacterial Infections

#### Hyejin Cho and Kwang-sun Kim\*

Department of Chemistry and Chemistry Institute of Functional Materials, Pusan National University

Treatment against multidrug-resistant (MDR) Gram-negative bacterial infections using last-resort antibiotics, polymyxins, has been hampered by the plasmid-borne mobilized colistin resistance (*mcr*) gene. Chemical compounds or nanocomposites mixed with antibiotics have been found as effective synergistic materials against MDR *Escherichia coli*; nevertheless, their delivery to the target and toxicity upon usage need the employment of novel bacteria-derived molecules as synergistic compounds. Here, we report the discovery of BAI22, a biofilm and antibiotic resistance inhibitor protein in *E. coli*, and its derived extracellular vesicle (EV), a nano-sized vesicles of lipids released from the outer membranes of bacteria, as a synergistic protein or carrier to polymyxins, respectively. In addition, we showed that the biological function of BAI22 by the EV is transmitted to other bacteria, according to further MIC determination and crystal violet testings. Furthermore, polymerase chain reaction (PCR), proteomic analysis, and MIC determination were used to identify and characterize the components of BAI22-derived EV implicated in the function. Because BAI22-derived EV increases the bacterial killing ability while retaining biological properties, it would be a promising antibacterial platform for controlling the undesired spread of Gramnegative pathogens without any side effect associated with pre-developed synergistic materials.

### A Prophylactic Effect of Lactic Acid Bacteria on Colorectal Cancer in an Orthotopic Syngeneic Murine Model

Hee Eun Jo<sup>1,2</sup>, Hyo Kyeong Park<sup>1</sup>, Hak-Jong Choi<sup>1</sup>, and Misun Yun<sup>1\*</sup>

<sup>1</sup>Technology Innovation Research Division, World Institute of Kimchi <sup>2</sup>Department of Biomedical Sciences, Chonnam National University Medical School

Colorectal cancer that tends to increase especially among young people highlight the importance of lifestyle modification as a complement for colorectal cancer (CRC) prevention. As a key metabolic and immune regulator, gut microbiota has been recognized to play an important role in colorectal tumorigenesis. Recently, a number of studies have indicated that probiotics consumption can effectively improve gut health, and reduce incidence of cancer development by manipulating the gut microbiota. In this study, we explored the effect of Lactic acid bacteria (LAB), which was isolated from Kimchi, as a prophylactic during chemotherapy, by analyzing the cecal microbiome in CRC orthotopic mouse model. BALB/c mice were fed orally with LAB for 2 months, and luminescent murine colorectal cancer cell line were injected orthotopically. Mice were imaged in the IVIS® Spectrum in vivo imaging system one week after the tumor implantation. We found that LAB administration retarded tumor growth and also had a synergistic effect with the prominent anti-cancer drug, 5-Fluorouracil (5-FU). Analysis of gut microbiota revealed a compositional dissimilarity among the groups. Using linear discriminant analysis (LDA) effect size (LEfSe), we assigned, as microbial markers, Alistipes and Clostridiaceae to the microbiota of tumorbearing mice. Among the mice co-treated with 5-FU and LAB, we observed that the microbiomes were enriched with Bacteroides caccae and Bacteroides vulgatus that consequently reduced metabolism including biosynthesis of bacterial cell structure, carbohydrate, fatty acid, and lipid. Our results suggested that change of gut microbiome by administration of LAB might decrease the incidence of colorectal cancer.

### Primary Transcriptome Analysis on Intra-macrophage Salmonella Typhimurium Defined a New Role of LeuO in Virulence Regulation

#### Eunsuk Kim<sup>1</sup> and Hyunjin Yoon<sup>1,2\*</sup>

<sup>1</sup>Department of Molecular Science Technology, Ajou University <sup>2</sup>Department of Applied Chemistry and Biological Engineering, Ajou University

Salmonella has become one of the most studied bacterial pathogens due to its exclusive nature of manipulating the host, eventually leading to systematic infections. Despite the ongoing effort of extensive studies, the detailed strategies used by Salmonella to survive inside macrophages have not been fully understood. In order to investigate the comprehensive transcriptional regulatory circuits during intramacrophage survival, intracellular Salmonella cells were isolated from murine RAW 264.7 macrophage cells and subjected to RNA-Seq. DESeq2-mediated analysis revealed 1,013 differentially expressed genes (DEGs) with a threshold of at least 3-fold change, which consisted of 596 up-regulated and 417 downregulated genes. Among the DEGs, genes enrolled in transcriptional regulation were further investigated in consideration of their potential to orchestrate the transcription of multiple genes associated with the intracellular survival. Six Salmonella mutant strains lacking (putative) regulatory genes (STM14 0016, nhaR, leuO, ydhB, yneJ, and envR), respectively, were attenuated in survival inside macrophages and only  $\Delta$ *leuO* strain was complemented by the introduction of leuO in trans. Overexpression of LeuO repressed the transcription of Salmonella pathogenicity Island (SPI-2) genes, which are critical virulence determinants required for Salmonella intracellular survival. ChIP-Seq analysis predicted the presumable LeuO-binding motif in SPI-2 loci as well as in well-known cognate targets. These results give insight into the new role of LeuO in Salmonella virulence regulation inside host cells.

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### Taxonomic and Functional Diversity of the Chemoautotrophic Microbiome in the *Pillucina pisidium* (Bivalvia: Lucinidae) Occurring on a Seagrass Meadow in Jeju Island

Jong-Seop Shin<sup>1</sup>, Kae Kyoung Kwon<sup>2</sup>, Seong-il Eyun<sup>3</sup>, and Kwang-Sik Choi<sup>1\*</sup>

<sup>1</sup>Department of Marine Life Science (BK21 FOUR), Jeju National University <sup>2</sup>Marine Biotechnology Research Center, Korea Institute of Ocean Science and Technology <sup>3</sup>Department of Life Science, Chung-Ang University

Unlike most bivalves, which acquire energy through filter feeding, the family Lucinidae uses organic matter synthesized by the chemoautotrophic microbiome in their gills as the main energy source. The host clams provide the gill filament as a habitat for the symbiotic microbes and supply sulfate from the ambient environment to the symbionts. The sulfur-oxidizing symbionts produce organic substances using the energy generated through sulfide oxidation and deliver it to the host. These functional symbiotic relationships have been reported in various environments, from deep-sea hydrothermal vents to coastal mangroves and seagrass beds in which sulfides are continuously supplied. Especially In the seagrass bed, lucinid clams and the symbiotic sulfur-oxidizing bacteria play an important role in seagrass growth by removing the toxic sulfide. This study explored molecular characteristics of the sulfur-oxidizing microbes in the gills of lucinid clam *Pillucina pisidium* occurring in the *Zostera marina* seagrass bed on Jeju Island. 16S rRNA metagenome sequencing was carried out on ten clams collected from the Z. marina bed to identify the gill symbionts. After sequencing the RNA expressed in the gills, the expressed genes were classified through the taxonomic independent binning technique to identify the species and understand their roles in the host. 16S metagenomic sequencing revealed that two bacterial OTUs, Thiodiazotropha (90.17%) and Alkalispirochaeta (8.68%), dominate the gill tissues. *Thiodiazotropha*-like bacteria sequences were clustered with other symbionts groups extracted from Codakia and Lucina clams in the Family Lucinidae in the phylogenetic tree using the 16S rRNA. On the other hand, Alkalispirochaeta is rarely reported in Lucinidae clam. RNA sequencing identified chemosynthesis-related genes from Thiodiazotropha-like bacteria, including the Iron-sulfur cluster insertion protein ErpA, Sulfur carrier protein TusA, and Adenylylsulfate reductase subunit beta, strongly suggesting that they are symbiotic sulfur-oxidizing microbes, which play a crucial role in the coastal seagrass bed.

### Pullulan Nanoparticles Inhibit the Pathogenicity of *Candida albicans* by Regulating Hypha-Related Gene Expression

Sujin Hong<sup>1</sup>, Seo-Kyung Kim<sup>2</sup>, Chong-Su Cho<sup>3</sup>, and Won-Ki Huh<sup>1,4\*</sup>

<sup>1</sup>School of Biological Sciences, Seoul National University <sup>2</sup>Department of Agricultural Biotechnology, Seoul National University <sup>3</sup>Research Institute of Agriculture and Life Sciences, Seoul National University <sup>4</sup>Institute of Microbiology, Seoul National University

*Candida albicans* is a common opportunistic pathogenic fungus that resides on the skin and gastrointestinal tract of humans. Under certain conditions, *C. albicans* cells undergo a transition from commensal to pathogenic, leading to superficial and invasive infections. Although systemic candidiasis is a life-threatening disease, only a few antifungal drugs are used as treatment for the disease. In addition, due to the occurrence of resistant strains to antifungal agents, the need for a new treatment is emerging. One promising strategy is therapy using nanomaterials. In this study, we synthesized phthalic pullulan nanoparticles (PPNPs) and examined their ability to inhibit the pathogenicity of *C. albicans*. We observed that PPNPs are internalized into *C. albicans* cells through endocytosis. Internalized PPNPs inhibit *C. albicans* adhesion to abiotic surface and biofilm formation in a dose-dependent manner. This inhibitory effect is mediated by transcriptional modulation, particularly down-regulation of hypha-related genes and up-regulation of stress-responsive genes. Furthermore, we observed that PPNPs inhibit the adhesion of *C. albicans* to human epithelial cells without toxicity to human cells. Taken together, our findings suggest that PPNPs have an inhibitory effect on *C. albicans* pathogenicity and thus have potential as a novel therapeutic agent for candidiasis.

### Quorum Sensing Protein VqmA Senses Glucose-induced Host Signals through HPr in *Vibrio vulnificus*

#### Gahee Park, Jaehun Lee, Kyoo Heo, and Yeong-Jae Seok\*

School of Biological Sciences and Institute of Microbiology, Seoul National University

Bacteria recognize and respond to a variety of extracellular environments. Quorum sensing is a cell-tocell communication process that allows bacteria to regulate genes in response to changes in surrounding bacterial cell density and species composition. In *Vibrio cholerae*, VqmA-VqmR quorum sensing pathway regulates pathogenesis and senses host-derived signals. Here, I identify the structural importance of VqmA to VqmA-VqmR pathway in *Vibrio vulnificus*. Transcription factor VqmA activates expression of *vqmR* and affects pathogenicity in the mouse infection model. The expression level of *vqmR* is increased in the presence of glucose because dephosphorylated HPr interacts with VqmA and increases the transcriptional activity of VqmA. The Interaction between HPr and VqmA is highly species-specific. Also in *Vibrio vulnificus*, VqmA PAS domain has some different amino acid residues compared to other *Vibrio* species, which can be considered to form a specific structural form with HPr. VqmA amino acid mutant in PAS domain loose its binding affinity to HPr. And mutations of other amino acids, expected to interact with HPr histidine residue, loose their repulsion power to phosphoryl residue in HPr. These structural features of VqmA in *Vibrio vulnificus* give advantages to virulence regulation in different environment. I propose that *Vibrio vulnificus* recognizes the infection niche through the interaction between VqmA and HPr and regulates pathogenicity.

### Single-cell Classification in Synthetic Community Using Machine Learning Models and Flow Cytometry

#### In Jae Jeong, Young Jun Bae, and Tae Kwon Lee\*

Department of Environmental & Energy Engineering, Yonsei University

Flow cytometry allows to characterize the properties of single cell using multiple parameters including cell size, cell complexity and nucleic acid contents. It is still challenging to identify how the flow cytometric phenotype of the interested populations changes due to environmental changes or microbial interaction. In this study we apply machine learning models on multidimensional flow cytometry data to identify the bacterial proportion in the synthetic bacterial communities.

We collected the flow cytometric data (400,000 events) for 4 different genera (*Acinetobacter johnsonii, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis*), and a synthetic microbial community (SynCom) was builded in silico with different ratios. Regardless of the ratio of each strain in the SynCom. Random forest could predict each strain with high accuracy with approximately 92%. The highest accuracy of 98% was obtained for *A. johnsonii* by the proposed approach whereas *M. luteus* was as classified as the lowest accuracy with 87%. In addition, these approaches made it possible to calculate the abundances of each genus in SynCom at an error rate of 6–10% compared to the predicted results.

Our result show that applying Random Forest classification with Flow cytometry analysis can identify the quantitative amount of Syncom and rapidly recognize the change of Syncom due to environment changes.

### Characterization of a *Vibrio vulnificus* Mutant Deficient in Putative *rcsB* Gene Which Only Present in Clinical Genotype (Biotype 1C)

#### Joon-Gi Kwon, Jeong-Eun Kwak, and Ju-Hoon Lee\*

Department of Agricultural Biotechnology, Seoul National University

Vibrio vulnificus is the pathogen isolated from the estuarine waters and variety of seafood. It has been subdivided into three biotypes. Biotype 1 is associated with the majority of human infection, and is divided into two genotypes: a clinical genotype (biotype 1C), and an environmental genotype (biotype 1E). To identify their virulence factors and clinical characteristics, complete genome sequences of clinical genotype and environmental type were obtained from the NCBI database and compared genome sequences using pan-genome approach. Pan-genomic analysis revealed that only biotype 1C had specific gene cluster, not had in biotype 1E. The specific gene cluster consists of a response regulator transcription factor, GGDEF domain containing protein, EAL domain containing response regulator, and histidine kinase (VV2 1508-1512). Mutations of all genes in a specific gene cluster were generated to determine the phenotype of each gene. The response regulator transcription factor gene (VV2\_1508) deficient mutant shows a colonial morphology change from opaque form to translucent form. To shed light on the function of VV2 1508 and its implication on promoter recognition, the protein structure was analyzed using Alphafold2. As a result, the VV2\_1508 is structurally very similar to the regulator capsule synthesis B (rcsB) gene of Salmonella Typhimurium and consists of two domains: a receiver domain (REC) and a DNA binding domain (DBD). In addition, this protein bind promoter of wza (VV1 0786) gene which belong to the capsular polysaccharide (CPS) cluster and the promoter of VV2 1512 gene which belong to the specific gene cluster. Furthermore, the mutant type displayed significantly reduced the CPS and relevant gene expression. A virulent opaque form produces CPS and a translucent phenotype produces little or no CPS. CPS reduction of mutant type shows that the viability was reduced by Raw 264.7 macrophage, and the mutant type formed significantly more biofilm than wild type because the CPS reduction increases the biofilm formation in V. vulnificus. These results suggest that putative rcsB gene affect the CPS production of clinical strains and indicate that this gene may be associated with virulence factor of clinical strains.

### Maternal High-fat Diet Intake Modulates the Gut Microbial Composition of 16-week-old Rat Offspring

Soo-Min Kim<sup>1,2</sup>, Sunwha Park<sup>1</sup>, Abuzar Ansari<sup>1</sup>, Gain Lee<sup>1,2</sup>, Young-Ah You<sup>1\*</sup>, and Young-Ju Kim<sup>1,2\*</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, College of Medicine, Ewha Medical Research Institute, Ewha Womans University Mokdong Hospital <sup>2</sup>System Health & Engineering Major in Graduate School (BK21) Plus Program

Maternal high-fat diet during pregnancy plays an important role in offspring's health, such as obesity and metabolic diseases. It is known that the gut microbial composition of the offspring is influenced by that of the mother. Here, we report that a high-fat diet during pregnancy affects gut microbiome composition by 16 weeks of age, even when rat offspring eat a normal diet. Sprague-Dawley (SD) rats were divided into three groups by maternal diet during pregnancy and after birth. From the ninth day of pregnancy, (1) the Control group (control, Ad-Libitum/Ad-Libitum) was provided a chow diet, and (2) the High-fat group (HF, 45% high-fat/Ad-Libitum) and (3) Obese group (OB, 45% high-fat/45% high-fat) fed 45% high-fat diet. After birth, the Control and High-fat groups were fed chow Ad-Libitum, and the Obese group was given a 45% high-fat diet during lactation. Body weight, weight gain, and food intake as well as blood lipid indices (total cholesterol, triglyceride, and free fatty acid) and glucose were measured in offspring. We conducted the 16S rRNA sequencing analysis in the colon of maternal and 16-week-old rat offspring to compare the gut microbial composition. Glucose and triglyceride levels were significantly increased in the OB compared to the control group. Also, the free fatty acid level was significantly higher in both HF and OB groups. HF and OB groups of both the maternal and 16-week-old rat offspring showed lower alpha diversity compared with the control group. The control group had a significantly higher relative abundance of several genera belonging to Lachnospiraceae, known as the butyrate producers, than the other two groups. Thus, our results suggest that the maternal high-fat diet during pregnancy may affect the gut microbiome of the offspring, leading to metabolic disorders later in life.

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### The Field-scale Study to Understand the Performances of Dechlorination Bacteria and Functional Genes in a TCE Contaminated Industrial Site

Jeongwon Kim<sup>1,2</sup> and Keunje Yoo<sup>1,2\*</sup>

<sup>1</sup>Department of Environmental Engineering, Korea Maritime & Ocean University <sup>2</sup>Interdisciplinary Major of Ocean Renewable Energy Engineering, Korea Maritime & Ocean University

Trichloroethylene (TCE) are mainly found in industrial complex areas where high concentrations of pollutants and low concentrations of pollutants exist in large areas. Dechlorinating microorganisms play an essential role in the effective natural degradation of TCE. Therefore, the degree of dechlorination capability were investigated in groundwater contaminated with TCE for about half a year from Ik-san industrial complex. TCE concentrations decreased significantly in August compared to March, up to 90%. In addition, cis-DCE and VC concentrations remained high during the monitoring period suggesting preferential enrichment of indigenous partial dechlorination. From qPCR analysis, the *Dehalococcoides* 16S rRNA recorded an average of 10<sup>10</sup> copies/L, exceeding the level known to be biodegradable for TCE (10<sup>6</sup> copies/L). The microbial community additionally showed that the relative abundances of dechlorination-associated bacteria, such as *Geobacter* and *Gallionella*, were similar between March and August samples. This suggests that a sufficient pool of dechlorination performances to understand how they can potentially enhance or inhibit augmented TCE degradation near future.

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### Unveiling the Mechanism of Bactericidal Activity by an Engineered Endolysin LNT103

#### Kyungah Park<sup>1</sup> and Hyunjin Yoon<sup>1,2\*</sup>

<sup>1</sup>Department of Molecular Science Technology, Ajou University <sup>2</sup>Department of Applied Chemistry and Biological Engineering, Ajou University

Bacteriophages encode endolysins at the end of their lytic cycle to degrade the peptidoglycan layer of the host bacterium, leading to release of progeny phages. In virtue of the bacteriolytic activity, endolysins have been explored as an alternative antibacterial agent. However, the outer membrane present in Gram-negative bacteria obstructs the access of exogenous endolysins to the peptidoglycan lying beneath the outermost membranous structure. In order to overcome the restriction of intrinsic endolysins, we engineered an endolysin to improve its ability to penetrate the membranous structures in Gram-negative bacteria. An endolysin encoded by *Pseudomonas aeruginosa* phage PBPA90 was engineered by substituting the 15 amino acids (mtPA90) and further by fusing the antimicrobial peptide cecropin A to its N-terminus (LNT103). Lipopolysaccharides (LPS) destabilization, bactericidal activity, membrane permeability and LPS neutralization were compared between mtPA90 and LNT103. Cecropin A of LNT103 improved the interaction with LPS and accelerated the disruption of bacterial membrane, leading to faster killing of Gram-negative bacteria. An LPS mutant strain with an altered lipid A structure was more susceptible to both endolysins, suggesting that the integrity of lipid A is important to dampen endolysin penetration into bacterial membrane. This study defined the molecular mechanism of action in destructing Gram-negative pathogens by LNT103.

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### Particulate Matter Regulates Lipid and Inflammation Cytokine Production in *Cutibacterium acnes*-induced Human Sebocyte Cell Line (*SZ95*)

Yong Hee Choi<sup>1</sup>, Yoon Jin Roh<sup>1</sup>, Mi-Kyung Lee<sup>2</sup>, Seong Jun Seo<sup>2</sup>, and Kui Young Park<sup>2\*</sup> <sup>1</sup>Department of Dermatology, Chung-Ang University Hospital, <sup>2</sup>Department of Laboratory Medicine, Chung-Ang University Hospital

The skin is one of the main organs exposed to airborne particulate matter (PM), which is a complex mixture including harmful solid and liquid particles linked to a wide range of adverse health endpoints. Recent some studies have demonstrated that PM is associated with inflammatory response and various skin diseases. *Cutibacterium acnes (C. acnes)* are commensal bacterium, which is involved in acne inflammation. Little is known concerning the potential effects of PMs on acne inflammation. Therefore, we conducted experiments to analyze the effects of PMs on *C. acnes* treated *SZ95* cells.

We injected Heat-killed *C. acnes* (500MOI) in *SZ95* cell to induce acne-like status. 1649b (PMB), which mainly comprised polycyclic aromatic hydrocarbons was used as reference PMs. After 1 h, SZ95 cells was treated with PM ( $10 \mu g/cm^2$ ). Cell viability was measured by WST-8 assay. The reactive oxygen species (ROS) generation was measured by DCFH-DA assay. The gene expression was investigated by real-time PCR and protein expression was assessed by Western blot. Levels of inflammatory cytokines were measured with ELISA. The results of this experiment will be shared and discussed at the time of presentation.

#### A002

### Detection and Isolation of a Single Colony of Hydrogen Peroxide-producing *Streptococcus oralis* Strain from Saliva

Thuy Tran Thi Dieu, Ha Pham, and Yong-Hak Kim<sup>\*</sup> Department of Microbiology, Daegu Catholic University School of Medicine

Hydrogen peroxide  $(H_2O_2)$  is produced by alpha-hemolytic streptococci in aerobic conditions. However, a suitable method for the detection of  $H_2O_2$ -producing streptococci in oral microbiota has not been set up. Here we show that *o*-dianisidine and horseradish peroxidase were useful in tryptic soy agar medium to detect and isolate only one single colony of the  $H_2O_2$ -producing strain HPO1 from a saliva sample with more than  $10^6$  colony-forming units. Further tests showed that the strain HPO1 belongs to *Streptococcus oralis in* the Mitis group and characteristically is able to form very short-chain cells with a high capacity of acid tolerance and biofilm formation. The genome analysis revealed the heterogeneity of the strain HPO1 with the type strains of *S. oralis*. The genome sequence shows different rates of variation in some essential proteins, including thioredoxins and glutaredoxins against oxidative damage caused by  $H_2O_2$ , as well as various antibiotic resistance genes and horizontally transferred genes.

### **Polyphasic Characterization of Two Novel** *Ornithinimicrobium* **Species Isolated from Korean Indigenous Birds** So-Yeon Lee, Hojun Sung, and Jin-Woo Bae<sup>\*</sup>

Department of Biology and Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University

Phenotypic and genomic analyses were performed to characterize two novel strains, H23M54<sup>T</sup> and AMA3305<sup>T</sup>, isolated from the faeces of the Oriental stork (*Ciconia boyciana*) and the cinereous vulture (*Aegypius monachus*), respectively. Strains H23M54<sup>T</sup> and AMA3305<sup>T</sup> showed the highest 16S rRNA gene sequence similarity with *Ornithinimicrobium cavernae* KCTC 49018<sup>T</sup> (98.54%) and *O. pekingense* JCM 14001<sup>T</sup> (98.49%), respectively. Both the strains were Gram-stain-positive, obligate aerobes, non-motile, non-spore forming, and coccoid to rod shaped. Colonies of the both strains were smooth, opaque, and convex and had an intact margin. Both the strains had iso- $C_{15:0}$ , iso- $C_{16:0}$ , and summed feature 9 (iso- $C_{17:1}$   $\omega$ 9c and/or  $C_{16:0}$  10-methyl) as their major cellular fatty acids. Strains H23M54<sup>T</sup> and AMA3305<sup>T</sup> possessed diphosphatidylglycerol and phosphatidylglycerol as their major polar lipids. Moreover, strains H23M54<sup>T</sup> and AMA3305<sup>T</sup> commonly contained ribose and glucose as their whole-cell sugar components and l-ornithine, l-alanine, glycine, and aspartic acid as their whole-cell amino acid components. We propose the name *Ornithinimicrobium avium* sp. nov. for strain AMA3305<sup>T</sup> (= KCTC 49180<sup>T</sup> = JCM 32873<sup>T</sup>). [This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean government (MSIT) (NRF-2022M3A9F3082331).]

#### A004

#### Photobacterium halophilum sp. nov. and a Salt-loving Bacterium Isolated from Marine Sediment

Minji Kim<sup>1</sup>, Ki-Eun Lee<sup>2</sup>, In-Tae Cha<sup>2</sup>, and Soo-Je Park<sup>1\*</sup>

<sup>1</sup>Department of Biology, Jeju National University, <sup>2</sup>Microorganism Resources Division, National Institute of Biological Resources

A Gram-stain-negative, rod-shaped, and facultatively anaerobic bacterium named strain  $GJ3^T$  was isolated from coastal sediment of Jeju Island, South Korea. Catalase and oxidase activity were detected in the cell of strain  $GJ3^T$ , as well as white pigmented colony and motility with polar flagellum. The cell grew optimally at 30°C, pH 7.0, in the presence of 4% (w/v) sodium chloride. Phylogenetic analysis using the 16S rRNA gene sequence indicated that strain  $GJ3^T$  was classified to the genus *Photobacterium*, with high sequence similarity to *Photobacterium galatheae*  $S2753^T$  (98.30%), *Photobacterium halotolerans* MACL01<sup>T</sup> (97.90%), and *Photobacterium panuliri* LBS5<sup>T</sup> (96.55%). Strain  $GJ3^T$  possessed only ubiquinone-8 (Q-8) as a respiratory quinone and summed feature 8 as the major fatty acid (38.18%). Additionally, the dominant polar lipids phosphatidylglycerol and phosphatidylethanolamine were identified. The complete genome size and G + C content of strain  $GJ3^T$  was estimated to be 3,603,274 bp in length and 50.70%, respectively. Polyphasic approach and genomic analyses (e.g., ANI and digital DDH) revealed that strain  $GJ3^T$  represented a novel species within the genus *Photobacterium*, and the name *Photobacterium halophilum* sp. nov., is proposed for this novel bacterium.

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#### Mushroom Research on the Hwasun Gotjawal

Dea-Ju Oh, Weon-Jong Yoon, Yong-Hwan Jung, and Seung-Hak Lee<sup>\*</sup> Biodiversity Research Institute, Jeju Technopark

This study was conducted to provide the academic foundation for the mushroom species diversity studies on Hwasun Gotjawal, which has not been investigated compared to other Gotjawal in Jeju Island. It was performed for 8 months from April to November 2020. To identify the collected mushrooms, morphological analyses of macroscopic and microscopic features and molecular analysis of the nuclear ribosomal internal transcribed spacer region were conducted. As a result, a total of 17 families, 25 genera, and 33 species of mushrooms were investigated. Also, one species each of the genera *Candolleomyces* and *Mycena*, are introduced as unrecorded species. The genus *Candolleomyces*, originally described as *Psathyrella*, have 26 species worldwide. *Mycena* is one of the largest genera with 1714 species recorded worldwide, and some species have characteristic luminescence. Our results indicated that the newly found specimens were *C. subsingeri* and *M. seminau*. Herein, we describe the species and basic data of the mushroom species' diversity in Gotjawal.

#### A006

*Falsiroseomonas oryzae* sp. nov., Isolated from Oxidized Layer of Rice Paddy Soil Hyo-Jin Lee<sup>1,2</sup>, Dan-Bi Kim<sup>2</sup>, and Kyung-Sook Whang<sup>1,2\*</sup> <sup>1</sup>Institute of Microbial Ecology and Resources, Mokwon University, <sup>2</sup>Department of Microbial Biotechnology, Mokwon University

Researchers have been tried to improve cultivation methods to isolate diverse and novel PPB in rice paddy soil. We succeeded in isolating purple phototrophic bacteria belonging to diversity of genera. In this study, we propose that strain MO-31<sup>T</sup> represent a novel species of the genus *Falsiroseomonas* based on polyphasic taxonomic approach. Cells are Gram-staining-negative, aerobic, non-motile and coccobacilli-shaped. The strain grew at 4-45 °C (optimum, 30-37 °C), at pH 4.0-11.0 (optimum, pH 7.0-9.0) and in 0-7.0% (w/v) NaCl (optimum, 0-1.0 % NaCl). The 16S rRNA gene sequence of strain MO-31<sup>T</sup> was highest in similarity to that of *F. bella* CQN31<sup>T</sup>, *F. terricola* EM0302<sup>T</sup> and *F. chloroacetimidivorans* BUT-13<sup>T</sup> at 96.1~96.5%, respectively. The genome size of strain MO-31<sup>T</sup> was 6.4 Mb with a 72.1 mol% G+C content. The average nucleotide identity and DNA–DNA hybridization values between strain MO-31<sup>T</sup> and type strains of *Falsiroseomonas* species. The major fatty acids were  $C_{18:1} \omega 7c$  and  $C_{18:1} 2$ -OH (>10%). Based on the phenotypic, phylogenetic, and chemotaxonomic characteristics, the strain MO-31<sup>T</sup> represent novel species of the genus *Falsiroseomonas*, for which the name for which the name *Falsiroseomonas oryzae* sp. nov. is proposed. The type strain is MO-31<sup>T</sup> (=KACC 22465<sup>T</sup> =JCM 35532<sup>T</sup>).

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*Erythrobacter oryzae* sp. nov., a Novel Plant Growth-promoting Bacterium Isolated from Rice Paddy Soil Hyo-Jin Lee<sup>1,2</sup>, Ji-Soo Hwang<sup>1</sup>, Eun-Kyung Lee<sup>1,2</sup>, and Kyung-Sook Whang<sup>1,2\*</sup> <sup>1</sup>Institute of Microbial Ecology and Resources, Mokwon University, <sup>2</sup>Department of Microbial Biotechnology, Mokwon University

We have isolated a large number of purple phototrophic bacteria (PPB) from rice paddy soils (oxidized and reduced soils) by using the soil suspension, inoculation method, culture medium and extended incubation times described previously. We obtained 500 isolates of PPB, which were detected by PCR amplification of the *pufLM* gene and pigment analysis. Twenty-three of the total PPB isolates were able to produce indole-3-acetic acid (IAA), and production ranged from 171 to 970  $\mu$ M OD540<sup>-1</sup>. All of the isolates showed the same germination quality in cucumber as the non-treated control. These isolates were initially screened by employing seed germination and seedling vigor index (SVI) to evaluate their potential as inoculants. The *Erythrobacter* sp. COR-2 was the most effective in improving the seedling vigor index (121%), rooting ability (102%), compared to the non-treated control. Phylogenetic analysis based on 16S rRNA gene and whole-genome sequences revealed that strain was most closely related to *Erythrobacter colymbi* TPW-24<sup>T</sup> within the cluster of the genus *Erythrobacter*. The ANI and dDDH values between strain COR-2<sup>T</sup> and most closely related species of the genus *Erythrobacter* were 79.3~85.5% and 24.1~29.1%, respectively. Based on phylogenetic and phenotypic considerations, it is proposed that strain COR-2<sup>T</sup> be classified as a new species, named *Erythrobacter oryzae* sp. nov. The type strain is COR-2<sup>T</sup> (=JCM 34624<sup>T</sup>).

#### A008

*Nocardiopsis composti* **sp. nov., an Ammonia-oxidizing Bacterium Isolated from Livestock Manure Compost** Hyo-Jin Lee<sup>1,2</sup>, Ye-Jin Kim<sup>2</sup>, So-Yeong Min<sup>2</sup>, Yun-Ji Park<sup>2</sup>, Na-Ri Shin<sup>2</sup>, Eun-Kyung Lee<sup>1,2</sup>, and Kyung-Sook Whang<sup>1,2\*</sup> <sup>1</sup>Institute of Microbial Ecology and Resources, Mokwon University, <sup>2</sup>Department of Microbial Biotechnology, Mokwon University

A novel ammonia-oxidizing bacterium, designated strain ATB16-24<sup>T</sup> was isolated from livestock manure compost. The strain showed morphological, chemotaxonomic and phylogenetic characteristics consistent with its classification in the genus *Nocardiopsis*. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain ATB16-24<sup>T</sup> belonged to the genus *Nocardiopsis* and showed the highest sequence similarities to *Nocardiopsis algeriensis* B32<sup>T</sup> (98.0%) and *Nocardiopsis lucentensis* DSM 44048<sup>T</sup> (97.8%). The genomic size of strain ATB16-24<sup>T</sup> was 5.3 Mb with a 69.2 mol% G+C content. The phylogenetic placement was supported by lower than species delineation threshold average nucleotide identity (ANI) ( $\leq$  84.7%), and digital DNA-DNA hybridization (dDDH) ( $\leq$  28.4%) values between strain ATB16-24<sup>T</sup> and closely related members in the genus *Nocardiopsis*. The major fatty acids (>10% of total) were iso-C<sub>16:0</sub> and anteiso-C<sub>17:0</sub>. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid, alanine, glutamic acid and aspatic acid. The polar lipids included DPG, PE, PG, PC, two unknown lipids and two unknown phospholipids. The results of phenotypic, chemotaxonomic, genotypic and phylogenetic analyses revealed that strain ATB16-24<sup>T</sup> represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis compoti* sp. nov. is proposed. The type strain is ATB16-24<sup>T</sup> (=KACC 22507<sup>T</sup>=JCM 35555<sup>T</sup>).

#### Halomonas antri sp. nov., a Carotenoid-producing Bacterium Isolated from Surface Seawater

Yoonseop So, Geeta Chhetri, Inhyup Kim, Minchung Kim, and Taegun Seo<sup>\*</sup> Department of Life Science, Dongguk University-Seoul

A Gram-negative, moderately halophilic bacterium, designated as strain Y3S6<sup>T</sup>, was isolated from a surface seawater sample collected from the Dongangyoeng cave, Udo-myeon, Jeju-do, in the Republic of Korea. The cells of strain Y3S6<sup>T</sup> were aerobic, rod shaped, non-sporulated and yellow. The cells were catalase negative, oxidase negative and motile with one polar flagellum. The growth of strain Y3S6<sup>T</sup> occurred at a temperature of 15–40°C at pH 6.0–9.0 and in the presence of 0–13% NaCl. The novel strain was able to produce carotenoids. Its chemotaxonomic and morphological characteristics were consistent with those of members of the genus *Halomonas*. Phylogenetic analysis of the 16S rRNA gene sequence revealed that strain Y3S6<sup>T</sup> formed clade with *H. pellis* L5<sup>T</sup> (98.97%), *H. saliphila* LCB169<sup>T</sup> (98.90%). The ANI and dDDH values of strain Y3S6<sup>T</sup> with the most closely related strains whose whole genomes are publicly available were 82.3–85.2% and 62.8–66.1%, respectively. The major fatty acids in strain Y3S6<sup>T</sup> were C<sub>16:0</sub>, C<sub>19:0</sub> cyclo *w*8*c* and summed feature 8, and the predominant quinone was Q-9. Its polar lipid profile consisted of DPG, PG, PE, two UPGLs, one UPAGL and one UPL. The genomic DNA G + C was 64.2 mol%. The results of physiological and biochemical tests and 16S rRNA sequence analysis clearly revealed that strain Y3S6<sup>T</sup> represents a novel species in the genus *Halomonas*, for which the name *H. antri* sp. nov. has been proposed.

[This work was supported by NIBR.]

#### A010

Isolation and Characterization of *Neoroseomonas alba* sp. nov., *Neoroseomonas nitratireducens* sp. nov., *Pararoseomonas indoligenes* sp. nov. and *Pararoseomonas baculiformis* sp. nov.

Yoonseop So, Geeta Chhetri, Inhyup Kim, Sunho Park, Yonghee Jung, and Taegun Seo<sup>\*</sup> Department of Life Science, Dongguk University-Seoul

Four novel Gram-negative bacteria designated as HAJ6<sup>T</sup>, PWR1<sup>T</sup>, SG15<sup>T</sup>, and SSH11<sup>T</sup> strains were isolated from the soil of paddy field Goyang in the Republic of Korea. The isolated strains were Gram-negative, aerobic, short-rod and rod shaped, non-sporulated. They grew optimally at 30°C, pH 7 and 0% NaCl (w/v). Phylogenetic analysis based on 16S rRNA gene sequence revealed that they belonged to *Neoroseomonas* and *Pararoseomonas* and closely related to *Neoroseomonas terrae* DS-48<sup>T</sup> (97.50%), *Neoroseomonas rubea* MO17<sup>T</sup> (99.37%), *Pararoseomonas pecuniae* N75<sup>T</sup> (97.33%) and *Pararoseomonas rosea* 173-96<sup>T</sup> (97.80%). The average nucleotide identity (ANI) and digital DNA–DNA hybridiation (dDDH) values of isolates having the most closely related strains with publicly available whole genomes were 72.9–90.9% and 19.1–26.8%, respectively. The major fatty acids in the isolates were C<sub>16:0</sub>, C<sub>19:0</sub> cyclo  $\omega 8c$ , C<sub>18:1</sub>2-OH and summed feature 8 (composed of C<sub>18:1</sub> $\omega 7c$  and/or C<sub>18:1</sub> $\omega 6c$ ), and the predominant quinone was ubiquinone 10. The polar lipid profile constisted of DPG, PE, PC and other unidentified polar lipids. Based on the draft genome sequence, the genomic DNA G + C was 69.5%, 72.0%, 70.8% and 69.7%. All isolates produced indole-3-acetic acid in the presence of L-tryptophan. Physiological and biochemical tests and 16S rRNA sequence analysis clearly revealed that isolates were novel species belonging to Neoroseomonas and Pararoseomonas. [This work was supported by NIBR and NRF.]

#### Runella salmonicolor sp. nov. and Dyella lutea sp. nov., Isolated from Paddy Field Soil in South Korea

Sunho Park, Inhyup Kim, Geeta Chhetri, Yoonseop So, Yonghee Jung, and Taegun Seo<sup>\*</sup> Department of Life Science, Dongguk University-Seoul

Strains S5<sup>T</sup> and Sa<sup>T</sup> were collected from the soil of a paddy field around Dongguk University in Goyang, South Korea. Two Gram-stain-negative, rod-shaped, aerobic or facultatively anaerobic bacterial strains were designated S5<sup>T</sup> and Sa<sup>T</sup>. A phylogenetic tree analysis based on 16S rRNA and whole-genome sequences suggested that these two strains belong to the genus *Runella* and the genus *Dyella*, respectively. Strain S5<sup>T</sup> exhibited 99.22%, 98.10%, and 97.68% similarity to *Runella rosea* HYN0085<sup>T</sup>, *Runella aurantiaca* YX9<sup>T</sup>, and *Runella slithyformis* DSM19594<sup>T</sup>, while strain Sa<sup>T</sup> exhibited 99.18%, 98.36%, 97.82%, and 97.68% similarity to *Dyella thiooxydans* ATSB10<sup>T</sup>, *Frateruia defendens* HyOG<sup>T</sup>, *Fulvimonas yonginensis* 5HGs31-2<sup>T</sup>, and *Dyella ginsengisoli* Gsoil 3046<sup>T</sup>. The average nucleotide identity difference values of strains S5<sup>T</sup>, Sa<sup>T</sup>, and the species reference strains were 92.16–92.62% and 92.71–93.43%, which confirms that the S5<sup>T</sup> and Sa strains stand for two new species of the genera *Runella* and *Dyella*. The draft genome of strain S5<sup>T</sup> consisted of 7,048,502 bp, with a G+C content of 44.9%, and that of strain Sa<sup>T</sup> of 4,398,720 bp with a G + C content of 67.9%.

[Supported by grants from NIBR and NRF.]

#### A012

#### Ideonella oryzae sp. nov., a Novel Bacterium Isolated from Soil, South Korea

Yonghee Jung, Geeta Chhetri, Inhyup Kim, Yoonseop So, Sunho Park, and Taegun Seo<sup>\*</sup> Department of Life Science, Dongguk University-Seoul

Strain NS12-5<sup>T</sup> was Gram-stain-negative, aerobic, rod-shaped, cream-white color, and motile by means of two or more polar or subpolar flagella. Growth occurred in the temperature range of 15–40°C, and at pH 5.0–11.0, and cells were tolerant to 0–1% NaCl concentration. Strain NS12-5<sup>T</sup> was positive for both catalase and oxidase activities. cells were positive for hydrolysis of casein, Tween 20, and Tween 80. cells are susceptible to ( $\mu$ g/disc) ceftazidime (30), erythromycin (15), gentamicin (10), kanamycin (30), neomycin (30), novobiocin (30), rifampicin (5), tetracycline (30), and resistance to ampicillin (10), cefazoline (30). In the API 20NE test, cells are positive for Nitrate reduction, gelatin hydrolysis, and assimilation of D-glucose and negative for indole production, glucose fermentation, Arginine dihydrolase, urea, esculin hydrolysis, and β-galactosidase (PNPG), and assimilation of Larabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid. In the API ZYM test, Positive for C4 esterase, C8 esterase lipase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and  $\alpha$ -glucosidase activities. The major fatty acids (> 10%) were summed feature 3 (comprising C<sub>16:1</sub> $\omega$ 7*c* and/or C<sub>16:1</sub> $\omega$ 6*c*), C<sub>16:0</sub>, and C<sub>10:0</sub> 3-OH. The major polar lipids were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and diphosphatidylglycerol (DPG).

#### Spirosoma liriopis sp. nov., a Novel Bacterium Isolated from Fruits of Liriope Muscari

Yonghee Jung, Geeta Chhetri, Inhyup Kim, Yoonseop So, Sunho Park, and Taegun Seo<sup>\*</sup> Department of Life Science, Dongguk University-Seoul

Strain RP8<sup>T</sup> was Gram-stain-negative, yellow-colored, facultative anaerobic, rod-shaped, and non-motile. Strain RP8<sup>T</sup> grew in the temperature range of 15–37°C, pH range of 5–11, and 0–1% NaCl concentration on R2A agar. Cells were positive for catalase, oxidase, hydrolysis of CM-cellulose. Strain RP8<sup>T</sup> was susceptible to ( $\mu$ g/disc) erythromycin (15), gentamicin (10), kanamycin (30), neomycin (30), novobiocin (30), rifampicin (5), tetracycline (30), and resistance to ampicillin (10), cefazoline (30), and ceftazidime (30). In the API 20NE test, cells are positive for esculin hydrolysis, gelatin hydrolysis, β-galactosidase (PNPG), but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. In API ZYM test, cells are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase. The major fatty acids were summed feature 3, C<sub>16:1</sub>ω5c, and iso-C<sub>15:0</sub>. The major polar lipids are phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG).

#### A014

### *Flavobacterium cyclinae* sp. nov. and *Flavobacterium channae* sp. nov., Isolated from the Intestines of Corb Shell and Northern Snakehead

Seomin Kang, Jae-Yun Lee, Jeong Eun Han, Yun-Seok Jeong, Do-Hun Gim, June-Young Lee, Hyun Sik Kim, Euon Jung Tak, Hojun Sung, Jee-Won Choi, Su-Won Jeong, Ji-Ho Yoo, So-Yeon Lee, In Chul Jeong, Do-Yeon Kim, and Jin-Woo Bae<sup>\*</sup>

### Department of Biology, Department of Biomedical and Pharmaceutical Sciences, and Department of Life and Nanopharmaceutical Sciences, Kyung Hee University

Two novel bacterial strains, KSM-R2A25<sup>T</sup> and KSM-R2A30<sup>T</sup>, were isolated from intestines of corb shell and northern snakehead, respectively, which were collected in Korea. According to phylogenetic analyses based on 16S rRNA gene sequences, strains belonged to the genus *Flavobacterium* within the family *Flavobacteriaceae*. 16S rRNA gene sequences of strains KSM-R2A25<sup>T</sup> and KSM-R2A30<sup>T</sup> were closely related to *Flavobacterium cucumis* DSM 18830<sup>T</sup> and *Flavobacterium aquaticum* JC164<sup>T</sup> with sequence similarities of 97.77% and 98.54%, respectively. Further genomic analyses suggested them as novel species of the genus *Flavobacterium*. The strains were Gram stainnegative, non-motile, and strictly aerobic. The major polar lipid in both strains was phosphatidylethanolamine (PE). Both strains contained menaquinone with six isoprene units (MK-6) as a major isoprenoid quinone and iso-C<sub>15:1</sub>G, iso-C<sub>15:0</sub>, and iso-C<sub>16:0</sub> as major cellular fatty acids. The genomic G + C contents of strains KSM-R2A25<sup>T</sup> and KSM-R2A30<sup>T</sup> were 31.7 and 31.9%, respectively. Based on the polyphasic taxonomic analyses presented in this study, strains KSM-R2A25<sup>T</sup> and KSM-R2A30<sup>T</sup> represent novel species of the genus *Flavobacterium*, for which the names *Flavobacterium cyclinae* sp. nov and *Flavobacterium channae* sp. nov are proposed.

[This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation of Korea (NRF) & funded by the Korean government (MSIT) (No. NRF-2022M3A9F3082331)]

# Description of Luteibacter aegosomatis sp. nov., Luteibacter aegosomaticola sp. nov. and Luteibacter aegosomatissinici sp. nov. Isolated from the Intestines of Aegosoma sinicum sinicum larva Hae-In Joe, Jee-Won Choi, and Jin-Woo Bae<sup>\*</sup>

Department of Biology, Department of Biomedical and Pharmaceutical Sciences, and Department of Life and Nanopharmaceutical Sciences, Kyung Hee University

Three novel bacterial strains,  $321^{T}$ ,  $335^{T}$ , and  $353^{T}$ , were isolated from intestines of *Aegosoma sinicum sinicum* larva collected from Paju-Si in Korea. The strains were Gram-negative, aerobic, rod-shaped bacterium with a single flagellum. These three strains, which belonged to the genus *Luteibacter* in the family *Rhodanobacteraceae*, shared < 99.2% in 16S rRNA gene sequence and < 83.56% in whole-genome sequence with each other. strains  $321^{T}$ ,  $335^{T}$ , and  $353^{T}$  formed a monophyletic clade with *Luteibacter yeojuensis* R2A16-10<sup>T</sup>, *L. anthropi* CCUG 25036<sup>T</sup> and *L. rhizovicinus* LJ96<sup>T</sup>, with sequence similarities of 98.77–98.91%, 98.44–98.58%, and 97.88–98.02%, respectively. Three strains contained Q-8 as a major quinone and  $C_{15:0}$  iso, Summed feature 9 as their major cellular fatty acids. The genomic DNA G + C contents of strains  $321^{T}$ ,  $335^{T}$ , and  $353^{T}$  were 66.3, 64.8, and 64.5%, respectively. Based on the multiphasic classification indicated here, strains  $321^{T}$ ,  $335^{T}$ , and  $353^{T}$  were classified into the genus *Luteibacter*, for which the names *Luteibacter aegosomatis* sp. nov., *Luteibacter aegosomaticola* sp. nov. and *Luteibacter aegosomatissinici* sp. nov. are proposed.

[This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean government (MSIT) (NRF-2022M3A9F3082331).]

#### A016

#### Chryseobacterium foetidum sp. nov., Isolated from the Han River

Miryung Kim, Yong-Seok Kim, and Chang-Jun Cha<sup>\*</sup> Department of Systems Biotechnology and Center for Antibiotic Resistome, Chung-Ang University

A novel bacterial species  $CJ63^{T}$  belonging to the genus *Chryseobacterium* was isolated from the Han River, South Korea. Cells of the strain were Gram-stain-negative, aerobic, non-motile, rod-shaped, and catalase- and oxidase-positive. The strain was shown to grow optimally at 30°C and pH 7 in the absence of NaCl on tryptic soy agar. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain  $CJ63^{T}$  belonged to the genus *Chryseobacterium* and were most closely related to *Chryseobacterium piscicola* VQ-6316s<sup>T</sup> with 98.46% 16S rRNA sequence similarity. The major fatty acids of strain  $CJ63^{T}$  were iso- $C_{17:0}$  3-OH and summed feature 9 ( $C_{16:0}$  10-methyl and/or iso- $C_{17:1}\omega$ 9c). Menaquinone 6 (MK-6) was identified as the primary respiratory quinone. The major polar lipids of strain  $CJ63^{T}$  were phosphatidylethanolamine and several unidentified amino lipids and lipids. Based on polyphasic taxonomy data, strain  $CJ63^{T}$  represents novel species of the genus *Chryseobacterium*, for which name *Chryseobacterium foetidum* sp. nov. is proposed. The type strain is  $CJ63^{T}$  (= KACC 22750<sup>T</sup>). [This work was supported by the National Institute of Biological Resources funded by the Ministry of Environment (MOE) as 'the Environmental Health Action Program (2016001350004)']

#### Microbacterium caeni sp. nov., a Novel Species Isolated from Sludge in Yeosu, Korea

You-Jung Jung, Seungki Lee, Sungho Choi, Hyeokjun Yoon, Yoonyong Yang, Garam Jeong, Byoung-Hee Lee, and Moonsuk Hur<sup>\*</sup>

National Institute of Biological Resources, Environmental Research Complex

A Gram-stain-positive, white-pigmented, aerobic, rod-shaped bacterium, designated strain NIBRBAC000506063<sup>T</sup>, was isolated from a sludge sample from Yeosu, Korea. Optimal growth was observed at 25°C and pH 8.0, in the absence of NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain NIBRBAC000506063<sup>T</sup> belongs to the genus *Microbacterium* and is closely related to *Microbacterium invictum* DC-200<sup>T</sup> (98.3% sequence similarity). The genome size of strain NIBRBAC000506063<sup>T</sup> was 3.4 Mb with 4,046 coding sequences, 47 tRNAs, and 4 rRNAs. Average nucleotide identity (ANI) values and G + C content were 75.89% and 69.6 mol%, respectively. The major respiratory isoprenoid quinone was MK12, and the major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, aminophospholipids, and glycolipids. The major fatty acids of strain NIBRBAC000506063<sup>T</sup> were C<sub>16:0</sub> iso and C<sub>15:0</sub> anteiso. Based on the polyphasic taxonomic data obtained in this study, it is proposed that strain NIBRBAC000506063<sup>T</sup> (= KCTC49453<sup>T</sup> = DSM111108<sup>T</sup>).

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#### A018

#### Brevundimonas sanguinis sp. nov., Isolated from Patient Blood in South Korea

Ji Hee Lee, Yewon An, Young Sill Choi, and Su Yeon Kim<sup>\*</sup>

Division of Pathogen Resource Management, National Institute of Infectious Diseases, National Institute of Health, Korea Disease Control and Prevention Agency (KDCA)

The genus *Brevundimonas* have been isolated from diverse environments, whereas some species have also been isolated in clinical samples and found to be opportunistic pathogens.

We confirmed Gram-stain-negative, aerobic, rod-shaped bacteria *Brevundimonas* sp. KBN06P01209<sup>T</sup> from the patient blood sample. It has been collected in National Culture Collection for Pathogens (NCCP).

A phylogenetic tree constructed using 16S rRNA gene sequences revealed that the isolate was a member of the *Brevundimonas* with 99.9% similarity to *B. naejangensis*. The DNA-DNA hybridization (DDH) between KBN06P01209<sup>T</sup> and *B. naejangensis* was 52.3 ± 1.7%. The digital DDH determined by Genome-to-Genome Distance Calculator was 49.5%. These results showed that the isolate represents a distinct species based on DDH value cutoff of 70%.

The fatty acids of summed feature 8 ( $C_{18:1} \omega 7 c / C_{18:1} \omega 6 c$ ) and  $C_{16:0}$  were presented in KBN06P01209<sup>T</sup> and *B*. *naejangensis*. However, KBN06P01209<sup>T</sup> contained a higher proportion of  $C_{17:0}$  and  $C_{18:1} \omega 5 c$  compared with those of *B*. *naejangensis*. The isolate had polar lipids and quinone were phosphatidylglycerol, 1,2-di-O-acyl-3-O-[D-

glycopyranosyl (1 $\rightarrow$ 4)- $\alpha$ -D-glucopyranuronosyl] glycerol, and ubiquinone-10, respectively, which were similar to the *Brevundimonas*.

Thus, we suggest that KBN06P01209<sup>T</sup> represents a novel species of the *Brevundimonas*, for which name *Brevundimonas sanguinis* sp. nov. is proposed.

[This research was supported by a grant from Korea National Institute of Health (2019-NG047-02)]

### Bioplastic (Poly-3-hydroxybutyrate) Producing *Massilia endophytica* sp. nov., Isolated from *Cannabis sativa* L. 'Cheungsam'

Doeun Jeon and Jiyoung Lee\*

Korean Collection for Type Cultures (KCTC), Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology

A light pink-coloured, motile, Gram-negative bacterium, designated DM-R-R2A-13<sup>T</sup> was isolated from *Cannabis sativa* L. 'Cheungsam' in Andong, Republic of Korea. The Phylogenetic analysis based on 16S rRNA gene sequence revealed, the strain DM-R-R2A-13<sup>T</sup> formed a lineage within the family *Oxalobacteraceae* and clustered as members of the genus *Massilia*. The closet members were *Massilia flava* KCTC 23585<sup>T</sup> (97.58% sequence similarity) and *Massilia armeniaca* DSM 104676<sup>T</sup> (97.37% sequence similarity). The dominant quinone is ubiquinone-8 and the major cellular fatty acids (> 10%) are C<sub>16:0</sub> and summed feature 3 (C<sub>16:1</sub>  $\omega$ 7*c* and/or C<sub>16:1</sub>  $\omega$ 6*c*). The major polar lipids are diphosphatidyglycerol (DPG), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG). Genome annotation predicted the presence of PHA synthase gene (PhaC and PhaR). The isolated strain was able to produce PHB up to 22.35 and 22.64% of its dry cell weight employing succes and lactose as a carbon source. The produced biopolymer was characterized and identified by using nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) techniques. The results of phylogenetic analyses and polyphasic features, revealed that strain DM-R-R2A-13<sup>T</sup> represents a novel species of the genus *Massilia*, for which the name *Massilia endophytica* sp. nov., is proposed. The type strain is DM-R-R2A-13<sup>T</sup> (= KCTC 92072<sup>T</sup> = GDMCC 1.2920<sup>T</sup>). [Supported by grants from NRF-2020R111A2072308 and KGM5382212]

#### A020

# The Study of CTM<sup>™</sup>-advance Transport Medium and Specific Saliva Swab for the Clinical Specimen Collection, Preservation and Transportation Compared to General Swab Kits

Younghwa Roh, Eunju Kim, Sujin Lee, Woonjeong Kim, Kyeseung Baek, and Changnam Hwang<sup>\*</sup> Noble Biosciences Institute

Regarding the new and mutated infectious disease of the COVID-19 pandemic that has terrorized the world. We have developed a specimen collection kit for the safety of medical staff, preventing secondary infection from the specimens and a user-centered swab that can collect specimen without pain. We have evaluated transportation of live virus using various medium including CTM<sup>™</sup>-Advance medium for the transportation of inactivated bacteria or virus by lysing cells and stabilizing nucleic acids for extended period of time. Several viruses were used for the medium evaluation, and each virus was inoculated into each transport medium and stored at 4°C, 25°C and 37°C for 0 to 14 days followed by each assay method, quantitative Polymerase Chain Reaction and Plaque assay method for evaluating survival efficiency and cell inactivation in the medium. In addition, we used the quantitative elution method with recommended strains, as described in CLSI (Clinical and Laboratory Standards Institute) M40-A2, to evaluate the various swabs for the absorption and release capacities for 0,24, and 48 h at room and refrigerator temperature. And protein quantification method was used to evaluate the absorption capacities of various saliva swab samples. Overall, CTM<sup>™</sup>-Advance medium maintains nucleic acid of virus with high efficacy under different storage conditions and all swabs in the experiment conform to CLSI standards and are suitable for use as a sampling tool.

#### Parabacteroides faecalis sp. nov., Isolated from Swine Feces

Jeong Eun Bak, Byeong Seob Oh, Seoung Woo Ryu, Seung Yeob Yu, Won Jung Choi, and Ju Huck Lee<sup>\*</sup> Korean Collection for Type Cultures, Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology

A Gram-negative, obligately anaerobic, non-motile, and non-spore-forming rod-shaped bacterial strain designated AGMB00274<sup>T</sup> was isolated from swine feces. The 16S rRNA gene analysis indicated that strain AGMB00274<sup>T</sup> was highly close to the members of the genus *Parabacteroides*, especially with *Parabacteroides johnsonii* DSM 18315<sup>T</sup> (sequence similarity of 94.9%). The dominant cellular fatty acids (>10%) of strain AGMB00274<sup>T</sup> were anteiso-C<sub>15:0</sub> (23.2%), iso-C<sub>15:0</sub> (16.6%), C<sub>18:1</sub>  $\omega$ 9c (16.4%), summed feature 11 (12.5%), and C<sub>16:0</sub> (11.3%). The genome size of strain AGMB00274<sup>T</sup> was 4,308,683 bp with DNA G + C content of 42.5 mol%. From the biochemical analysis of strain AGMB00274<sup>T</sup>, it was positive for gelatin hydrolysis,  $\alpha$ -fucosidase; and was negative for D-glucose, D-mannitol, D-maltose, salicin, glycerol, D-cellobiose, D-mannose, D-melezitose, D-sorbitol, D-trehalose,  $\alpha$ -arabinosidase, glutamic acid decarboxylase, and pyroglutamic acid arylamidase. Based on the phylogenetic, chemotaxonomical, genetic, and physiological analyses, as a novel species of the genus *Parabacteroides*, strain AGMB00274<sup>T</sup> is proposed with the name *Parabacteroides faecalis* sp. nov. The type strain is AGMB00274<sup>T</sup> (= KCTC 25286<sup>T</sup> = GDMCC 1.2742<sup>T</sup>).

#### Agarivorans sediminis sp. nov., an Alginate-degrading Bacterium Isolated from Seawater

Seung Seob Bae, Yoon-Hee Jung, Yong Min Kwon, Dawoon Chung, Woon-Jong Yu, and Grace Choi<sup>\*</sup> National Marine Biodiversity Institute of Korea

A bacterium with the ability to degrade alginate, designated strain TSD2052<sup>T</sup> was isolated from a tidal flat sediment sample collected at Taean County, Republic of Korea. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain TSD2052<sup>T</sup> formed a monophyletic clade with members of the genus *Agarivorans*, showing 96.2–97.8% sequence similarity. Strain TSD2052<sup>T</sup> has a single circular chromosome of 4.62 Mb with a DNA G + C content of 44.3%. The values of average nucleotide identity (ANI), average amino acid identity (AAI), and digital DNA-DNA hybridization (dDDH) between strain TSD2052<sup>T</sup> and all genome-sequenced species of the genus *Agarivorans* were below 76.7, 81.7%, and 18.9% respectively, indicating low values than the standard cut-off for species delineation. Growth was observed at 10–35°C (optimum 25°C), at pH 6–10 (optimum pH 8), and with 1–5% NaCl (optimum 3). The major fatty acids (> 10%) were C<sub>12:0</sub>, C<sub>16:0</sub>, summed feature 3 (C<sub>16:1</sub> $\omega$ 6c and/or C<sub>16:1</sub> $\omega$ 7c), and summed feature 8 (C<sub>18:1</sub> $\omega$ 6c and/or C<sub>18:1</sub> $\omega$ 7c). The respiratory quinone was Q-8. The major polar lipids were identified as phosphatidylethanolamine, unidentified aminolipid, and four unidentified lipids. Based on the results of phenotypic characterization, phylogenetic analysis, and genome-based comparison, strain TSD2052<sup>T</sup> represents a novel species in the genus *Agarivorans*, for which we propose the name *Agarivorans sedimins* sp. nov. Type strain is TSD2052<sup>T</sup> (= KCTC 92288<sup>T</sup> = JCM 35392<sup>T</sup>).

#### A024

**Isolation and Characterization of** *Chitinibacter bivalviorum* sp. nov., Isolated from the Gut of Freshwater Mussel Jee-Won Choi and Jin-Woo Bae<sup>\*</sup>

Department of Biology, Kyung Hee University

A *Chitinibacter*-like Gram-stain-negative, aerobic, rod-shaped bacterium with a single polar flagellum, designated strain  $2T18^{T}$ , was isolated from the gut of the freshwater mussel *Anodonta arcaeformis* collected in the Republic of Korea. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain formed a monophyletic clade with all species of the genus *Chitinibacter*. The G + C content of the genomic DNA was 50.6 mol%. The average nucleotide identity and digital DNA–DNA hybridization values between strains  $2T18^{T}$  and *C. fontanus* KCTC  $42982^{T}$  were below the thresholds used for the delineation of a novel species. The polar lipids comprised phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, one unidentified lipid, three unidentified phospholipids and two unidentified aminophospholipids. The predominant fatty acids were summed feature 3 ( $C_{16:1}\omega6c$  and/ or  $C_{16:1}\omega7c$ ) and  $C_{16:0}$ . The major isoprenoid quinone was ubiquinone-8 (Q-8). Based on the phenotypic, phylogenetic, genotypic, and chemotaxonomic analyses, strain  $2T18^{T}$  represents a novel species of the genus *Chitinibacter*, for which the name *Chitinibacter bivalviorum* sp. nov. is proposed. The type strain is  $2T18^{T}$  (= KCTC 72821<sup>T</sup> = CCUG 74764<sup>T</sup>).

[This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean government (MSIT) (NRF 2022M3A9F3082331).]

#### *Microbacterium salitolerans* sp. nov., Isolated from Agricultural Soil in Yongin-si, Republic of Korea Gracia Pradnya Lolita and Chang-Jun Cha<sup>\*</sup>

Department of Systems Biotechnology and Center of Antibiotic Resistome, Chung-Ang University

Strain CJ85<sup>T</sup> was isolated from an agricultural soil in Yongin-si, Republic of Korea. Cells were Gram-staining-positive, short rods and forming yellowish-white colonies on Tryptic Soy agar. Growth was observed at pH 5.0–9.0 (optimum pH 6.0–7.0), at 10–45°C (optimum 37°C), and at NaCl 0–14% (optimum 0–2%). Phylogenetic analysis of 16S rRNA gene sequences showed that strain CJ85<sup>T</sup> belonged to a clade represented by the genus *Microbacterium* of the family *Microbacteriaceae* and had the highest 16S rRNA gene sequence similarities with *Microbacterium agarici* DSM 21798<sup>T</sup> (97.50%), *M. lindanitolerans* MNA2<sup>T</sup> (97.16%) and *M. humi* DSM 21799<sup>T</sup> (96.74%). The average nucleotide identity values between strain CJ85<sup>T</sup> and the three closely related type strains were 80.24%, 80.25%, and 80.14%, respectively. Moreover, digital DNA-DNA hybridization values between strain CJ85<sup>T</sup> and the three reference strains were 20.4, 20.6, and 20.3, respectively. Whole genome sequencing revealed that strain CJ85<sup>T</sup> had a genome of 3.17 Mb with 64.8% G + C content. Strain CJ85<sup>T</sup> was shown to be resistant to amoxicillin, cephalexin, meropenem, rifampicin, erythromycin, clindamycin, and tylosin, and resistant genes related to these phenotypes were found in the genome of strain CJ85<sup>T</sup>. Based on the polyphasic taxonomic study, it is proposed that strain CJ85<sup>T</sup> belongs to a novel species of the genus *Microbacterium*, for which the name *Microbacterium salitolerans* sp. nov. is proposed.

[Supported by grants from RDA and NIBR]

#### A026

**Description of Novel Strain** *Aureibaculum* **sp. from** *Hermit crab* **of** *Crustaceans* Yea Lin Moon and Jin Sook Park<sup>\*</sup> Department of Biological Science and Biotechnology, Hannam University

A Gram-stain-negative, yellow colored bacterium (designated strain 208BN30-3<sup>T</sup>) was isolated from *Hermit crab* of *Crustaceans* collected on baengnyeong island, Nampo-ri, Baengnyeong-myeon, Ongjin-gun, Incheon, Republic of Korea. On the basis of 16S rRNA gene sequencing results, strain 208BN30-3<sup>T</sup> clustered with species of the genus *Aureibaculum* appeared closely related to *Aureibaculum marinum* BH-SD17<sup>T</sup> (95.18%), *Lutibacter litoralis* CL-TF09<sup>T</sup> (92.05%), *Lutibacter crassostreae* TYO-8<sup>T</sup> (91.91%). Growth occurred at 25–37°C on MA medium in the presence of 1.0–8.0% NaCl (w/v) and at pH 7.0–10.0. The ANI values calculated between strain 208BN30-3<sup>T</sup> and *Aureibaculum marinum* were 74.0%. The DNA G + C content of thegenomic DNA was 33.84 mol%, and Menaquinone was the major respiratory quinone. Physiological and biochemical characteristics indicated that strain 208BN30-3<sup>T</sup> represents a novel species of the genus *Aureibaculum*, for which the name *Aureibaculum* sp. nov. is proposed. [This research was supported by National Marine Biodiversity Institute of Korea (2022M01100), and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R111A3046479).]

#### Seonamhaeicola citrea sp. nov., a Marine Bacterium Isolated from Tidal Flat Sediment

Sung-Hyun Yang, Mi-Jeong Park, and Kae Kyoung Kwon<sup>\*</sup> Korea Institute of Ocean Science and Technology

Gram-negative, facultatively anaerobic, rod-shaped  $(1.6 \pm 2.1 \ \mu m \times 0.3 \pm 0.5 \ \mu m)$  and non-motile marine bacterium, designated as MEBiC01930<sup>T</sup> was isolated from a marine sediment collected from Dokdo island, Korea, in October 2004. The 16S rRNA gene sequence analysis revealed that strain MEBiC1930<sup>T</sup> showed high similarity with members of the genus *Seonamhaeicola* (97.0–98.4%). Growth was observed at 20-32°C (optimum 28°C), at pH 6.5– 8.5 (optimum pH 7.5) and with 0-4% (optimum 0.5%) NaCl. The predominant cellular fatty acids are C<sub>12:0</sub> (5.2%), iso-C<sub>15:0</sub> (14.8%), anteios-C<sub>15:0</sub> (4.6%), iso-C<sub>15:0</sub> 3-OH (9.8%), iso-C<sub>17:0</sub> 3-OH (12.2%), and summed feature 3 (C<sub>16:1</sub>  $\omega$ 7*c* and/or C<sub>16:1</sub>  $\omega$ 6*c*; 4.0%). The DNA G+C contents is 33.6 mol%. The major respiratory quinone is MK-6. Several phenotypic characteristics such as utilization of gluconate, malate, adipate, arabinose etc., DNA G + C ratio, composition of cellular fatty acids, and growth range of pH and salinity differentiate strain MEBiC11861<sup>T</sup> from members of the genus *Nisaea*. On the basis of this polyphasic taxonomic data, strain MEBiC11861<sup>T</sup> should be classified as a novel species in the genus *Nisaea* and it is proposed as *Nisaea* acidiphila sp. nov. The type strain is MEBiC11861<sup>T</sup> (= KCCM 43219<sup>T</sup> = JCM 31589<sup>T</sup>). Emended description of the genus *Nisaea* Urios *et al.* 2008 is also given.

[Supported by the KIOST in-house program and the National Marine Biodiversity Institute of Korea.]

#### A028

#### Parasphingorhabdus cellanae sp. nov, Isolated from the Intestine of a Korean Limpet, Cellna toreuma

Ji-Ho Yoo, Jeong Eun Han, June-Young Lee, Su-Won Jeong, Yun-Seok Jeong, Jae-Yun Lee, So-Yeon Lee, JeeWon Choi, Hojun Sung, Euon Jung Tak, Hyun Sik Kim, Pil Soo Kim, Do-Hoon Kim, Seo Min Kang, In Chul Jeong, and Jin-Woo Bae<sup>\*</sup> Department of Biomedical and Pharmaceutical Sciences and Department of Biology, Kyung Hee University

A novel Gram-stain-negative, rod-shaped, non-spore-forming, obligately aerobic, non-motile bacterium, yellowpigmented, designated strain JHSY0214<sup>T</sup>, was isolated from the gut of a Korean limpet, *cellana toreuma*. The 16S rRNA gene sequences-based phylogenetic analysis revealed that strain JHSY0214<sup>T</sup> showed 98.71, 98.14 and 97.28% similarity to the type strains of *Parasphingorhabdus litoris*, *P. marina*, and *P. flavimaris*, respectively. Strain JHSY0214<sup>T</sup> grew at 10–30°C (optimum temperature: 30°C), with 1–6% (w/v) NaCl (optimum salinity: 2%) and at pH 6–8 (optimum pH 7). The main cellular fatty acid was C<sub>16:0</sub>. The predominant isoprenoid quinone was Q-10. The major poly lipid components were phosphatidylethanolamine, sphingoglycolipid. The genomic DNA G + C content was 52.8 mol%. Based on phenotypic analyses and genotypic results indicated that strain JHSY0214<sup>T</sup> represents a novel species of the genus *Parasphingorhabdus*, for which the name *Parasphingorhabdus cellanae* sp. nov. is proposed. The type strain is JHSY0214<sup>T</sup> (= KCTC 82387<sup>T</sup> = DSM 112279<sup>T</sup>).

### *Chryseobacterium tagetis* sp. nov., a Plant Growth Promoting Bacterium with an Antimicrobial Activity Isolated from the Roots of Medicinal Plant (*Tagetes patula*)

Geeta Chhetri, Inhyup Kim, Jiyoun Kim, Yoonseop Seo, Sunho Park, Yonghee Jung, Haejin Woo, and Taegun Seo<sup>\*</sup> Department of Life Science, Dongguk University-Seoul

A novel plant growth-promoting and indole acetic acid (IAA) producing strain designated RG1<sup>T</sup> was isolated from the roots of *Tagetes patula* (marigold) collected from Goyang, South Korea. The Cells of strain RG1<sup>T</sup> is aerobic, yellow, Gram-stain-negative, pleomorphic and non-motile. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain RG1<sup>T</sup> belongs to the genus *Chryseobacterium* and is closely related to *Chryseobacterium gwangjuense* THG-A18<sup>T</sup> (98.6%). The strain produced IAA (70.5 µg/ml) in the presence of Ltryptophan and showed antimicrobial activity against Gram-negative bacterium *Xanthomonas campestris* pv. *campestris* KACC 10377<sup>T</sup>. The isolate had a significant positive effect on rice plant shoot and root growth. The novel strain RG1<sup>T</sup> had a draft genome size of 4,430,189 bp, with 10 scaffolds and 3,969 protein-coding genes. The digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) values between strain RG1<sup>T</sup> and other closely related members ranged from 21.5 to 36.6% and from 79.2 to 86.6%, respectively. Furthermore, anti-SMASH analysis of the whole genome revealed six putative biosynthetic gene clusters responsible for various secondary metabolites. Based on the genotypic, chemotaxonomic and physiological data, strain RG1<sup>T</sup> represents a novel species, for which the name *Chryseobacterium tagetis* sp. nov. is proposed. The type strain is designated as RG1<sup>T</sup> (= KCTC 82696<sup>T</sup> = NBRC 115057<sup>T</sup>) as the type strain.

#### A030

Paenibacillus flumini sp. nov. and Paenibacillus bambusae sp. nov., Isolated from Eunpa Lake and Bamboo Root in Gunsan, Respectively

Ye Jin Gwak and Hyo Jung Lee<sup>\*</sup> Department of Biological Science, Kunsan National University

Two Gram-staining-positive, strictly aerobic bacterium, designated strain KS-LC4<sup>T</sup> and KS-SF5<sup>T</sup>, was isolated from the water of Eunpa lake and Bamboo root in Gunsan, respectively. Cells of strains were motile, formed colonies on agar and showing oxidase- and catalase-positive reaction. Strain KS-LC4<sup>T</sup> growth was observed at 10–37°C (optimum, 30°C), with 0–3% (w/v) NaCl (optimum, 0%) and pH 6.0–10.0 (optimum, pH 9.0). Strain KS-SF5<sup>T</sup> growth was observed at 10–40°C (optimum, 30°C), with 0–6% (w/v) NaCl (optimum, 0–3%) and pH 6.0–9.0 (optimum, pH 6.0). Comparative analysis of the 16S rRNA gene sequence showed that isolates KS-LC4<sup>T</sup> and KS-SF5<sup>T</sup> belonged phylogenetically to the genus *Paenibacillus*, and was most closely related to *Paenibacillus liaoningensis* LNUB461<sup>T</sup> and *Paenibacillus frigoriresistens* YIM016<sup>T</sup> with 98.9% and 97.6% 16S rRNA gene sequence similarities, respectively. Additional experiments will be performed and discussed. Based on phenotypic, chemotaxonomic and molecular features, strain KS-LC4<sup>T</sup> and KS-SF5<sup>T</sup> represent novel species of the genus *Paenibacillus*, for which the name *Paenibacillus flumini* sp. nov. and *Paenibacillus bambusae* sp. nov. are proposed.

### Description of Novel Strain *Thalassotalea* sp. 208BN3-11<sup>T</sup> Isolated from Sea Water

Soo Bin Kim and Jin Sook  ${\rm Park}^{\ast}$ 

Department of Biological Science and Biotechnology, Hannam University

Gram-stain-negative, round-shaped, purple-pigmented bacterium, designated strain 208BN3-11<sup>T</sup>, was isolated from see water from the Dumujin Coast, Baengnyeong-do, Republic of Korea (33°59'25.0"N 126°14'58.6"E). The Strain 208BN3-11<sup>T</sup> was able to grow at pH 7.0–10.0, in the presence of 1–3% (w/v), and at 25–30°C. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain 208BN3-11<sup>T</sup> belonged to the family Colwelliaceae, and was most closely related to *Thalassotalea fusca* G-MB1<sup>T</sup> (97.37%). The major respiratory quinone was menaquinone. The genomic DNA G + C content of strain 208BN3-11<sup>T</sup> was 39.21%. The average nucleotide identity values compared to all other related species was below 89.4%. Phenotypic, phylogenetic, genomic and chemotaxonomic characteristics showed that strain 208BN3-11<sup>T</sup> represents a novel species of the genus *Thalassotalea*, 208BN3-11<sup>T</sup> sp. nov. is proposed.

#### A032

**Taxonomic and Genomic Characterization of a Novel** *Massilia* **sp. FBCC-B171, Isolated from Freshwater** Hyangmi Kim, Ye-Ji Hwang, Ju-Hyung Jeon, Jun Sung Kim, and Ji Young Jung<sup>\*</sup> *Microbial Research Department, Nakdonggang National Institute of Biological Resources (NNIBR)* 

*Massilia* sp. FBCC-B171, was isolated from frewhwater of the Geum River, in Boeun, Korea. The cells were Gramstain-negative, aerobic, motile, rod-shaped bacterium. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain FBCC-B171 belongs to the genus *Massilia* and is most closely related to *Massilia jejuensis* 5317J-18<sup>T</sup> (98.7%). The genome comprises 5,570,563 bp with a G + C content of 64.4%. It contained 4,925 protein-coding genes, 21 rRNA genes and 78 tRNA genes. The average nucleotide identity and digital DNA-DNA hybridization values between strain FBCC-B171 and related *Massilia* species were below 77.03–77.55% and 20.5–20.8%, respectively. Based on data obtained from this genomic information and polyphasic taxonomic study, strain FBCC-B171 represents a novel species belong to genus *Massila*.

#### Bacillus sp. nov., Isolated from the Unmun Mountain Soil, Korea

Yelim You and Jaisoo Kim\*

Microbiology Laboratory, Department of Life Science, College of Natural Sciences, Kyonggi University

A Gram staining negative, white color and rod-shaped, oxidase-negative, and catalase-negative bacterium, isolated from the Unmun mountain soil sample, Korea. Strain R4-4<sup>T</sup> can hydrolysed DNA but not starch and casein. The optimum temperature for growth is 30°C–40°C and maximum growth temperature lies between 45°C and 47°C. A phylogenetic analysis based on 16S rRNA gene sequence revealed that strain R4-4 clustered together with other species of the genus *Bacillus* and showed similarities with *Bacillus drentensis* LMG 21831<sup>T</sup> (97.8%), *Bacillus vireti* LMG 21834<sup>T</sup> (97.46%) and *Bacillus cucumis* AP-6<sup>T</sup> (97.22%). Polyphasic analysis results revealed that R4-4<sup>T</sup> represents a novel species of the genus *Bacillus*, with the proposed name *Bacillus* sp. nov. The type strain is R4-4<sup>T</sup>.

#### A034

### Performance Evaluation of Modified Chromogenic Medium to Validate *Salmonella* or *Shigella* Strains for Clinical Fecal Specimen

Eunju Kim, Woonjeong Kim, Younghwa Roh, Sujin Lee, Kyeseung Baek, and Changnam Hwang<sup>\*</sup> Noble Biosciences Institute

Infections due to *Salmonella* and *Shigella* species (below, two species) continue to be a major health problem of food and restaurant industrial worker, and their hygiene must be confirmed by the state. The hygiene test is sampled from fecal and testes the presence of two species which are indicators of typhoid and bacterial dysentery with a chromogenic medium. Their diagnosis most often relies on direct detection of bacteria in fecal specimen by culture or by PCR after enrichment. Contrary to PCR, isolation of two species on selective culture media allows identification. Hektoen enteric agar (HE agar; no proteus) has been widely used for this purpose since its introduction in 1968, and because of its good sensitivity, it remains the standard primary plating medium in our routine screening for two species. However, most of which turn out to be false-positive normal flora, such as *Proteus* and *Pseudomonas* species. To alleviate these labor-intense workups, Noble HE agar allows for the selective isolation and differentiation of two species from non-pathogenic enteric bacteria (both lactose and non-lactose-fermenting organisms). Noble HE agar is a new selective chromogenic medium that allows the detection of *Salmonella* as green and black-centered colonies after 15 h of incubation, whereas other members of the family *Shigella* grow as green. The results of this study show potential to develop a chromogenic medium for identifying each strain of the two species definitely.

#### Taxonomic Study of a Novel Species of the Genus Barnesiella Isolated from the Healthy Korean Faeces

Min Kuk Suh<sup>1,2</sup>, Hyo Eun Do<sup>1,3</sup>, Han Sol Kim<sup>1,2</sup>, Ji-Sun Kim<sup>1</sup>, Mi Kyung Eom<sup>1</sup>, Se Won Kang<sup>1</sup>, Ju Huck Lee<sup>1</sup>, Seung-Hwan Park<sup>1</sup>, and Jung-Sook Lee<sup>1,4\*</sup>

<sup>1</sup>Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>Department of Lifestyle Medicine, Jeonbuk National University, <sup>3</sup>Department of Oriental Medicine Resources, Jeonbuk National University, <sup>4</sup>Department of Biotechnology, University of Science and Technology

Strain SMK-766-005<sup>T</sup> was isolated from a faecal sample of the healthy Korean. Bacterial Cells of strain SMK-766-005<sup>T</sup> were anaerobic, Gram-positive, non- flagellated, straight rods and approximately 0.8–1.1 × 0.5–0.7 μm in size. Colonies on Reinforced Clostridial medium agar with 5% horse blood are round, smooth and brown. The complete 16S rRNA gene sequence of the isolate was determined and compared with species of the genus *Barnesiella*. *Barnesiella intestinihomonis* YIT 11860<sup>T</sup> showed the highest 16S rRNA gene sequence similarity (98.62%). The phylogenetic trees indicated that strain SMK-766-005<sup>T</sup> was formed separate lineage within genus *Barnesiella*. Strain SMK-766-005<sup>T</sup> was positive for catalase activity and negative for oxidase. Based on phylogenetic, physiological and chemotaxonomic characteristics, strain SMK-766-005<sup>T</sup> represent a novel specis of the genus *Barnesiella*. [This research was supported by the National Research Foundation of Korea (NRF-2016M39A9F3947962) funded by the Ministry of Science and ICT (MIST) of the Republic of Korea and a grant from the Korea Research Institute of Bioscience & Biotechnology (KRIBB) Research Initiative Program.]

#### A036

#### A Novel Species of Genus Phocaeicola Isolated from Healthy Korean Faeces

Hyo Eun Do<sup>1,2</sup>, Young Bong Ha<sup>1</sup>, Han Sol Kim<sup>1,3</sup>, Ji-Sun Kim<sup>1</sup>, Min Kuk Suh<sup>1,3</sup>, Mi Kyung Eom<sup>1</sup>, Ju Huck Lee<sup>1</sup>, Seung-Hwan Park<sup>1</sup>, Se Won Kang<sup>1</sup>, and Jung-Sook Lee<sup>1,4\*</sup>

<sup>1</sup>Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>Department of Oriental Medicine Resources, Jeonbuk National University, <sup>3</sup>Department of Lifestyle Medicine, Jeonbuk National University, <sup>4</sup>Department of Biotechnology, University of Science and Technology

An obligately anaerobic, non-motile, Gram-negative and rod-shaped strain (KGMB11183<sup>T</sup>) was isolated from Korean human faeces. Growth of strain KGMB11183<sup>T</sup> occurred at 30–45°C (optimum, 37°C), at pH 6–9 (optimum, pH 7) and in the presence of 0–0.5% NaCl (optimum, 0). Strain KGMB11183<sup>T</sup> showed 16S rRNA gene sequence similarities of 95.4% and 94.2% to the nearest recognized species, *Phocaeicola plebeius* M12<sup>T</sup> and *Phocaeicola faecicola* KCTC25014<sup>T</sup>. Based on phylogenetic analysis, this strain was included within the Bacteroidaceae family. Strain KGMB11183<sup>T</sup> was able to utilize D-glucose, salicin, D-xylose,  $\alpha$ -galactosidase,  $\alpha$ -glucosidase, and  $\alpha$ -fucosidase. The major end product of fermentation was acetate. The major cellular fatty acids (> 10%) of this isolate were anteiso-C<sub>15:0</sub> and summed feature 11 (iso-C<sub>17:0</sub> 3OH and/or C<sub>18:2</sub> DMA). The assembled draft genome sequences of strain KGMB11183<sup>T</sup> consisted of 4,100,144 bp, with a G + C content of 41.4 mol%. On the basis of the biochemical, chemotaxonomic, phenotypic and phyogenetic data, strain KGMB11183<sup>T</sup> represents a novel species of the genus *Phocaeicola*. The type strain is KGMB11183<sup>T</sup>.

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#### A Novel Gordonibacter Species, KHS-420-004<sup>T</sup>, Isolated from Faeces of Healthy Korean

Han Sol Kim<sup>1,2</sup>, Ji-Sun kim<sup>1</sup>, Min Kuk Suh<sup>1,2</sup>, Hyo Eun Do<sup>1,3</sup>, Mi Kyung Eom<sup>1</sup>, Ju Huck Lee<sup>1</sup>, Seung-Hwan Park<sup>1</sup>, Se Won Kang<sup>1</sup>, and Jung-Sook Lee<sup>1,4\*</sup>

<sup>1</sup>Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>Department of Lifestyle Medicine, Jeonbuk National University, <sup>3</sup>Department of Oriental Medicine Resources, Jeonbuk National University, <sup>4</sup>Department of Biotechnology, University of Science and Technology (UST)

A novel Gram-stain-positive, anaerobic, and short rod-shaped bacterial strain, designated KHS-420-004<sup>T</sup>, was isolated from healthy Korean faecal sample. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain KHS-420-004<sup>T</sup> was closely related to members of the genus *Gordonibacter*, and had the highest sequence similarity to *G. pamelaeae* 7-10-1-b<sup>T</sup> and *G. urolithinfaciens* DSM 27213<sup>T</sup> (95.2 and 94.5%, respectively). The genome-derived average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values among closely related strains of the novel strain KHS-420-004<sup>T</sup> were 80.1 and 25.3% respectively for *G. pamelae* 7-10-1-b<sup>T</sup>, and *G. urolithinfaciens* DSM 27213<sup>T</sup> was 79.5 and 24.5% respectively. The genome comprises 33 contigs with a chromosome length of 3,326,705 bp, N 50 of 195,068, and 59.9% GC content. The draft genome contains 2,744 protein-coding genes and contains 48 tRNAs and 3 rRNAs. On the basis of the results of a polyphasic taxonomic study, it is concluded that KHS-420-004<sup>T</sup> represents a novel species of the genus *Gordonibacter*. The type strain is KHS-420-004<sup>T</sup>.

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#### A038

#### Morphological Classification of Bacteria Single Colonies Using Optical Coherence Tomography Images

Daewoon Seong<sup>1</sup>, Youngae Gu<sup>1</sup>, Seung-Yeol Lee<sup>2</sup>, Hee-Young Jung<sup>2</sup>, Mansik Jeon<sup>1</sup>, and Jeehyun Kim<sup>1\*</sup> <sup>1</sup>School of Electronic and Electrical Engineering, College of IT Engineering, Kyungpook National University, <sup>2</sup>School of Applied Biosciences, Kyungpook National University

Morphology of bacteria single colony is a significant phenotypic feature of bacterial species and strains, which is used for the identification of taxonomic grouping. The elevation type of a single colony on an agar medium is useful for the identification and preliminary assessment. However, conventional methods for observing morphological characteristics, such as stereoscopic microscopy, electron microscopy, and using spectral light scattering, have the incapability to acquire lateral view images. In this study, optical coherence tomography (OCT) was utilized to inspect the morphology of single bacterial colonies in four different genera. All bacterial samples were provided by the Bacterial Plant Pathology Laboratory at Kyungpook National University in South Korea, and four different genera were presented *Hymenobacter*, *Spirosoma*, *Bacillus*, and *Deinococcus*. OCT cross-section images, en face images, and reconstructed three-dimensional volumetric images were obtained and compared between every colony sample. As a result, this study suggests a new approach to assessing the morphology of a bacterial single colony including an elevation type from the lateral view image using OCT.

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### **Description of Novel Strain** *Bizionia* **sp. 2012CJ3-1 Isolated from Marine Sponge** *Cliona celata* **Grant** Ji Yoon Shin and Jin Sook Park<sup>\*</sup>

Department of Biological Science and Biotechnology, Hannam University

Gram-stain-negative, rod-shaped, aerobic, yellow-pigmented bacterium, designed strain 2012CJ3-1<sup>T</sup>, was isolated from the marine sponge *Cliona celata Grant* from the Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°59'25.0"N 126°14'58.6"E). The strain 2012CJ3-1<sup>T</sup> was able to grow at pH 6–10 (optimum, pH 8), in the presence of 1–3% NaCl (w/v) (optimum, 2.0%), and at 25–30°C (optimum, 30°C). The strain 2012CJ3-1<sup>T</sup> was most closely related to *Bizionia echini* DSM 23925<sup>T</sup> (93.44%). The ANI value of strain 2012CJ3-1<sup>T</sup> and its related species *Bizionia echini* DSM 23925<sup>T</sup> was 75.8%. The major respiratory quinone was menaquinone. The genomic DNA G + C content was 32.61 mol%. Phenotypic, genomic and chemotaxonomic characteristics showed that strain 2012CJ3-1<sup>T</sup> represents a novel species of the genus *Bizionia*, 2012CJ3-1<sup>T</sup> sp. nov., is proposed.

#### A040

Light-inducible Carotenoid Producing Agromyces soybeansoli sp. nov., Isolated from Soybean Cultivated Soils Ji Yoon Seo<sup>1,2</sup> and Ji Young Lee<sup>1\*</sup>

<sup>1</sup>Korean Collection for Type Cultures (KCTC), Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>Department of Agriculture and Life Sciences, Chonnam National University

A novel endophytic bacterial strain, designated S2-1-8<sup>T</sup>, was isolated from soybean cultivated soils. 16S rRNA gene sequences showed that S2-1-8<sup>T</sup> formed a lineage within genus *Agromyces* and most closely to *Agromyces mediolanus* DSM 20152<sup>T</sup> (97.88%), *Agromyces italicus* DSM 16388<sup>T</sup> (97.73%), *Agromyces soli* MJ21<sup>T</sup> (97.72%). The whole genome of strain S2-1-8<sup>T</sup> was 4,099,562 bp in length with a G + C content of 71.1%. This strain S2-1-8<sup>T</sup> was Gram-positive, weak-motile and rod-shaped. Growth of strain S2-1-8<sup>T</sup> was found to occur at pH 5.0–10.0 (optimum pH 8.0), 10–40°C (optimum 25–30°C). The major respiratory quinones were menaquinone 12 (MK12) and MK13. The predominant polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phospholipid and glycolipid. The major fatty acids (> 10%) of strain S2-1-8<sup>T</sup> were antensio-C<sub>15:0</sub> (37.25%), iso-C<sub>15:0</sub> (19.33%), anteiso-C<sub>17:0</sub> (18.29%), and iso-C<sub>16:0</sub> (16.27%). Genome annotation indicated that there is a terpene cluster associated with carotenoid biosynthesis. Strain S2-1-8<sup>T</sup> produced a yellow pigment under light condition, but not in dark conditions. Based on its phenotypic, genotypic, and chemotaxonomic characteristics strain S2-1-8<sup>T</sup> is proposed to represent a novel light-inducible carotenoid producing species of genus *Agromyces*, for which the name is *Agromyces soybeansoli* sp. nov. The type strain is S2-1-8<sup>T</sup> (KCTC 49457<sup>T</sup> = CCTCC AF 2020002<sup>T</sup>).

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#### Tessaracoccus palaemonis sp. nov., Isolated from the Gastrointestinal Tract of the Lake Prawn

Do-Yeon Kim<sup>1</sup>, In-Chul Jeong<sup>1</sup>, So-Yeon Lee<sup>1</sup>, Yun-Seok Jeong<sup>1</sup>, Jeong Eun Han<sup>1</sup>, Euon Jung Tak<sup>2</sup>, June-Young Lee<sup>2</sup>, Pil Soo Kim<sup>2</sup>, Dong-Wook Hyun<sup>1</sup>, and Jin-Woo Bae<sup>1,2\*</sup>

<sup>1</sup>Department of Biology and Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University, <sup>2</sup>Department of Biology and Department of Life and Nanopharmaceutical Sciences, Kyung Hee University

A novel Gram-stain-positive, non-motile, and non-spore-forming bacterial strain, designated strain J1M15<sup>T</sup>, was isolated from the gastrointestinal tract of the lake prawn *Palaemon paucidens*. Bacterial isolates were Gram-stain-positive, non-motile, and non-spore-forming. Strain J1M15T was a facultatively aerobic bacterium that formed orangish-yellow-colored colonies and showed rod-shaped cells. Strain J1M15<sup>T</sup> showed the highest 16S rRNA gene sequence similarity (98.08%) and OrthoANI value with *Tessaracoccus flavescens* SST-39<sup>T</sup>. The whole-genome sequence of strain J1M15<sup>T</sup> was 3.20 Mb in size and the genomic G + C content directly calculated from the genome sequence of the strain J1M15<sup>T</sup> was 69.6%. Strain J1M15<sup>T</sup> showed the highest similarity with *T. flavescens* (77.2% OrthoANI, 23.4% dDDH, and 73.5% AAIm values). We analyzed the genome sequences of strain J1M15T about Carbohydrate-active enzymes, antibiotic resistance genes, and virulence factor genes. The multiple taxonomic analyses indicated that strain J1M15<sup>T</sup> represents novel species of the genus *Tessaracoccus*, respectively. We propose the names *Tessaracoccus palaemonis* sp. nov. for strain J1M15<sup>T</sup> (= KCTC 49462<sup>T</sup> = CCUG 74766<sup>T</sup>). [Supported by a grant from the Mid-Career Researcher Program (NRF-2020R1A2C3012797)]

#### A042

Description of Chryseobacterium fluminis sp. nov. Isolated from Freshwater River

Moonsoo Kim and Seung Bum Kim\*

Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University

In this study, a Gram-negative bacterial strain designated MMS21-OT14<sup>T</sup> isolated from freshwater river, and analyzed by means of a polyphasic taxonomic approach. The 16S rRNA gene sequence analysis revealed that strain MMS21-OT14<sup>T</sup> belongs to the genus *Chryseobacterium* of the family *Weeksellaceae* and is closely related to *C. hagamense* RHA2-9<sup>T</sup> (97.45% sequence similarity), *C. gwangjuense* THG-A18<sup>T</sup> (97.39%) and *C. gregarium* P 461/12<sup>T</sup> (97.22%). The optimal growth of MMS21-OT14<sup>T</sup> occurred at 25–30°C, pH 6.0–7.0 and in the absence of NaCl. Strain MMS21-OT14<sup>T</sup> was capable of hydrolyzing casein, starch, DNA, Tween 20 and tyrosine. The strain also showed decolourising activity of remazol brilliant blue R (RBBR) and keratinolytic activity with keratin azure. The main polar lipids of strain MMS21-OT14<sup>T</sup> were aminophospholipid, aminolipid, phospholipid, and several unidentified lipids. The predominant fatty acids of strain MMS21-OT14<sup>T</sup> were iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3-OH, Summed Feature 9 (C<sub>16:1</sub>  $\omega$ 7*c* and/or C<sub>16:1</sub>  $\omega$ 6*c*) and Summed Feature 3 (iso-C<sub>17:1</sub>  $\omega$ 9*c* and/or 10-methyl C<sub>16:0</sub>). The major isoprenoid quinone was menaquinone-6 (MK-6). It is evident from this study that strain MMS21-OT14<sup>T</sup> should be classified as a novel species of *Chryseobacterium*, for which the name *Chryseobacterium fluminis* sp. nov. (type strain= MMS21-OT14<sup>T</sup>=KCTC 92255<sup>T</sup>=LMG 32529<sup>T</sup>) is proposed.

[This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE).]

### *Pseudorhodobacter turbinis* sp. nov., Isolated from the Gut of the Korean Turban Shell, *Turbo cornutus* Yun-Seok Jeong<sup>1</sup> and Jin-Woo Bae<sup>1,2\*</sup>

<sup>1</sup>Department of Biology, Kyung Hee University, <sup>2</sup>Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University

A novel aerobic, Gram-negative, coccus-shaped and motile bacterial strain designated S12M18<sup>T</sup> was isolated from the gut of the Korean turban shell, *Turbo cornutus*. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain S12M18<sup>T</sup> belonged to the genus *Pseudorhodobacter* and had the highest 16S rRNA gene sequence similarity with *Pseudorhodobacter aquimaris* HDW-19<sup>T</sup> (98.63%). The phylogenomic tree verified that strain S12M18<sup>T</sup> occupies a taxonomic position within the genus *Pseudorhodobacter*. The OrthoANIu value between strain S12M18<sup>T</sup> and *P. aquimaris* HDW-19<sup>T</sup> was 87.22%. The major cellular fatty acid of strain S12M18<sup>T</sup> was summed feature 8 ( $C_{18:1} \omega 7c$  or  $C_{18:1} \omega 6c$ ). The major components of the polar lipids were phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine. The predominant isoprenoid quinone was Q-10. The DNA G + C content was 57.8%. The polyphasic analyses indicated that strain S12M18<sup>T</sup> represents a novel species of the genus *Pseudorhodobacter*, for which the name *Pseudorhodobacter turbinis* sp. nov. is proposed. The type strain is S12M18<sup>T</sup> (= KCTC 62742<sup>T</sup> = JCM 33168<sup>T</sup>).

#### A044

# Specific Mass Peaks for Discriminating *Lacticaseibacillus* Species Using Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass with In-house Database

Eiseul Kim, Seung-Min Yang, and Hae-Yeong Kim<sup>\*</sup> Department of Food Science and Biotechnology, Kyung Hee University

The *Lacticaseibacillus casei, L. paracasei, L. rhamnosus, L. chiayiensis*, and *L. zeae* are phylogenetically closely related species. The nomenclature and taxonomic status of five species have been used with insufficient resolution methods, leading to the mislabeling of these species in some products and publicly available genome sequences. This study was conducted to validate the feasibility and accuracy of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in identifying five *Lacticaseibacillus* species and discovering mass peaks to discriminate these species. From the mass spectra of 130 isolates aligned with databases, 118 strains were correctly identified. On the other hand, databases could not accurately differentiate 12 isolates such as *L. casei, L. rhamnosus*, and *L. chiayiensis* because the same colony was identified as different species with a similar score. To overcome the database's limitations, the mass spectra were analyzed to identify specific mass peaks. The peaks at  $6731 \pm 1$ ,  $6849 \pm 1$ ,  $7008 \pm 1$ ,  $7376 \pm 1$ , and  $2593 \pm 1$  m/z were specifically found in the *L. casei, L. paracasei, L. rhamnosus, L. chiayiensis*, and *L. zeae*, respectively. These mass peaks confirmed that the five peaks were consistently present in each species using strains isolated from food samples. Our results demonstrate the high-resolution of MALDI-TOF MS for rapid and accurate classification of five species when used with databases coupled to specific peaks.

### Description of *Polaribacter batillariae* sp. nov., *Polaribacter cellanae* sp. nov., and *Polaribacter pectinis* sp. nov., Novel Bacteria Isolated from the Gastrointestinal Tract of Three Types of South Korean Shellfish

Su-Won Jeong, Jeong Eun Han, June-Young Lee, and Jin-Woo Bae<sup>\*</sup>

Department of Biomedical and Pharmaceutical Sciences and Department of Biology, Kyung Hee University

Three bacterial strains, designated G4M1<sup>T</sup>, SM13<sup>T</sup>, and L12M9<sup>T</sup>, were isolated from the gastrointestinal tract of shellfish collected from the Yellow Sea in South Korea. The 16S rRNA gene sequence analysis showed that all these three strains belong to the genus *Polaribacter* in the family Flavobacteriaceae. According to the phylogenetic tree, *P. haliotis* KCTC 52418<sup>T</sup> (< 98.68%) and *P. litorisediminis* KCTC 52500<sup>T</sup> (< 98.13%) were the closest related type strains of *Polaribacter*. The three strains shared < 98.8% in the 16S rRNA gene sequence and < 86.68% in the whole-genome sequence with each other. The G + C content of the genomic DNA of strains G4M1<sup>T</sup>, SM13<sup>T</sup>, and L12M9<sup>T</sup> were 31.0, 30.4, and 29.7 mol%, respectively. All three strains are aerobic, Gram-negative, rod-shaped bacterial strains, and grew optimally at 25°C, in the presence of 2% (w/v) NaCl, and at pH 7. All the strains contained MK-6 as a predominant menaquinone, iso-C<sub>15:0</sub> as a major fatty acid, and phosphatidylethanolamine as a polar lipid component. Based on these results, each of the three strains G4M1<sup>T</sup> (= KCTC 82388<sup>T</sup> = DSM 112372<sup>T</sup>), SM13<sup>T</sup> (= KCTC 82389<sup>T</sup> = DSM 112373<sup>T</sup>), and L12M9<sup>T</sup> (= KCTC 62751<sup>T</sup> = DSM 112374<sup>T</sup>) represents a novel species of the genus *Polaribacter*, for which the name *Polaribacter batillariae* sp. nov., *Polaribacter cellanae* sp. nov., and *Polaribacter pectinis* sp. nov., respectively, have been proposed.

[This research was supported by a grant (22213MFDS537) from ministry of food and drug safety in 2022.]

#### A046

### Description of *Deefgea piscis* sp. nov., and *Deefgea tanakiae* sp. nov., Isolated from the Gut of Korean Indigenous Fish

Do-Hun Gim<sup>1</sup>, So-Yeon Lee<sup>1</sup>, Jeong Eun Han<sup>1</sup>, Jae-Yun Lee<sup>2</sup>, Seo Min Kang<sup>1</sup>, and Jin-Woo Bae<sup>1,2\*</sup> <sup>1</sup>Department of Biology and Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University, <sup>2</sup>Department of Life and Nanopharmaceutical Sciences and Department of Biology, Kyung Hee University

Three novel strains,  $(D17^{T}, D13, and D25^{T})$  isolated from the gut of the Korean indigenous fish were identified as two novel species. Strains  $D17^{T}$  and D13 showed the highest similarities in 16S rRNA gene and complete genome sequences to *Deefgea rivuli* WB 3.4-79<sup>T</sup> (98.0% and 97.9%, respectively, of 16S rRNA gene sequence similarity, 77.8% and 77.7%, respectively, of orthologous average nucleotide identity, OrthoANI, and 21.9% and 21.9%, respectively, of digital DNA-DNA hybridization, dDDH). Strain  $D25^{T}$  showed the highest similarities in 16S rRNA gene and complete genome sequences to *D. chitinilytica* Nsw-4<sup>T</sup> (98.2% of 16S rRNA gene sequence similarity, 82.4% of OrthoANI, and 25.1% of dDDH). Strains  $D17^{T}$  and D13 were Gram-stain-negative, facultative anaerobes, non-motile, non-flagellated, and rod-shaped. Strain  $D25^{T}$  was Gram-stain-negative, facultative anaerobe, rod-shaped, and motile by a single polar flagellum. These strains had C<sub>16:0</sub> and summed feature 3 (C<sub>16:1</sub>  $\omega$ 7*c* and/or C<sub>16:1</sub>  $\omega$ 6*c*) as the major cellular fatty acids and possessed Q-8 as a major respiratory ubiquinone. All three strains contained phosphatidylethanolamine and phosphatidylglycerol as the major polar lipids. Based on polyphasic taxonomic data, strains D17<sup>T</sup>, D13, and D25<sup>T</sup> represent two novel species of the genus *Deefgea*.

[This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean government (MSIT) (NRF-2022M3A9F3082331)]

#### Novel Species Isolated from the Gut of the Lake Shirmp

In-Chul Jeong and Jin-Woo Bae<sup>\*</sup>

Department of Biology and Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University

A novel Gram-positive, non-motile irregular rod (rod-coccus cell cycle), ivory and aerobic bacterial strain designated J2M5<sup>T</sup> was isolated from the gut of the lake shirmp. The isolate J2M5<sup>T</sup> was characterized by phenotypic, phylogenetic, chemotaxonomic analysis. The optimal growth condition of strain J2M5<sup>T</sup> was 30°C, 0% (w/v) NaCl and pH 9. The phylogenetic analysis based on 16S rRNA sequences showed that the strain J2M5<sup>T</sup> was associated to the genus *Nocardioides* and showed highest 16S rRNA gene sequence similarity with *Nocardioides ganghwensis* CGMCC 4.6875<sup>T</sup> (98.61%). The isoprenoid quinone was MK-8. The major component of cellular fatty acid was iso-C<sub>16:0</sub>. Amino acids were L-alanine, L-lysine, and whole cell sugars were ribose, galactose, glucose, mannose. Polar lipid consisted of diphosphatidylglycerol, phosphatidylgrecerol, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine. The DNA G + C content was 72.5 mol%. The polyphasic analysis showed that the strain J2M5<sup>T</sup> represents a novel species of the genus *Nocardioides*. The name *Nocardioides macrobrachii*sp. nov. is suggested. The type strain is J2M5<sup>T</sup> (= KCTC 49461).

[Supported by a grant from the Mid-Carrer Researcher Program (NRF-2020R1A2C3012797), Republic of Korea.]

#### A048

### Mosquito Distribution and Phylogenetic Analysis of Mosquito-borne Pathogen in Gwangju Metropolitan City in 2018–2021

A Yoon Kim, Mi Geum Ryu, Jin Jong Seo, and Jong Jin Park<sup>\*</sup> Health and Environment Research Institute of Gwangju

Owing to global climate change, vector-borne infectious, especially mosquitoes-borne diseases, have emerged as a major issue in twenty-first century. Thus, we investigated the distribution patterns of Mosquito and performed a phylogenetic analysis by identifying flavivirus and togavirus sequences in Gwangju metropolitan city, republic of Korea. From April to October in 2018–2021, a total of 9,399 female mosquitoes were collected in 3 sites (waterside, mountain and urban forests) using BG-Sentinel traps. The result of mosquitoes species classification, 12 species mosquitoes were trapped and the dominant species was *Aedes albopictus* (4,661, 49.7%) known to mediate Zika virus, followed by *Culex pipiens* (2,135, 22.8%) and *Armigeres subalbatus* (1,543, 16.5%). From the results of viral RNA (1,070 pools) detections, eleven Culex flavivirus strains were detected from genus *culex* pools and one Japanese encephalitis virus (JEV) strain was detected from Culex orientalis. The one JEV-positive pools phylogenetically grouped as genotype VI. Also, no togaviral infection was observed upon real-time RT-PCR. This is the first study on the distribution of mosquitoes and the phylogenetic analysis of flavivirus in Gwangju Metropolitan city. Our findings demonstrate that continous monitoring should be performed to control the entry of exogenous flavivirus into Korea and improve public health.

#### New Trichoderma Species Isolated from Lichen Cladonia

Yunhyeok Jang, Yehyeon Cha, and Seung-Yoon Oh<sup>\*</sup> Department of Biology and Chemistry, Changwon National University

*Trichoderma* lives in various environments as endophytes, saprophytes, or parasites. A lot of *Trichoderma* species interact with other organisms such as animals, plants, and mushrooms. However, the diversity and relationship of Trichoderma in lichens have not been studied well. In this study, we isolated the endolichenic fungi from lichen *Cladonia*, and found a strain CSC21B0361 that is a potentially new species in *Trichoderma*. According to the polyphasic analysis for morphology and multi-gene phylogeny (nuclear ribosomal internal transcribed spacer, translation elongation factor 1-alpha, and RNA polymerase II second largest subunit regions), this strain is close to *T. koningiopsis* belonged to *Viride* clade, but the cultural, morphological, and molecular characteristics were different to other *Trichoderma* species in this clade. Herein, we described detailed characteristics of this species. [This work was supported by a grant from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment (MOE) of the Korea (NIBR202203112) and the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT) (NRF 2021R1C1C1008045).]

#### A050

**Introducing the Software Enables both Reconstructing Phylogenetic Trees and Revealing Pathway Relationship** Inwoo Baek, Hui-Jeong Gwon, and Jong-Hyun Jung<sup>\*</sup> *Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute* 

Inferring phylogenetic trees is an essential step in studying microbial genomic analysis. Recently, concatenating bacterial universal genes has been utilized for the information of reconstructing phylogenetic trees. However, the topology of intra-species and intra-genus phylogenetic trees could vary depending on the universal gene set. Therefore, the users can easily modify the software's gene set by selecting a reasonable universal gene set (e.g. lineage-specific core gene set) or applying a custom gene set. Moreover, this software also enables the revealing of pathway evolution, by executing the software by modifying HMM files charged in a sequential pathway. Gene trees can be inferred by using this software, and it can be applied for unveiling horizontal gene transfer of those genes.

### **Genome Mining and Description of** *Chitinophaga horti* sp. nov., Isolated from Flower Garden Soil Mingyeong Park and Jaisoo Kim<sup>\*</sup>

Kyonggi University

A Gram staining negative, straw color and flexirubin test-positive bacterium, designated GS18<sup>T</sup> was isolated from soil sample at the garden, Korea. Strain GS18<sup>T</sup> hydrolysed gelatin but not starch and casein. Cells were aerobic and grew well at 32–37°C. Strain GS18<sup>T</sup> showed antimicrobial activity against Gram-negative pathogens (*Pseudomonas aeruginosa* and *Escherichia coli*). A phylogenetic analysis based on its 16S rRNA gene sequence revealed that strain GS18<sup>T</sup> formed a lineage within the family *Chitinophagaceae* and clustered as members of the genus *Chitinophaga*. The closest members were *Chitinophaga qingshengii* JN246<sup>T</sup> (98.76% sequence similarity), *Chitinophaga eiseniae* DSM 22224<sup>T</sup> (98.27%). The major cellular fatty acids were iso-C<sub>15:0</sub>, C<sub>16:1</sub>  $\omega$ 5*c*, C<sub>16:0</sub> and iso-C<sub>17:0</sub> 3-OH. The major respiratory quinone was MK-7. The principal polar lipids were phosphatidylethanolamine and aminolipids. The DNA G + C content of the type strain was 48.8mol%. The average nucleotide identity (ANI) and *in silico* DNA–DNA hybridization (dDDH) relatedness values between strain GS18<sup>T</sup> and phylogenetically closest members were below the threshold value for species delineation. Based on genomic, chemotaxonomic, phenotypic and phylogenetic analyses, strain GS18<sup>T</sup> represents a novel species in the genus *Chitinophaga*.

[This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. 2022R1A2C1010877).]

#### A052

#### Isolation, Identification, and Collection of Fungi from Nuruk

Jisu Lee, Dong Hyun Kim, Daseul Lee, and Seung-Beom Hong<sup>\*</sup> Korean Agricultural Culture Collection, Agricultural Microbiology Division, NAS, RDA

Fungal genus *Aspergillus* is one of the most important fungi for the fermentation of alcoholic drinks in East Asia. *Nuruk* and *Koji*, the traditional fermentation starter in Korea and Japan, respectively, mainly contain *Aspergillus* and influence on the taste and flavor of beverages. However, in the recent days, Korean traditional drinks are usually made by Japanese *Koji* molds such as *A. oryzae*, *A. luchuensis*, and *A. luchuensis* mut. *kawachii* due to their characteristics such as fast growth, ease to handle, and high amylase activity. Therefore, it is important to collect *Nuruk* in Korea and isolate fungi including *Aspergillus* to manufacture the Korean traditional drinks using Korean *Nuruk* molds. In this study, we isolated fungi from *Nuruk* and identified them as species levels by genetic and morphological analyses. As the results, a nubmer of fungi such as *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium*, *Cladosporium* were isolated. In order to observe the predominant fungis in each *Nuruk*, direct examination and dilution plating analyses were performed. For the application on fermentation, isolated fungal strains from *Nuruk* and conserved *Aspergillus* strains in Korean Agricultural Culture Collection (KACC) were examined for amylase and protease activity on starch agar media (pH 6) and skim milk agar media (pH 4 and 7), respectively. [This study was supported by the grant from National Institute of Agricultural Sciences (PJ01637).]

#### **Paenibacillus sangjiensis sp. nov., Paenibacillus wonjuensis sp. nov., Isolated from Agricultural Soil** Eun-Woo Choi<sup>1</sup>, Ki-Eun Lee<sup>2</sup>, In-Tae Cha<sup>2</sup>, Won-Jae Chi<sup>2</sup>, and Dong-Uk Kim<sup>1\*</sup> <sup>1</sup>Department of Life Environmental Sciences, Sangji University, <sup>2</sup>National Institute of Biological Resources

A Gram-stain-positive, aerobic, milk white coloured rod-shaped bacterium, designated GW78<sup>T</sup> (*Paenibacillus sangjiensis* sp. nov.). Growth occurs at 10–37°C (optimal at 37°C), and 0–1.0% (w/v) NaCl on R2A medium. A Gramstain-variable, aerobic, yellowish white coloured rod-shaped bacterium, designed dw9<sup>T</sup> (*Paenibacillus wonjuensis* sp. nov.). Growth occurs at 20–37°C (optimal at 28°C), and 0–2.0% (w/v) NaCl on R2A medium. Also, catalase positive, oxidase negative. Strains were isolated from soil, Republic of Korea. Phylogenetic analysis based 16S rRNA gene sequences indicated that strain GW78<sup>T</sup> was affiliated with the genus *Paenibacillus* in the family *Paenibacillaceae* and shared 93.77–98.26% sequence similarities with *Paenibacillus* species. The strain dw9<sup>T</sup> shared 94.70–97.44%. The strains contained C<sub>15:0</sub> anteiso as a major fatty acid. The polar lipids detected in the strains were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylethanolamine. On the basis of phylogenetic and phenotypic characteristics, strains GW78<sup>T</sup>, dw9<sup>T</sup> are considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus* sangjiensis sp. nov. (type strain GW78<sup>T</sup> = KCTC 43430<sup>T</sup>), *Paenibacillus wonjuensis* sp. nov. (type strain dw9<sup>T</sup> = KCTC 43431<sup>T</sup>), are proposed. [This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202203112).]

#### A054

#### *Bdellovibrio svalbardensis* sp. nov., a Novel Predator Isolated from Svalbard Norway Sumin Choi<sup>1</sup>, SeongYeol Choi<sup>1</sup>, Wonsik Moon<sup>1</sup>, Kyoung Lee<sup>2</sup>, and Robert J. Mitchell<sup>1\*</sup>

<sup>1</sup>Ulsan National Institute of Science and Technology, <sup>2</sup>Changwon National University

Identified as a novel species from a biocrust in Svalbard, Norway, isolate PAP01 has a different character from known predatory microorganisms. The isolate was vibrio-shaped, Gram-negative strain that employed flagellar motility. Phylogenetic analysis based on the 16S rRNA gene sequence revealed a clustered isolate within the genus *Bdellovibrio* in the phylum *Bdellovibrionaceae*. 16S rRNA gene sequence similarities between strain PAP01 and the type strain (*Bdellovibrio bacteriovorus* HD100) was 95.7%. The PAP01 genome has a size of 3.898 Mb and possesses 3732 genes and a G + C content of 45.7%. The results of genetic and physiological tests indicated phenotypic differentiation of strain PAP01 from other *Bdellovibrio* species with valid published names. Therefore, the PAP01 strain was classified in the genus *Bdellovibrio* as the type strain of a novel species, for which the name *Bdellovibrio svalbardensis* sp. nov. is proposed.

### Multilocus Sequencing Based Re-identification of *Aspergillus* Species in Korean Agricultural Culture Collection (KACC)

Anbazhagan Mageswari, Yunhee Choi, HyoRim Choi, and Seung-Beom Hong<sup>\*</sup> Korean Agricultural Culture Collection, Agricultural Microbiology Division, NAS, RDA

Conventional identification of fungi makes use of morphological characterisation and phylogenetic markers such as ITS and 18S rRNA gene sequencing which has drawbacks such as, misidentification and inability to delimit cryptic species. Recent developments in fungal taxonomy has identified further phylogenetic markers such as  $\beta$ -tubulin (BenA) and calmodulin (CaM) genes, characterization of fungal metabolites and use of next-genome sequencing. Korean Agricultural Culture Collection (KACC) is a major research organization specializing in identification and long term storage of fungal biodiversity which can serve as a potential source of useful fungal strains. The collection currently preserves 13,697 strains of fungi from 4,235 species covering all major fungal taxonomic groups. In the present study, 418 strains previously classified as *Aspergillus* species were taken and their BenA & CaM genes sequenced. Phylogenetic analyses using multiple sequence alignment of the sequences and tree construction using Neighbor-joining algorithm, maximum-likelihood model in MEGA XI indicated that 21 strains could be moved and re-assigned to different section of Aspergillus. Use of integrated taxonomic approach is expected to help better delimitation of fungi and reclassify misidentified fungal species as genetic ambiguity when using currently available markers pose a formidable challenge for the future identification of fungal species.

#### A056

A Novel Butyrate-producing Bacteria Isolated from Feces of a Patient with Crohn's Disease

Jae-Yun Lee<sup>1</sup> and Jin-Woo Bae<sup>1,2\*</sup>

<sup>1</sup>Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, <sup>2</sup>Department of Biology and Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University

Strain BG01<sup>T</sup> was rod-shaped, Gram variable, endospore-forming and strictly anaerobic bacterium. Strain BG01<sup>T</sup> grew at 15–45°C (optimum 37°C), pH 6–10 (optimum 7), and 0–4% (w/v) NaCl (optimum 0–1%), and had resistance to bile salt, but not to ampicillin, metronidazole, vancomycin and cefoperazone. Possessed *meso*-diaminopimelic acid (*meso*-DAP) as a diagnostic diamino acid and  $C_{12:0}$ ,  $C_{18:0}$  dimethyl aldehyde (DMA), and  $C_{18:1} \omega 9c$  DMA as predominant cellular fatty acids. Butyrate, propionate, oxalacetate and fumarate were produced as fermentation end products from Gifu Anaerobic Medium (GAM) broth. Strain BG01<sup>T</sup> showed 97.7% of 16S rRNA gene sequence similarity, and 92.0% and 48.5% of average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values, respectively, with *Anaerostipes caccae* KCTC 15019<sup>T</sup>. Genomic analysis pointed out that strain BG01<sup>T</sup> had a pathway for butyrate production, and genomic G + C content of the strain was 43.5 mol%. The polyphasic analyses indicated that strain BG01<sup>T</sup> represents a novel butyrate producing species of the genus *Anaerostipes*, for which the name *Anaerostipes hominis* sp. nov. is proposed. The type strain is BG01<sup>T</sup> (= KCTC 15617<sup>T</sup> = JCM 32275<sup>T</sup>). [This work was supported by a grant (22213MFDS537) from the Ministry of Food and Drug Safety (MFDS).]

### Phenotypic and Genomic Characterization of a Novel *Kingella* Species, Isolated from a Child with Osteomyelitis Ah-In Yang<sup>1,2</sup>, Min Ok Jun<sup>1</sup>, and Na-Ri Shin<sup>1\*</sup>

<sup>1</sup>Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>Department of Biology, Kyung Hee University

Strain SNUBH-2017<sup>T</sup> was isolated from vertebral body of the lumbar spine of 4-year-old male with subacute osteomyelitis. Cells are Gram-stain-negative, aerobic, catalase-negative and oxidase-positive, and the colonies grown on Gifu anaerobic medium agar plate are circular, smooth, glistening, translucent, dry, and flat-to-umbonate with a diameter of 2 mm. The isolate grows at 20–45°C (optimum 37°C) and 0% (w/v) NaCl concentration optimally. 16S rRNA gene sequence analysis of strain SNUBH-2017<sup>T</sup> showed the highest similarity to *Kingella potus* 3/SID/1128<sup>T</sup> with 97.3%, followed by *Neisseria bacilliformis* ATCC BAA-1200<sup>T</sup> (96.78%). Phylogenetic analysis based on 16S rRNA gene further suggested that strain SNUBH-2017<sup>T</sup> is closely associated to *Kingella* species. The genome size of strain SNUBH-2017<sup>T</sup> is 2,263,453 bp and G + C content is 59.1 mol%. The average nucleotide identity (ANI) values based on OrthoANIu algorithm between strain SNUBH-2017<sup>T</sup> and closely related species were 79.3% to *Neisseria bacilliformis* ATCC BAA-1200<sup>T</sup> and 77.3% to *Kingella potus* 3/SID/1128<sup>T</sup>. Based on the phenotypic and genotypic analyses in this study, strain SNUBH-2017<sup>T</sup> is considered to represent a novel species of the genus *Kingella*.

[This work was supported by KRIBB Research Initiative Programs (KGM5232022 and KGM1192211) and was performed with collaboration with Dr. Hyunju Lee, M.D. Ph.D (Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Republic of Korea)]

#### A058

**Eleven Novel Species in the Genus** *Flavobacterium*, Isolated from Nakdong River in Korea Hyunyoung Jo, Miri S Park, Ilnam Kang, and Jang-Cheon Cho<sup>\*</sup> *Department of Biological Sciences and Bioengineering, Inha University* 

Eleven Gram-negative, non-motile, catalase-positive, rod-shaped bacterial strains, designated strains IMCC34515<sup>T</sup>, IMCC34518<sup>T</sup>, IMCC34673<sup>T</sup>, IMCC34774<sup>T</sup>, IMCC34775<sup>T</sup>, IMCC34776<sup>T</sup>, IMCC34777<sup>T</sup>, IMCC34779<sup>T</sup>, IMCC36791<sup>T</sup>, IMCC36792<sup>T</sup>, and IMCC36793<sup>T</sup>, were isolated from Nakdong River in Korea. Phylogenetic analyses based on 16S rRNA gene sequences indicated that all eleven strains are affiliated with the genus *Flavobacterium*. The 16S rRNA gene sequence similarities between the 11 strains and type strains of the genus were < 98.33%. Optimal growth of the 11 novel strains occurred on R2A at 20–25°C, at pH 7.0–8.0 and without NaCl. Major fatty acid constituents of these strains were iso-C<sub>15:0</sub> and summed feature 3 (comprised C<sub>16:1</sub>  $\omega$ 6c and/or C<sub>16:1</sub>  $\omega$ 7c). Respiratory quinone detected in the strains was menaquinone-8 (MK-8). The draft genome sequences of the strains showed a rage of 3.4–5.8 Mb and average G + C contents of 33.41–37.32%. The genomes also showed ANI values of < 97.49% sequence similarities to each other and any currently described *Flavobacterium* species. This suggested that the 11 strains each represent different novel species of the genus *Flavobacterium*, therefore, we propose the establishment of 11 novel *Flavobacterium* species.

### Nibricoccus lotistagni sp. nov., Isolated from Lotus Wetland in Korea

Hyerim Cho, Miri s. Park, Ilnam Kang, and Jang-Cheon Cho<sup>\*</sup> Department of Biological Sciences and Bioengineering, Inha University

A novel strain characterized as being aerobic, Gram-negative, oxidase-positive, catalase-negative, non-motile and coccus-shaped bacterium, designated strain IMCC34717<sup>T</sup>, was isolated from a lotus wetland. Cells were 0.8–1.0 µm in diameter and colonies are circular, smooth and white colored on R2A agar plate grown at 30°C for 3 days. The 16S rRNA gene sequence similarity and phylogenetic analyses showed that strains IMCC34717<sup>T</sup> belonged to the genus *Nibricoccus*. Strain IMCC34717<sup>T</sup> was most closely related to *Nibricoccus aquaticus* HZ-65<sup>T</sup> (95.2%), followed by *Opitutus terrae* PB90-1<sup>T</sup> (94.6%) in the phylum *Verrucomicrobiota*. The draft genome of strain IMCC34717<sup>T</sup> genome was predicted to contain 2,979 protein coding genes (CDSs), 3 rRNA genes and 55 tRNA genes. Average nucleotide identity and digital DNA–DNA hybridization values between strain IMCC34717<sup>T</sup> and *N. aquaticus* HZ-65<sup>T</sup> were 73.6% and 20.7%, respectively, indicating that the strain represents a novel species. These results, strain IMCC34717<sup>T</sup> is proposed to be a novel species in the genus *Nibricoccus* with the following names: *Nibricoccus lotistagni* (type strain IMCC34717<sup>T</sup>).

#### A060

*Corynebacterium intestinalis* sp. nov., a Novel Bacterium Isolated from Healthy Human Feces Md Shamsuzzaman<sup>1</sup>, Ram Hari Dahal<sup>1</sup>, Shukho Kim <sup>1,2</sup>, Yoon-Jung Choi<sup>1</sup>, Bokyung Kim<sup>1</sup>, Yun-Jung Choi<sup>1</sup>, and Jungmin Kim<sup>1,2\*</sup>

<sup>1</sup>Department of Biomedical Sciences, The Graduate School, Kyungpook National University, <sup>2</sup>Department of Microbiology, School of Medicine, Kyungpook National University

*Corynebacterium* is a genus of Gram-positive and mostly aerobic bacteria, some species of which are essential for digesting food, preventing infection, and stimulating the immune system. To characterize the novel bacteria, a culture method with phenotypic and genomic analysis of the 16S rRNA gene sequencing was used to isolate previously uncultured strains. Therefore, we isolated an obligate, aerobic, Gram-positive, oxidase-negative, catalase-positive, non-motile, indole-negative, non-spore-forming coryneform bacterium named B5-R-101<sup>T</sup> from healthy human faces. It grew at temperatures between  $10-40^{\circ}$ C (optimum,  $20-37^{\circ}$ C), pH 4.5–11.0 (optimum, 5.5–8.5), and salinity (up to 10% NaCl) was observed. In phylogenetic analysis of 16S rRNA gene sequences, this strain belongs to the genus *Corynebacterium* and represents a novel and deep lineage within the genus *Corynebacterium aurimucosum* DSM 44532<sup>T</sup> (98.8% sequence similarity). G + C content (%) was 61.1% and genome size was 2,677,399 bp. Genome analysis has revealed genes involved in the production of H<sub>2</sub>O<sub>2</sub> and the synthesis of vital secondary metabolites. The polar lipids were di-phosphatidylglycerol, phosphatidylglycerol, glycolipids, amino lipids, aminophospholipid, and three unidentified lipids. Based on genomic, phenotypic, and phylogenetic analyses of strain B5-R-101<sup>T</sup> represents a novel species of the genus *Corynebacterium*, for which the name *Corynebacterium intestinalis* sp. nov., is proposed B5-R-101<sup>T</sup>.

#### Isolation and Characterization of Rotaviruses from Feces of Diarrheic Calves in Korea

Michelle Miguel, Seon-Ho Kim, Ye Pyae Naing, A-Rang Son, Yong-Il Cho, and Sang-Suk Lee<sup>\*</sup> Department of Animal Science and Technology, Sunchon National University

Bovine rotaviruses are among the common agents involved in diarrhea in calves. This study aimed to isolate and characterize group A rotavirus (RVA) in calves in Korea. Fecal samples of diarrheic calves were obtained from different farms in Korea. Rotavirus was propagated in MA104 cells and cytopathic effect (CPE) was observed daily. After several passages, the CPE was characterized by rounding, detachment, and destruction of monolayer cells. Virus supernatant was then harvested and an aliquot of the supernatant was used for the RNA extraction. A reverse transcriptase polymerase chain reaction (RT-PCR) assay was used to confirm the presence of rotavirus nucleic acid from isolates. Bovine rotavirus G and P serotypes isolates were analyzed using the VP7 and VP4 genes by PCR. For the determination of G and P serotypes, a 1,062 bp fragment of the VP7 gene and 864 bp fragment of VP4 gene was first amplified, respectively. In a second PCR amplification, the 5' generic primer and different typing primers, each one specific to one of the G (G3, G5, G6, G8, G10) and P (P[1], P[3], P[11]) serotypes, were used to generate fragments whose sizes served to identify the G and P serotypes. Genotyping using VP7 and VP4 revealed that the bovine rotaviruses were serotypes of G6P[5] and G6P[11]. These assays were able to identify the rotavirus G and P serotypes.

[This work was supported by a research grant from the National Research Foundation of Korea (NRF) (Project number: 2021R1C1C2014102)]

#### A062

#### A Novel Veillonella Species, Isolated from Feces of an Infant

Haneol Yang<sup>1,2</sup>, Hana Jo<sup>1</sup>, Yeong Rak Son<sup>1</sup>, Seung Hyun Kim<sup>1</sup>, Chan-Seok Yun<sup>1</sup>, Yu-ri Lee<sup>1</sup>, Jin-Woo Bae<sup>2</sup>, and Doo-Sang Park<sup>1\*</sup>

<sup>1</sup>Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>2</sup>Department of Biology, Kyung Hee University

An anaerobic, Gram-negative, catalase negative and cocci-shaped bacterial strain designated as DS1651 was isolated from feces of an infant in South Korea. Phylogenetic analysis based on 16S rRNA gene sequences indicated that DS1651 was most closely related to *Veillonella nakazawae* CCUG 74597<sup>T</sup> (99.86%), followed by *Veillonella infantium* ATCC TSD-88<sup>T</sup> (99.80%), and *Veillonella dispar* ATCC 17748<sup>T</sup> (99.73%) in the family *Veillonellaceae*. ANI values between DS1651 and reference species were 95.48% for *Veillonella nakazawae* CCUG 74597<sup>T</sup>, 94.46% for *Veillonella infantium* ATCC TSD-88<sup>T</sup> and 92.81% for *Veillonella dispar* ATCC 17748<sup>T</sup>. Major end products of fermentation were acetic and propionic acid in TPGY broth with 1% (v/v) sodium lactate. The major cellular fatty acids (> 10%) were summed in feature 8 (31.45%) and C13:0 FAME (17.51%). The DNA G+C content of the DS1651 was 38.58 mol%. Based on the phenotypic, chemotaxonomic and phylogenetic data, DS1651 represents a novel species in genus *Veillonella*.

[This work was supported by a Korea Innovation Foundation (INNPOLIS) grant funded by the Korean government (MSICT) through a science and technology project that opens the future of the region (2021-DD-UP-0380), the Center for Women In Science, Engineering and Technology (WISET) Program for Returners into R&D (WISET-2022-444) and a grant from the KRIBB Research initiative program (KGM5232221)]

### Glutamate Decarboxylase Gene Detection and Glutamine and Glutamate Activities of Glutamic Acid-producing Microorganisms Isolated from Ruminants

Lovelia Mamuad, Seon-Ho Kim, Eun-Jeong Ko, and Sang-Suk Lee<sup>\*</sup> Department of Animal Science and Technology, Sunchon National University

Glutamic acid-producing (GAP) microorganisms containing the glutamate decarboxylase gene could enhance the gamma-aminobutyric acid (GABA) production that eventually could be used as a feed additive for ruminants. Hence, we isolated potential GAP microorganisms in ruminants with different rumen pH. Also, we detected the glutamate decarboxylase gene and determined the glutamine and glutamate activities of the isolates. Cannulated Holstein cows in 6 × 3 Latin square design were fed with different proportions of Klein grass, concentrate, and total mixed ration to achieve the approximately 5, 6, and 7 rumen pH. Rumen fluids were collected after 14 days of the feeding. Of 198 isolates, 50, 113, and 35 were isolated at approximately pH 5, 6, and 7, respectively. With these isolates, a total of 22 species were identified as potential GAP microorganisms, which were subjected to glutamate decarboxylase gene detection using GAD and CORE primers. Eight species were positive for either CORE or GAD genes, which are all lactic acid bacteria. Four species (F1-4, F6-3, M17, and 39-1) were positive for CORE while five species (64, C4, C42, E4-3, and M17) were positive for GAD genes. Interestingly, one species (M17) was both positive for GAD and CORE genes. Isolates 39-1, 64, FI-4, and M17 had the highest glutamate, and/or glutamine activities, which could be used as feed additives for ruminants in enhancing GABA production. [Supported by National Research Foundation of Korea: 2020R1I1A1A01075615]

#### A064

**Molecular Identification of** *Fusarium* **Strains in Korean Agricultural Culture Collection (KACC)** Yunhee Choi, Anbazhagan Mageswari, Hyorim Choi, and Seung-Beom Hong<sup>\*</sup> *Korean Agricultural Culture Collection, Agricultural Microbiology Division, NAS, RDA* 

The species-rich genus *Fusarium* is one of the representative fungal pathogens causing diseases in plants. In order to prevent the plant from *Fusarium* diseases, accurate identification is extremely necessary. Nowadays, molecular analyses are preferred to morphological analysis for identification. Three protein-coding genes, translation elongation factor 1- $\alpha$  (*tef1*), RNA polymerase II largest subunit (*rpb1*) and RNA polymerase II second largest subunit (*rpb2*) are used to differentiate *Fusarium* at species level. However, the *Fusarium* strains preserved in KACC were primarily identified by morphological and pathological characteristics. Hence, in this study, 331 Korean strains of *Fusarium* in KACC were re-identified by sequencing the *tef1*, *rpb1* and *rpb2* genes. As a result, 306 strains were reassigned to 8 species complex and 25 strains were transferred to fungal genera *Necosmospora*, *Albonectria*, and *Bisifusarium* from genus *Fusarium*. In particular, the most taxonomically difficult species complex *Sambucinum* were classified to contain 3 clades, 7 species, and 57 strains. In conclusion, phylogenetic positions could be confirmed for *Fusarium* strains distributed in Korea.

[Supported by grants from National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea (PJ016307)]

#### Brachybacterium sillae sp. nov., Isolated from Hot Spring

Ji Yeong Park<sup>1</sup>, Dariimaa Ganbat<sup>1</sup>, Sondor Ganbat<sup>1</sup>, Beom Su Kim<sup>1</sup>, Dong-Woo Lee<sup>2</sup>, Seong-Bo Kim<sup>3</sup>, Yong-Jik Lee<sup>4</sup>, Jung-Sook Lee<sup>5</sup>, and Sang-Jae Lee<sup>1\*</sup>

<sup>1</sup>Department of Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University, <sup>2</sup>Department of Biotechnology, Yonsei University, <sup>3</sup>Bio-Living Engineering Major, Global Leaders College, Yonsei University, <sup>4</sup>Department of Bio-Cosmetics, Seowon University, <sup>5</sup>Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology

A novel Gram-positive, non-motile and moderately halophilic rod-shaped bacterium EF45031<sup>T</sup> was isolated from hotspring in Chungju, Republic of Korea. The strain was able to grow at concentrations of 0–5% (w/v) NaCl (optimum 3% NaCl), at pH 6.0–9.0 (optimum pH 7.0) and in a temperature range of 20–50°C (optimum 45°C). On the basis of 16S rRNA gene sequence analysis, strain EF45031<sup>T</sup> was closely related to *Brachybacterium nesterenkovii* CIP 104813<sup>T</sup> (97.71%), *Brachybacterium huguangmaarense* M1<sup>T</sup> (97.43%), *Brachybacterium phenoliresistens* phenol-A<sup>T</sup> (97.12%), *Brachybacterium zhongshanense* JB<sup>T</sup> (97.10%), *Brachybacterium sacelli* LMG 20345<sup>T</sup> (97.09%), *Brachybacterium squillarum* M-6-3<sup>T</sup> (97.01%), and *Brachybacterium rhamnosum* LMG 19848<sup>T</sup> (96.94%). The DNA G+C content of the strain was 70.95 mol%. On the bases of chemotaxonomic, phenotypic and genotypic data, strain EF45031<sup>T</sup> represents a novel species of the genus *Brachybacterium*, for which the name *Brachybacterium sillae* sp. nov. is proposed.

[This work was supported by National Research Foundation of Korea (NRF) grant (2020R1F1A1076624), the Technology Innovation Program (20015807) funded by the Ministry of Trade, Industry & Energy, and by grants from the Ministry of Ocean and Fisheries (PM62830)]

#### A066

# *Flavobacterium rhizosphaerae* sp. nov., a Novel Plant Growth-promoting Bacterium from Biofilm of Aquatic Plants

Hyun-Soo Roh and Song-Ih Han<sup>\*</sup> Department of Microbial Biotechnology, Mokwon University

The Two hundred-fifteen strains were isolated from Aquatic Plant Biofilms (*Potamogeton distinctus*, *Nelumbo nucifera*, *Trapa natans*) in the Bukhan River. In the course of screening for new bioactive compounds from bacteria, 14 strains that appeared to have extracellular enzymes (protease, amylase, cellulose, *θ*-glucosidase) production was selected for further studies. These isolates were confirmed as plant-growth promoting activity (siderophores, N2-fixing, IAA production, phosphorus solubilization). Based on the analysis of the 16S rRNA gene sequence, extracellular enzyme-producing and plant growth promoting bacteria were assigned to the genus *Chryseobacterium* (1 isolate), *Arthrobacter* (3 isolates), *Pelomonas* (2 isolates), *Sphingopyxis* (1 isolate), *Rhodobacter* (1 isolate), *Terrabacter* (1 isolate), *Phycicoccus* (1 isolate), *Flavobacterium* (2 isolates), and *Janthinobacterium* (1 isolate). During a course of study, a novel bacterial strain was isolated and classified in the *Flavobacterium*. In this study, we have characterized one of these isolates, strain IMH1S 5-3. The data obtained in this study suggest that the isolate represents a novel species of the genus *Flavobacterium*, and the name *Flavobacterium rhizosphaerae* sp. nov. is proposed.

#### Microbacterium neungamense sp. nov. Isolated from Hot Spring

Ji U Im<sup>1</sup>, Dariimaa Ganbat<sup>1</sup>, Joo Young Yang<sup>1</sup>, Min-Kyeong Kim<sup>1</sup>, Dong-Woo Lee<sup>2</sup>, Seong-Bo Kim<sup>3</sup>, Yong-Jik Lee<sup>4</sup>, Jung-Sook Lee<sup>5</sup>, and Sang-Jae Lee<sup>1\*</sup>

<sup>1</sup>Department of Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University, <sup>2</sup>Department of Biotechnology, Yonsei University, <sup>3</sup>Bio-Living Engineering Major, Global Leaders College, Yonsei University, <sup>4</sup>Department of Bio-Cosmetics, Seowon University, <sup>5</sup>Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology

Gram-positive, nonmotile, rod-shaped bacterium, designated EF45044<sup>T</sup>, was isolated from a hot spring in Chungju, Republic of Korea. Strain EF45044<sup>T</sup> contained menaquinone-12 and menaquinone-13 (MK-12, MK-13) as the predominant respiratory lipoquinone and C<sub>17:0</sub> anteiso as the major fatty acid. A neighbor-joining tree based on 16S rRNA gene sequences showed that the isolate falls within the evolutionary radiation enclosed by the genus *Microbacterium*. Strain EF45044<sup>T</sup> showed the highest 16S rRNA gene sequence similarities with *Microbacterium ketosireducens* DSM 1251<sup>T</sup> (98.2%). The DNA G + C content of strain EF45044<sup>T</sup> was 71.4 mol%. On the basis of phenotypic, phylogenetic, chemotaxonomic data, strain EF45044<sup>T</sup> (= KCTC 49703<sup>T</sup>) presents a novel species of the genus *Microbacterium*, for which the name *Microbacterium neungamense* sp. nov. is proposed. [This work was supported by National Research Foundation of Korea (NRF) grant (2020R1F1A1076624), the Technology Innovation Program (20015807) funded by the Ministry of Trade, Industry & Energy, and by grants from the Ministry of Ocean and Fisheries (PM62830).]

#### A068

#### Characterization of Thermophilic Bacteria Isolated from Hot Springs in South Korea

Dariimaa Ganbat<sup>1</sup>, Bo Hyeon Park<sup>1</sup>, Min-Kyeong Kim<sup>1</sup>, Sondor Ganbat<sup>1</sup>, Dong-Woo Lee<sup>2</sup>, Seong-Bo Kim<sup>3</sup>, Sung Haeng Lee<sup>4</sup>, Yong-Jik Lee<sup>5</sup>, and Sang-Jae Lee<sup>1\*</sup>

<sup>1</sup>Department of Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University, <sup>2</sup>Department of Biotechnology, Yonsei University, <sup>3</sup>Bio-Living Engineering Major, Global Leaders College, Yonsei University, <sup>4</sup>Department of Cellular and Molecular Medicine, Chosun University School of Medicine, <sup>5</sup>Department of Bio-Cosmetics, Seowon University

Thermophiles that produce extracellular hydrolases are of great importance due to their applications in various industries. Thermophilic enzymes are of interest for industrial applications due to their compatibility with industrial processes and the availability of the organisms is essential to developing their full potential. In this study, characterization, taxonomic identification, and extracellular hydrolase (amylase, lipase, and protease) activity of 32 thermophilic bacterial isolates from Neungam carbonate, Mungang sulfur, Deokgu, Baegam, and Dongnae hot springs were investigated. Identification based on 16S rRNA gene sequence revealed that strains belonged to phylum *Bacillota* and were divided into *Aeribacillus, Bacillus, Caldibacillus, Geobacillus,* and *Thermoactinomyces* genera. It was found that 26 isolates could produce at least one extracellular enzyme. *Geobacillus,* representing 46.9% of the isolates, was the most abundant. The highest amount of proteolytic and lipolytic enzymes were secreted by strains of the genus *Geobacillus,* whereas *Caldibacillus* species produced the highest amount of amylolytic enzyme. The *Geobacillus* species producing hydrolytic extracellular enzymes appeared to be the most promising.

[This work was supported by National Research Foundation of Korea (NRF) grant (2020R1F1A1076624), the Technology Innovation Program (20015807) funded by the Ministry of Trade, Industry & Energy, and by grants from the Ministry of Ocean and Fisheries (PM62830).]

#### Description of Arthrobacter humidisoli sp. nov., Isolated from Riverside Soil

Jeong Mu Shin and Seung Bum Kim<sup>\*</sup>

Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University

An actinobacterial strain, designated MMS18-M83<sup>T</sup>, was isolated from riverside soil and analyzed by polyphasic taxonomy. Based on 16S rRNA gene sequence similarity, strain MMS18-M83<sup>T</sup> was closely related to species of the genus *Arthrobacter*. The most closely related strains to MMS18-M83<sup>T</sup> were *Arthrobacter bambusae* GM18<sup>T</sup> (98.27%), *Arthrobacter gyeryongensis* DCY72<sup>T</sup> (98.19%), and *Arthrobacter ramosus* CCM 1646<sup>T</sup> (97.98%). The strain showed an optimal growth in nutrient agar with pH 8 at 25–30°C and 0% of NaCl. Strain MMS18-M83<sup>T</sup> showed hydrolytic activity of casein, hypoxanthine and tyrosine. Strain MMS18-M83<sup>T</sup> possessed chemotaxonomic properties consistent with those of members of the genus *Arthrobacter*, including lysine as cell wall diamino acid, MK-9 as major quinone and anteiso-C<sub>15:0</sub> and anteiso-C<sub>17:0</sub> as major cellular fatty acids. The polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, unidentified glycolipids and unidentified aminolipid. The results of characterization enabled the differentiation of strain MMS18-M83<sup>T</sup> from other species of the genus *Arthrobacter*, for which the name *Arthrobacter humidisoli* sp. nov. is proposed (type strain = MMS18-M83<sup>T</sup>). [This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE), and also by the Basic Science Research Program of the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2020R1F1A1076440)]

#### A070

**Re-identification of** *Colletotrichum* Species in Korea Based on Multi-locus Genetic Sequence Typing Hyorim Choi, Yunhee Choi, Anbazhagan Mageswari, and Seung-Beom Hong<sup>\*</sup> *Korean Agricultural Culture Collection, Agricultural Microbiology Division, National Institute of Agricultural Science, Rural Development Administration* 

The genus *Colletotrichum* hosts pathogenic fungi that causes anthracnose, often leading to huge agricultural losses in a wide variety of plants. Researchers have isolated and studied a number of *Colletotrichum* spp. from Korea and have deposited them in the Korean Agricultural Culture Collection (KACC). Currently, a total of 252 different strains of *Colletotrichum* are preserved in KACC. To stay update with the progress in fungal taxonomy, the KACC *Colletotrichum* strains need to be re-confirmed of their taxonomic position or be re-identified with respect to the changing trends. When morphology based identification has been conventionally used for fungal identification in past, current methods include combining gene sequencing based phylogenetic analysis. In the current study, reidentification of *Colletotrichum* strains from KACC was carried by sequencing and phylogenetic analyses of their ITS, *6-tublin* and *GAPDH* genes. A total of 251 strains were studied for their ITS, *6-tublin* and *GAPDH* sequences and 75 strains were transferred from former species complex (gloeosporioides, acutatum, obiculare, dematium etc.) to new complex (acutatum, destructivum, orchidearum, boninense etc.), followed by 49 strains being re-identified at species level. Our results help us to stay update with the current fungal taxonomic standings. [Supported by grants from National Institute of Agricultural Sciences, Rural Development Administration, Republic

of Korea (PJ0135492022)]

#### Description of Microcella daejeonensis sp. nov., Isolated from Riverside Soil

Katya Molina and Seung Bum Kim\*

Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University

A novel Gram-positive, yellow-pigmented, aerobic and catalase-positive bacterial strain designated MMS21-STM12<sup>T</sup>, was isolated from riverside soil. The optimal growth was confirmed in nutrient agar after 3 days at 30°C, pH 8 and with 0% NaCl. The diagnostic polar lipids were diphosphatidylglycerol phosphatidylglycerol, and three unidentified glycolipids were also present. Major fatty acids were anteiso- $C_{15:0}$  and iso- $C_{16:0}$ . The genome of MMS21-STM12<sup>T</sup> was 2.72 Mb, and the DNA G + C content was 72.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences and the digital DNA-DNA hybridization indicated that strain MMS21-STM12<sup>T</sup> shared the highest similarity of 98.34% with *Microcella indicus* CTD02-10-2<sup>T</sup> and 39.5% with *Microcella flavibacter*, which were below the suggested cutoff for species distinction. The phenotypic characteristics also differentiated the strain from related species, and a new species, *Microcella daejeonensis* sp. nov. is now proposed (type strain = MMS21-STM12<sup>T</sup> = KCTC 49750<sup>T</sup> = LMG 32523<sup>T</sup>).

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## A072

## Diversity of Mushroom-forming Saprotrophic Fungi in Mudeungsan National Park

Minseo Cho<sup>1</sup>, Sun Lul Kwon<sup>1</sup>, Yeonjae Yoo<sup>1</sup>, Chul-Hwan Kim<sup>1</sup>, Sang Hyun Lee<sup>1</sup>, Young Min Lee<sup>1</sup>, Changmu Kim<sup>2</sup>, and Jae-Jin Kim<sup>1\*</sup>

<sup>1</sup>Division of Environmental Science & Ecological Engineering, College of Life Sciences & Biotechnology, Korea University, <sup>2</sup>Microorganism Resources Division, National Institute of Biological Resources

Saprotrophic fungi are important decomposers in nature. They decompose organic matters such as leaf litters and woody debris, which contribute to nutrient cycling. Saprotrophic fungi are mainly divided into microfungi and mushroom-forming fungi. The microfungi are well studied due to its industrial uses. However, mushroom-forming saprotrophic fungi (MFSF) got less attention than microfungi. MFSF were collected in Mudeungsan National Park from May 2020 to July 2022. The collected specimens were identified based on morphological, molecular, and phylogenetic approach. Internal transcribe spacer (ITS) region and nuclear large subunit rRNA (LSU) region were used for molecular analyses. As a result, 62 specimens were collected, and 43 species, 22 genus, 13 family, and four orders were identified. The most collected species was *Gymnopus bicolor*. In the genus level, many species belonged to *Collybiopsis, Entoloma, Gymnopus, Marasmius,* and *Mycena*. Only one species (*Leotia lubrica*) belonged to ascomycetes, and the other species belonged to basidiomycetes. Among a total of 62 specimens, 45% of the specimens were occurred on woody debris, 29% on leaf litters, and 26% on soil. In this study, we provided macro- and microscopic figures with phylogenetic trees to support four species as new to Korea. This is the first study which analysed the diversity of MFSF in Mudeungsan National Park, so further researches are required to better understand the diversity.

[Supported by grants from NIBR.]

#### Cutibacterium equinum sp. nov., Isolated from Horse Feces

Jeong Ui Yun, Hye Su Jung, Mi-Ja Jung, Hye Seon Song, Yeon Bee Kim, Yujin Kim, Tae Woong Whon, and Se Hee Lee<sup>\*</sup> Kimchi Functionality Research Group, World Institute of Kimchi

Two Gram-stain-positive, creamy white, and anaerobic bacteria, designated strains CBA3107<sup>T</sup> and CBA3108, were isolated from horse feces. Both strains were non-flagellated rods with non-motility and grew at 25–45°C (optimum, 35°C) and pH 6.0–7.0 without NaCl. Menaquinone-9 was detected from both strains. The major fatty acids of strains CBA3107<sup>T</sup> and CBA3108 were iso-C<sub>15:0</sub> and iso-C<sub>15:0</sub> DMA. The major polar lipids of both strains were diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylcholine. Strains CBA3107<sup>T</sup> and CBA3108 shared 99.79% of the 16S rRNA gene sequence similarity, indicating that the strains belong to one species. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains CBA3107<sup>T</sup> and CBA3108 together formed a distinct phylogenetic lineage within the genus *Cutibacterium; C. avidum* ATCC 25577<sup>T</sup> was the most closely related strain with strains CBA3107<sup>T</sup> (98.13%) and CBA3108 represent a novel species of the genus *Cutibacterium*, for which the name *Cutibacterium* equinum sp. nov. is proposed.

## A074

## Weissella fermenti sp. nov., Isolated from Fermented Vegetables

Jae Kyeong Lee<sup>1</sup>, Jeong Min Kim<sup>1</sup>, Se Hee Lee<sup>2</sup>, and Che Ok Jeon<sup>1\*</sup> <sup>1</sup>Department of Life Science, Chung-Ang University, <sup>2</sup>Microbiology and Functionality Research Group, World Institute of Kimchi

Two Gram-stain-positive and facultatively anaerobic bacterial strains, designated KCKM0189<sup>T</sup> and LMG11983, were isolated from fermented cabbage (kimchi) and grass silage, respectively. Both strains grew optimally at 30°C, pH 7, and in the presence of 1% (w/v) NaCl. The major polar lipids of strains KCKM0189<sup>T</sup> and LMG11983 consisted of phosphatidylglycerol, an unidentified aminolipid, two unidentified phospholipids, and seven unidentified lipids. The genomic DNA G+C contents of both strains KCKM0189<sup>T</sup> and LMG11983 were 44.7%. Strains KCKM0189<sup>T</sup> and LMG11983 shared 100% 16S rRNA gene sequence similarity, 99.9% average nucleotide identity (ANI), and 99.7% digital DNA-DNA hybridization (DDH) values, indicating that they are the same species Phylogenetic analysis based on 16S rRNA gene sequences revealed that both strains formed a phylogenetic lineage with *Weissella confusa* KACC11841<sup>T</sup> with a 100% 16S rRNA gene sequence. Both strains KCKM0189<sup>T</sup> and LMG11983 were members of the genus *Weissella* and were most closely related to *Weissella confusa* KACC11841<sup>T</sup>, *Weissella cibaria* JCM12495<sup>T</sup>, *Weissella viridescens* DSM20410<sup>T</sup>, and *Weissella muntiaci* 8H-2<sup>T</sup> with 92.3, 78.4, 70.8, and 70.5% ANI values, respectively. Based on phenotypic, chemotaxonomic, and molecular properties, strains KCKM0189<sup>T</sup> and LMG11983 represent a novel species of the genus *Weissella*, for which the name *Weissella fermenti* sp. nov. is proposed. The type strain is KCKM0189<sup>T</sup>.

[Supported by the grants from RDA]

#### Salinimicrobium tongyeongense sp. nov., Isolated from Seawater

KwangSoo Jung<sup>1</sup>, Dariimaa Ganbat<sup>1</sup>, Bo Hyeon Park<sup>1</sup>, Hae Rang Lee<sup>1</sup>, Dong-Woo Lee<sup>2</sup>, Seong-Bo Kim<sup>3</sup>, Yong-Jik Lee<sup>4</sup>, Jung-Sook Lee<sup>5</sup>, and Sang-Jae Lee<sup>1\*</sup>

<sup>1</sup>Department of Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University, <sup>2</sup>Department of Biotechnology, Yonsei University, <sup>3</sup>Bio-Living Engineering Major, Global Leaders College, Yonsei University, <sup>4</sup>Department of Bio-Cosmetics, Seowon University, <sup>5</sup>Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology

A novel Gram-negative, non-motile, and moderately halophilic rod-shaped bacterium HN-2-9-2<sup>T</sup> was isolated from seawater in Tongyeong, South Korea. The strain was able to grow at concentrations of 0.5-7% (w/v) NaCl (optimum 3% NaCl), at pH 5.5–8.5 (optimum pH 7.0–7.5) and in a temperature range of 18–45°C (optimum 37°C). On the basis of 16S rRNA gene sequence analysis, strain HN-2-9-2 was closely related to *Salinimicrobium xinjiangense* BH206<sup>T</sup> (98.22%), *Salinimicrobium terrae* YIM C338<sup>T</sup> (97.70%), *Salinimicrobium soli* CAU 1287<sup>T</sup> (97.16%), *Salinimicrobium sediminis* CGMCC 1.12641<sup>T</sup> (96.95%), *Salinimicrobium gaetbulicola* BB-My20<sup>T</sup> (96.54%), *Salinimicrobium marinum* KMM 6270<sup>T</sup> (96.12%), *Salinimicrobium flavum* X7<sup>T</sup> (95.29%) and *Salinimicrobium catena* HY1<sup>T</sup> (94.81%). The DNA G+C content of the strain was 43.01 mol%. Strain HN-2-9-2<sup>T</sup> contained MK-6 as the predominant respiratory quinone and iso-C<sub>15:0</sub> as a major fatty acid. Phosphatidylethanolamine was detected as a major polar lipid. Based on the polyphasic data, strain HN-2-9-2<sup>T</sup> (= KCTC 82934<sup>T</sup>) presents a novel species of the genus *Salinimicrobium*, for which the name *Salinimicrobium tongyeongense* sp. nov. is proposed. [This work was supported by National Research Foundation of Korea (NRF) grant (2020R1F1A1076624), the Technology Innovation Program (20015807) funded by the Ministry of Trade, Industry & Energy, and by grants from the Ministry of Ocean and Fisheries (PM62830)]

## A076

# Peptoniphilus faecicola sp. nov. Isolated from Faeces of Korean Cow

Seung-Hyeon Choi, Jam-Eon Park, Gyeong Bin Cho, and Seung-Hwan Park<sup>\*</sup> Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology (KRIBB)

A novel bacterial isolate designated as strain AGMB12026<sup>T</sup> was isolated from faeces of Korean cow received from the National Institute of Animal Science (Wanju, Republic of Korea). The bacterium was an obligately anaerobic, Gram-stain-positive, coccoid-shaped. Flagella weren't observed. Growth occurred between 20–40°C (temperature optimum of 35°C), at pH 7–9 (pH optimum of 8), and in the presence of 0.5–4.0% (w/v) NaCl. Based on the 16S rRNA gene sequence analysis, the strain belonged to the genus *Peptoniphilus* and was most closely related to *P. ivorii* DSM 10022<sup>T</sup> (= KCTC 15407<sup>T</sup>, similarity, 93.0%). The DNA G + C content was 37.6 mol%, determined by the whole-genome sequence. The average nucleotide identity value between strain AGMB12026<sup>T</sup> and *P. ivorii* DSM 10022<sup>T</sup> was 65.03%. The dDDH and AAI values between AGMB12026<sup>T</sup> and *P. ivorii* DSM 10022<sup>T</sup> were 26.5 [24.2–29.0%] and 58.65%, respectively. The major cellular fatty acids (> 10%) of strain AGMB01083<sup>T</sup> were C<sub>16:0</sub>, C<sub>18:2</sub> *cis* 9, 12, and C<sub>14:0</sub>. Based on the phylogenetic, phenotypic, biochemical, chemotaxonomic, and genomic characteristics, strain AGMB12026<sup>T</sup> is proposed to be a novel species, named *Peptoniphilus faecicola*, in the genus *Peptoniphilus*. The type strain is AGMB12026<sup>T</sup> (= KCTC 25270<sup>T</sup> = GDMCC 1.2726<sup>T</sup>).

[Supported by grants from the Ministry of Trade, Industry & Energy (MOTIE, Korea)]

# *Roseicyclus maritimus* sp. nov. and *Roseicyclus halotolerans* sp. nov., Two Novel Species Isolated from Seawater Heeyoung Kang<sup>1</sup>, Haneul Kim<sup>2</sup>, and Kiseong Joh<sup>2\*</sup>

<sup>1</sup>Division of Microbiology, Honam National Institute of Biological Resources, <sup>2</sup>Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

Two halotolerant bacteria, designated as HME9570<sup>T</sup> and HME9693<sup>T</sup>, were isolated from seawater of the Yellow Sea, Republic of Korea. They were Gram-stain-negative, strictly aerobic, non-motile, rod-shaped. Phylogenetic analyses based on 16S rRNA gene and 92 core genes sequences indicated that both strains belonged to the genus *Roseicyclus*. The 16S rRNA gene sequence similarity of strains HME9570<sup>T</sup> and HME9693<sup>T</sup> showed the highest similarity with *Roseibacterium elongatum* OCh 323<sup>T</sup> (96.5 and 95.3%, respectively) and *Roseibacterium persicicum* KMU-115<sup>T</sup> (96.5 and 95.9%), and shared 98.4% sequence similarity with each other. The average nucleotide identity and the DNA-DNA hybridization estimate values for strain HME9570<sup>T</sup> and HME9693<sup>T</sup> with their related type strains were below the respective threshold for species differentiation. Both strains shared common chemotaxonomic characteristics comprising MK-6 as the main isoprenoid quinone, summed feature 8 ( $C_{18:1}\omega7c$  and/or  $C_{18:1}\omega6c$ ) and iso- $C_{16:0}$  as the predominant fatty acids, phosphatidylglycerol as the principal polar lipid. The DNA G+C contents of strains HME9570<sup>T</sup> and HME9693<sup>T</sup> were 65.26 and 66.56 mol%, respectively. Based on phenotypic, genotypic and chemotaxonomic characteristics, two novel species are proposed, *Roseicyclus maritimus* sp. nov. with HME9570<sup>T</sup> (= KCTC 82472<sup>T</sup> = MCCC 1K06529<sup>T</sup>) and *Roseicyclus halotolerans* sp. nov. with HME9693<sup>T</sup> (= KACC 17691<sup>T</sup> = CECT 8459<sup>T</sup>) as the type strains.

#### A078

# Effects of Exposure to Whole-body 900 MHz CDMA Cell Phone Signal on Microbiome Composition in Hsd Sprague Dawley SD Rats; Preliminary Results

HyeSun Kim<sup>1</sup>, Yong-Bum Kim<sup>2</sup>, Kang-Hyun Han<sup>2</sup>, Sang Bong Jeon<sup>3</sup>, Hyung Do Choi<sup>3</sup>, Ae-Kyoung Lee<sup>3</sup>, and Young Hwan Ahn<sup>1\*</sup>

## <sup>1</sup>Ajou University School of Medicine, <sup>2</sup>Korea Institute of Toxicology, <sup>3</sup>Electronics and Telecommunications Research Institute (ETRI)

Microbiome composition is known to be important to physiologic and pathologic conditions and is influenced by a variety of environmental factors. In this study, we studied whether exposure to RFR can cause an alteration of microbiome composition in rats. A reverberation chamber system (KIT, Korea) was used. Male Sprague-Dawley rats (n = 30/group) were divided into cage-control, sham-exposed, and RF-exposed groups. Rats were exposed to 900 MHz CDMA RFR at a whole-body specific absorption rate of 0 (sham-exposed) and 4 W/kg (RF-exposed) for 225 days. Daily exposures occurred throughout 18 h and 20 min with continuous cycling of 10 min on and 10 min off. Fresh fecal samples were harvested. 16S rRNA gene-based microbiome taxonomic profiling was conducted to examine the microbiome compositional alteration. The higher  $\alpha$ -diversity indices including the Cho1 and Shannon indice were observed in the RF-exposure group. When the entire population was examined together on a PCOA plot, using both the Bray-Curtis distance, samples tended to cluster according to groups (p = 0.001). At the phylum level. *Firmicutes, Bacterodetes, Tenericutes, Actinobacteria,* and *Verruccomicrobia* were altered after exposure to RFR. We suggest that RFR exposure could be a factor influencing the microbiome composition of the animal. [This work was supported by the ICT R&D program of MSIT/IITP (2019-0-00102, A Study on Public Health and Safety in a Complex EMF Environment)].

### Hahella aquimaris sp. nov., Isolated from Aquaculture Seawater

Seung Yeol Shin, Heeyoung Kang, Yi Hyun Jeon, and Jaeho Song<sup>\*</sup> Division of Microbiology, Honam National Institute of Biological Resources

A Gram-staining-negative, aerobic, red-colored, non-gliding, motile with a single polar flagellum and rod-shaped bacterium, designated HNIBRBA332<sup>T</sup>, was isolated from a *Litopenaeus vannamei* aquaculture seawater sample in Jindo, Republic of Korea. Phylogenetic analysis based 16S rRNA gene sequences revealed that strain HNIBRBA332<sup>T</sup> belonged to the genus *Hahella* of the family *Hahellaceae* and shared 95.3–99.9% sequence similarities with *Hahella* species. Whole genome sequencing of strain HNIBRBA332<sup>T</sup> revealed genome size of 7.2 Mb and the G + C content of 53.8 mol%. The HNIBRBA332<sup>T</sup> genome shared 89.0% of average nucleotide identity and 36.6% of digital DNA-DNA hybridization values to the genome of *Hahella chejuensis* KCTC 2396<sup>T</sup>, the type species of the genus. The strain contained C<sub>17:0</sub> 10-methly, summed feature 3 (C<sub>16:1</sub>  $\omega$ 7c and/or C<sub>16:1</sub>  $\omega$ 6c), C<sub>18:1</sub>  $\omega$ 9c and C<sub>20:4</sub>  $\omega$ 6,9,12,15c as the major fatty acids and menaquinone-7 (MK-7) as the major respiratory quinone. The polar lipids detected in the strain were phosphatidylglycerol, phosphatidylethanolamine, and two unidentified aminolipids. On the basis of phylogenetic and phenotypic characteristics, strain HNIBRBA332<sup>T</sup> is considered to represent a novel species of the genus *Hahella* for which the name *Hahella aquimais* (type strain HNIBRBA332<sup>T</sup>) sp. nov., is proposed. [Supported by the research grant "Survey of Korean Indigenous Species HNIBR202101111"]

#### A080

# Isolation and Characterization of Two *Bifidobacterium longum* subsp. *infantis* Strains Which Can Decompose Sialyllactose in Human Milk Oligosaccharide

Yeong Rak Son<sup>1,2</sup>, Hana Jo<sup>1,2</sup>, Haneol Yang<sup>1</sup>, Seung Hyun Kim<sup>1</sup>, Yeon Jeong Choi<sup>1</sup>, Young Min Kim<sup>2</sup>, and Doo-Sang Park<sup>1\*</sup>

<sup>1</sup>Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>2</sup>Chonnam National University

Sialyllactose (SL) is the second most abundant component of human milk oligosaccharides, and it has resistance to intestinal hydrolysis, so its absorption rate in the small intestine is low, and it acts as a preciotics for specific intestinal microorganisms. SL are the representative sialyllactose components among HMOs and are known for their functions such as brain development, cognitive improvement, immune enhancement, and intestinal maturation. *Bifidobacterium longum* subsp. *infantis* (BI) is a representative strain that decomposes SL within the cell. For the screening of BI strains that effectively use 3'-SL and 6'-SL, growth of colony were tested against 98 BI strains isolated from infant feces on a solid medium containing 1% 3'-SL or 6'-SL as carbon source. Strains with excellent growth were primarily selected and two strains, DS2952 and DS2770 were selected which can utilize 3'-SL and 6'-SL, respectively, in a liquid media under the same condition. The DS2952 strain decompose 75% of the 3'-SL after 20 h culture in a same liquid media and the DS2770 strain decompose 95% 6'-SL after 40 h. For a probiotic characteration, antibiotic susceptibility, pH, and bile acid resistance were tested. Growth optimization experiment for industrialization of the strain showed OD<sub>600</sub>=19.4 and OD<sub>600</sub>=20.2 for DS2770 and DS2952, respectively. [This work was supported by a grant from the KRIBB Research initiative program (KGM5232221 and KGS1222221)]

# Physiological Differences between *Flavobacterium sedimentum* sp. nov. and *Flavobacterium fluvius* sp. nov., Isolated from Water Sediment

Ye Zhuo<sup>1,2</sup>, Chun-Zhi Jin<sup>1</sup>, and Hyung-Gwan Lee<sup>1,2\*</sup>

<sup>1</sup>Cell Factory Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>2</sup>Department of Biotechnology, KRIBB School of Bioscience, Korea University of Science and Technology (UST)

During a research project on screening native bacteria for the production of natural pigments, two Gram-stainnegative, non-motile, rod-shaped and aerobic strains, designated SUN046<sup>T</sup> and SUN052<sup>T</sup>, were isolated from Daechicheon, Korea. Strain SUN046<sup>T</sup> grew with 0.5–1.0% (w/v) NaCl, at 15–25°C (optimum, 20°C) and at pH 5.0–7.0 (optimum, 7.0), while strain SUN052<sup>T</sup> grew with 0.5–1.5% (w/v) NaCl, at 4–25°C (optimum, 15–20°C) and at pH 7.0– 9.0. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains SUN046<sup>T</sup> and SUN052<sup>T</sup> being grouped with members of the genus *Flavobacterium*. Strain SUN046<sup>T</sup> and SUN052<sup>T</sup> were highly related to *Flavobacterium aquatile* LMG 4008<sup>T</sup> 96.68% and 97.37%, respectively. They were considered as two novel species of genus *Flavobacterium*, for which the names *Flavobacterium sedimentum* sp. nov. (SUN046<sup>T</sup> = GenBank ON323500) and *Flavobacterium fluvius* sp. nov. (SUN052<sup>T</sup> = GenBank ON323499). Based on genome mining, strain SUN052<sup>T</sup> has carotenoid biosynthesis gene clusters for the bacterial yellow color, which could be useful for pigment industrial applications.

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## A082

# **Description of** *Pontixanthobacter jejuensis* **sp. nov., Isolated from Marine Alga** *Champia expansa* So Hyun Park<sup>1</sup>, Ji Young Kim<sup>1</sup>, and Moon Soo Heo<sup>2\*</sup>

<sup>1</sup>Research Institute for Basic Science, Jeju National University, <sup>2</sup>Department of Aquatic Life Medicine, Jeju National University

A marine bacterium, designated strain CEM42<sup>T</sup>, was isolated from marine alga *Champia expansa* collected in Jeju Island (Biyangdo), Republic of Korea. Cells of CEM42<sup>T</sup> were aerobic, Gram-negative, rod-shaped and non-motile. Strain CEM42<sup>T</sup> grew aerobically at 10–37°C (optimum, 25°C) and optimum at pH 7.0–8.0. Growth occurs in the presence of 1.0–5.0% (w/v) NaCl with an optimum 2.0% NaCl(w/v). Phylogenetic analysis based on 16S rRNA gene sequences, showed that strain CEM42<sup>T</sup> belonged to the genus *Pontixanthobacter* and was most closely related to *Pontixanthobacter aestiaquae* HDW-31<sup>T</sup>, *Pontixanthobacter aquaemixtae* JSSK-8<sup>T</sup> and *Pontixanthobacter luteolus* SW-109<sup>T</sup>. The predominant fatty acids were  $C_{18:1}\omega7c$ , summed feature3 ( $C_{16:1}\omega6c$  and/or  $C_{16:1}\omega7c$ ) and  $C_{16:0}$ . The major polar lipid included phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and sphingoglycolipid (SGL). The major respiratory quinone was Q-10. On the basis of the results of phenotypic and phylogenetic analyses, strain CEM42<sup>T</sup> represents a novel species of the genus *Pontixanthobacter*, for which the name *Pontixanthobacter jejuensis* sp. nov. is proposed. The type strain is CEM42<sup>T</sup> (KCCM 43386<sup>T</sup>).

#### Analysis of Soil Microbial Properties under Upland Soil in Gyeongnam Province

Eun-heui Han<sup>1</sup>, Dong-chun An<sup>1</sup>, Hee-jeong Je<sup>1</sup>, Jae-young Heo<sup>1</sup>, Jae-hyeok Choi<sup>1</sup>, Young-ho Chang<sup>1</sup>, and Hang-Yeon Weon<sup>2\*</sup>

<sup>1</sup>Gyeongsangnam-do Agricultural Research and Extension Services, <sup>2</sup>National Institute of Agricultural Sciences

Measuring diversity of soil microorganism was carried out on the agricultural upland in Gyeongnam area, 2021 by Rural Development Administration and Gyeongsangnam-do Agricultural Research and Extension Services according to the regulation of Act on the Promotion of Environment-friendly Agriculture and Fisheries and the Management and Support for Organic Foods. For the analysis of microbial community diversity, 25 upland soil in Gyeongnam area was investigated. The average molecular microbial mass of carbon in upland soil was 1.9 µg/g soil and the average dehydrogenase activity was 25.8 µg TPF/g soil/day. The bacteria community structure at phylum level was observed in the order of *Proteobacteria* (52.2%), *Acidobacteria* (16.9%), *Bacteroidetes* (12.2%), *Actinobacteria* (9.6%), *Firmicutes* (3.7%). The fungi community structure at phylum level was observed in the order of *Ascomycota* (65.0%), *Mortierellomycota* (25.6%), *Basidiomycota* (5.2%), *Mucoromycota* (1.4%), *Chytridiomycota* (2.2%). Bacterial species abundance estimates were Ace 5384, Chao 5095, mean Shannon 6.4, Invsimpson 157.9, and uniformity index, mean Simpson 0.041 and Shannon 0.783. Estimates of fungal species abundance were Ace 489, Chao 496, mean Shannon 3.4, Invsimpson 13.8, and uniformity index, mean Simpson 0.031 and Shannon 0.561. [This study was carried out with the support (Project No. PJ01558409) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.]

#### B002

# Comparative Analyzes of Alkane-degrading *Gordonia* and *Rhodococcus* Species Isolated from Activated Sludges Treated with Polyethylene and Polypropylene

Ha Pham, Thuy Tran Thi Dieu, and Yong-Hak Kim<sup>\*</sup> Department of Microbiology, Daegu Catholic University School of Medicine

Plastic waste increases sharply with rising plastic products of polyethylene (PE) and polypropylene (PP). We treated activated sludge samples obtained from the Yeosu petroleum industry complex of South Korea and a mixture of 23 hydrocarbon-degrading strains with low-density polyethylene (LDPE) and polypropylene (PP). In activated sludges, the dominance levels of 9 classes of the major phyla *Bacteroidetes, Proteobacteria, Firmicutes,* and *Actinobacteria* have changed greatly over time in the culture supernatants containing microplastics from the plastic films. Among isolated strains, 3 actinobacterial strains belonging to the genera *Gordonia* and *Rhodococcus* were able to utilize middle and long-chain *n*-alkanes ( $C_{16}$ - $C_{40}$ ) and branched squalane. The three isolates and a reference *R*. *rhodochrous* strain St116 grown with tryptic soy broth, ethanol, and eicosane showed no apparent differences in mycolic acid characteristics; however, when they used ethanol and eicosane, the cellular levels of tuberculostearic acid (10-methyl 18:0) significantly increased with the decrease of oleic acid (18:1  $\omega$ *9c*). The genome analysis revealed that the three strains shared the genes encoding the fatty acid methyltransferase BfaA. Further functional gene analysis shows that they all have a core gene set of extracellular MpaB oxygenases, steroid  $C_{26}$  monooxygenases, and Baeyer-Villiger monooxygenases, possibly involved in the extracellular and intracellular degradation pathway of various alkyl chains.

#### Arthrobacter alpinus BP31 from Antarctic Soil Reveals Antibiotic Resistance and Resistance to Heavy Metals

Fabian Davamani Amalraj<sup>1</sup>, Catherine Chong Sze Ling<sup>1</sup>, Stephen Ambu<sup>1</sup>, Yoke Kqueen Cheah<sup>2</sup>, Kok-Keong Chan<sup>1</sup>, Ebenezer Chitra<sup>1</sup>, Wan Loy Chu<sup>1,3</sup>, and Pete Convey<sup>4\*</sup>

<sup>1</sup>International Medical University, Kuala Lumpur, Malaysia, <sup>2</sup>University Putra Malaysia, Selangor, Malaysia, <sup>3</sup>National Antarctic Research Centre, University of Malaya, Malaysia, <sup>4</sup>British Antarctic Survey, UK

The flexibility and resilience of Antarctic strains of Arthrobacter were studied in correlation to their metabolic versatility and cold adaptation genes. A combinational approach of genomic and phenomics analyses was adopted and whole genome sequencing and BIOLOG's Phenotype Microarray (PM) analysis were carried out. Data obtained from both phenotype and whole genome sequence were analysed with DuctApe suite (v.0.17.13). This suite utilises KEGG database as well as data obtained from phenotype microarray experiment and mapped both data into the metabolic pathways. Linking both analyses enables species-specific metabolic profiles as it provides natural snapshot of the point-on-time cellular physiology of the strain BP31. This study provides insight into the genetic features and metabolic profiles of *Arthrobacter alpinus* strain BP31 isolated from Antarctica with a genome size of 4,059,912 bp. This strain tolerated salinity from 1% to 4%. Biodegradation pathways were identified for 24 substrates including 31 genes. For antibiotic resistance 37 genes were identified. About 104 genes were related to heavy metal resistance. The findings from the genomic data are highly attributed to resilience in different environmental niches.

#### B004

# Introduction to a Comprehensive Pipeline Developed for Analyzing Antibiotics Resistome and Assessing Dissemination of Antibiotics Resistance Genes

Hokyung Song<sup>1</sup> and Tatsuya Unno<sup>1,2\*</sup>

<sup>1</sup>Subtropical/Tropical Organism Gene Bank, Jeju National University, <sup>2</sup>Faculty of Biotechnology, School of Life Sciences, SARI, Jeju National University

Numerous tools have been developed for the analysis of antibiotics resistome so far, however, most of them describe only a single step in the whole analysis procedures and are not inter-connected with each other. Here, we are introducing a comprehensive pipeline which consists of bioinformatics tools and in-house scripts developed for analyzing antibiotics resistome and assessing dissemination of antibiotics resistance genes (ARGs). We downloaded publicly available metagenomic sequences obtained from fecal samples and wastewater treatment plant (WWTP)-related samples collected throughout Korea. After initial quality control, we analyzed the sequences in two tracks: 1) read-based quantitative analysis and 2) contig-based qualitative analysis. In quantitative analysis, small and large subunit rRNA genes were identified and classified. ARGs were annotated and their abundance was calculated. For qualitative analysis, sequence reads were first assembled into contigs. ARGs and mobile genetic elements in each contig were annotated. To find clues of dissemination, potential mobile ARGs were identified and compared between samples. Plasmids were also identified and typed. The pipeline developed in this study and resulting figures from examples would be useful not only for senior bioinformaticians, but also for beginners who are looking for a concise tutorial.

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# Distribution and Characteristics of *Legionella* Species Isolated from Environmental Water Systems of Public Facilities in Northern Gyeonggi Province

Ji-Hyun Lim, Chan-Mi Lee, Ah-Ra Jo, Su-Hyeon Yoo, Han-Byul Park, Da-Jung Ko, Jong Seong Son, Yong-Bae Park, Jo-Gyo Oh, and Sang-Heon Oh<sup>\*</sup>

Gyeonggi Province Research of Health & Environment Nothern Support Institute

Among *Legionella*-positive samples detected in northern Gyeonggi Province from 2017 to 2020, distribution and antibiotics susceptibility were investigated using the results of 90 facilities that confirmed *L. pneumophilla* serogroups and *Legionella* species. And 32 facilities of them were tested for antibiotic susceptibility (MICs) using E-strip. As a result of the distribution survey, a total of seven types of *Legionella* were detected in northern Gyeonggi Province, including seven serogroups of *L. pinumophilla* 1, 2, 3, 4, 5, 6, 10 and *L. anisa, L. rubrilucens, L. spiritensis, L. bozemanii* and *L. dumoffii, L. pneumophilla* accounted for 84% of the total detection, of which serogroups 1 (27%) and 5 (17%) were distributed at high rates.

We investigated the antimicrobial susceptibility (MICs) of seven types of antibiotics, including azithromycin, clarithromycin, doxycycline, erythromycin, imipenem, levofloxacin and rifampicin. The minimum inhibitory concentrations (MICs) of 7 antibiotics were determined by the gradient test using  $\alpha$ -BCYE. A high level of MIC values ( $\geq 1 \mu g/ml$ ) were observed in a total of 7 cases, including 1 in *L. phenumophilla* serogroups 1 and 3, 1 in *L. feleeii*, 3 in *L. rubrilucens*, and 1 in *L. spiritensis*. When the value was high in azithromycin, a high value of MICs was observed concurrently in erythromycin, and in one of *L. rubrilucens*, the value of clarithromycin was also high. [Supported by grants from Gyeonggi Province]

#### B006

# Seed-to-Seed Transmission and Dynamics of Rice-associated Microbial Communities

Hyun Kim<sup>1,2</sup>, Yeo Kyoung Yoon<sup>2</sup>, and Yong-Hwan Lee<sup>1,2,3\*</sup>

<sup>1</sup>Department of Agricultural Biotechnology, Seoul National University, <sup>2</sup>Research Institute of Agriculture and Life Sciences, Seoul National University, <sup>3</sup>Center for Plant Microbiome Research, Seoul National University

Vertical transmission of microbes is essential for the persistence of host-associated microbial communities. Although vertical transmission of microbes has been reported in diverse plants, knowledge of underlying ecological mechanisms and dynamics is still lacking. In this study, we reported ecological mechanism governing transmission of rice microbial communities across two seasons. We identified 29 bacterial and 34 fungal members transmitted from parent to progeny. Abundance-based regression models showed that seed-to-seed transmitted bacteria and fungi are late colonizers dominating each community at the late seed maturity stage. Ecological models further demonstrated that the observed temporal colonization patterns are affected by niche change and neutrality. Source-sink modeling revealed that parental seeds and stem endosphere are major origins of progeny seed microbes. This study will give empirical evidence for ecological mechanism and dynamics of seed microbial communities as an ecological continuum during vertical transmission.

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#### Investigating the Post-mortem Microbial Community Using 16S rRNA Gene Sequencing

Eun-Mi Hwang<sup>1</sup>, Miwha Chang<sup>2</sup>, Haeyoung Lee<sup>1</sup>, Kyu-Sik Jeong<sup>1</sup>, Min-Hee Kim<sup>1</sup>, Jung Eun Kim<sup>1</sup>, Hyeon Jin Bae<sup>1</sup>, Hyun Jung Lim<sup>1</sup>, and Chong Min Choung<sup>1\*</sup>

<sup>1</sup>Forensic DNA Division, National Forensic Service, <sup>2</sup>Division of Emerging Infectious Disease Response, Bureau of Infectious Disease Emergency Preparedness and Response, Korea Disease Control and Prevention Agency

Three years of sequencing data collection and analysis was proceeded in order to understand distribution and diversity of post-mortem microorganism in various conditions. Isolation of strains was conducted using two types of selective media. To isolate Gram positive bacteria, BAP (Blood Agar Plate) was used and MAC (MacConkey) was used for isolation of Gram-negative bacteria. Sequencing was proceeded using 'Fast MicroSeq<sup>™</sup> 500 16S rDNA PCR kit', which amplifies V1-V3 region of 16R rRNA gene. Identification of isolated strains was carried out using BLAST and MicroSEQ Microbial ID System, a database of 'Fast MicroSeq<sup>™</sup> 500 16S rDNA PCR kit'. Analyzing of collected data was done using R software v. 4.1.0. Total 406 autopsy specimens were used for microbial isolation and 827 strains were identified through 16S rRNA sequencing for three years. In order to investigate diversity of post-mortem microbial community, identified strains were analyzed according to Gram-staining, season, sampling sites, age and pathogenicity. Through this study, we provide useful information to help understand the diversity and distribution of post-mortem microbes. Furthermore, results can be used as basic data for the development of forensic microbiological applications and future post-mortem microbiological research.

[This work was supported by National Forensic Service (*NFS2022DNA01*), Ministry of the Interior and Safety, Republic of Korea.]

#### B008

# Microbiota Analysis of the Western Honeybee (*Apis mellifera* L.) Infested with the Mite Varroa destructor Reveals Altered Bacterial and Archaeal Community

Minji Kim<sup>1</sup>, Woo Jae Kim<sup>2</sup>, and Soo-Je Park<sup>1\*</sup>

<sup>1</sup>Department of Biology, Jeju National University, <sup>2</sup>Center for Life Science (HCLS), Harbin Institute of Technology

The western honeybee, *Apis mellifera* L., is a crop pollinator that makes royal jelly and other hive products. However, widespread concerns arise about opportunistic diseases (e.g., bacteria, fungi, or mites) or chemicals that have an effect on the health and number of colonies, as well as their activity. The relationships between the gut microbiota and its host are currently being researched extensively. The effects of *Varroa destructor* infestation on the gut microbial community, in particular, have received little investigation. This study utilized amplicon sequencing of the bacterial and archaeal 16S rRNA genes to assess the bacterial and archaeal communities of adult bee groups (healthy and affected by *Varroa* designed in NG and VG, respectively) and larvae from *Varroa destructor*-infested hives. Our results suggest that the genus *Bombella* was substantially dominant in larvae, while the genera *Gillamella*, unidentified *Lactobacillaceae*, and *Snodgrassella* were significantly dominant in adult bees. NG and VG, on the other hand, did not differ statistically significantly. Additionally, despite the complexity of the honeybee's bacterial community, all groups exhibited a straightforward archaeal community structure. In summary, it might be possible that the mite infestation induces the changes for the microbial community structure and function of honeybee.

[Supported by grants from the NRF of Korea (No. 2020R1I1A3062110) and Startup funds of HIT Center for Life Sciences.]

#### An Isolated Arthrobacter sp. Enhances Rice (Oryza sativa L.) Plant Growth

Inhyup Kim, Geeta Chhetri, Minchung Kang, Yoonseop So, and Taegun Seo<sup>\*</sup> Department of Life science, Dongguk University-Seoul

In this study, four bundles of root samples were collected from the same rice field located in Goyang, South Korea. A total of 504 culturable bacteria were isolated and evaluated for their plant growth-promoting abilities *in vitro*. Among them, *Arthrobacter* sp. GN70 was selected for inoculation into the rice plants under laboratory and greenhouse conditions. The results showed a significantly positive effect on shoot length, root length, fresh plant weight, and dry plant weight. Moreover, SEM images demonstrated the accumulation of bacterial biofilm networks at the junction of the primary roots, confirming the root-colonizing ability of the bacterium. The strain also exhibited a broad spectrum of in vitro antimicrobial activities against bacteria and fungi. Here, we first report the rice plant growth-promoting ability of the *Arthrobacter* species with the biofilm-producing and antimicrobial activities against plant and human pathogens. Genome analyses revealed features attributable to enhancing rice plant growth, including the genes involved in the synthesis of plant hormones, biofilm production, and secondary metabolites. This study revealed that the rhizobacteria isolated from the roots of rice plants have dual potential to be utilized as a plant growth promoter and antimicrobial agent.

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#### B010

# Protection of Cyanobloom-forming *Microcystis aeruginosa* by Extracellular Catalase-producing *Massilia aquatica* HC52

Yeji Cha, Yerim Park, and Woojun Park<sup>\*</sup> Department of Environmental Science and Ecological Engineering, Korea University

Cyanobacteria, especially *Microcystis aeruginosa*, have no catalase-encoding genes, which might be mitigated by extracellular catalase of symbiotes to defend *M. aeruginosa* from environmental stress. Among 40 symbiosis isolates, the highest concentration of O<sub>2</sub> in a cell-filtered culture was detected in *Massilia* sp. HC52 by using the microelectrode O<sub>2</sub> sensor. Extracellular catalase activity in the cell-free supernatant was observed via native PAGE. Further, two copies of katA-encoding genes were detected in extracellular protein of strain HC52 by whole genome sequencing and LC-MS-MS. Morphological changes and H<sub>2</sub>O<sub>2</sub> concentration of cell cultures were analyzed at different temperatures. Consequently, higher H<sub>2</sub>O<sub>2</sub> concentration was detected at 30°C incubated cells, which have longer cells, than 13°C cultured cells, supporting that catalase of strain HC52 could be induced at a high temperature. By unisense and PAGE analyses, extracellular catalase activity was confirmed in strain HC52 which was incubated at 30°C, not at 13°C. By co-culture experiment, we supported that releasing the extracellular catalase in strain HC52 might protect *M. aeruginosa* from H<sub>2</sub>O<sub>2</sub>. Our analysis suggested that the extracellular catalase KatA of strain HC52 might protect catalase-less *M. aeruginosa* under high temperature. [This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea [NIBR202221201]]

#### Postharvest-induced Alterations in the Phyllosphere Mycobiota of Broccoli Florets

Min-Soo Kim<sup>1</sup> and Eun-Jin Park<sup>2\*</sup>

<sup>1</sup>Department of Microbiology and Molecular Biology, Chungnam National University, <sup>2</sup>Department of Food Bioengineering, Jeju National University

The phyllosphere microbiota is important not only for enhancing crop production, but also maintaining the quality and safety of fresh vegetables. However, very little is known about changes to the fungal communities of fresh vegetables after harvest. This study explored the phyllosphere mycobiota of broccoli florets collected from 14 farms and 10 retail stores by culturing and ITS2 amplicon sequencing. Fungal populations exceeded bacterial populations in both total abundance and biodiversity, and fungal communities differed much more between preharvest and postharvest samples than bacterial communities. Both species richness and evenness were significantly higher in postharvest samples, and the *Ascomycota*-to-*Basidiomycota* ratio was significantly lower in postharvest samples. Microbial network analysis illustrated that preharvest interspecies interactions involving *Purpureocillium* were replaced with interactions involving *Sporobolomyces*, *Papiliotrema*, and *Bulleromyces* in postharvest samples, resulting in a decrease of network robustness, as corroborated by functional changes and enrichment of a postharvest pathogen. Overall, the fungal community is an important component of postharvest microbiome in the phyllosphere of fresh vegetables that has large potential impacts on fresh produce storage and spoilage.

#### B012

# Isolation and Identification of Microorganisms in Fresh Vegetables and Agricultural Environments for Antibiotic Resistance Confirmation

Inyoung Han<sup>1</sup>, Young Don Chin<sup>1</sup>, Dong-Wan Kang<sup>1</sup>, Seok min Lee<sup>1</sup>, Jae-Hyeuk Choi<sup>1</sup>, and Kwang Kyo Oh<sup>2\*</sup> <sup>1</sup>Gyeongsangnam-do Agricultural Research and Extension Services, <sup>2</sup>Microbial Safety Division, National Institute of Agricultural Science, Rural Development Administration

Antibiotic resistance refers to the ability of microorganisms to resist and survive after exposure to antibiotics. The improper use of antibiotics in agriculture and livestock contributes to the problem. There is a high risk of transmitting antibiotic-resistant bacteria to humans through consumption of food (meat and crops). Antibiotic-resistant salmonella is linked to the death of many people after consuming contaminated turkey. According to the European Centre for Disease Prevention and Control, it has been reported that the antibiotic treatment had a minor effect on antibiotic-resistant *Salmonella*. In Korea, there are many studies on the presence and absence of antibiotic-resistant food poisoning bacteria in agricultural products. However, there are few studies on microbial resistance to the antibiotics uses in crops. In current study, microbes were isolated from onion, tomato plants and agricultural antibiotics. *E. coli* was isolated from tomato, propagation cube and soil. *Klebsiella* sp., was isolated from the propagation cube, tomatoes and onions. From pool water and soil *Enterococcus* sp., and *Staphylococcus* sp., were isolated. In order to confirm the antibiotic resistance of the isolated strains disk diffusion method will be followed.

# Characterization of Chitinolytic and Antifungal Activities in *Trichoderma bissettii* Isolated from Marine Environments

Dawoon Chung, Yong-Min Kwon, Woon-Jong Yu, Seung Sub Bae, and Grace Choi<sup>\*</sup> National Marine Biodiversity Institute of Korea

*Trichoderma* species are well known for their mycoparasitic activities and chitinase production. In this study we isolated two *Trichoderma* strains from marine sponge and algae, respectively, and elucidated their chitinolytic and antifungal activities. Based on the morphological and molecular analysis, these isolates (designated GJ-Sp1 and TOP-Co8) were identified as *Trichoderma bissettii*, which is closely related species to *Trichoderma longibrachiatum*. The culture filtrate of GJ-Sp1 and TOP-Co8 showed both exochitinase (chitobiosidase and beta-N-acetylglucosaminidase) and endochitinase (chitotriosidase) activities. The maximum chitinase activity of the culture filtrate was observed at 50°C, pH 5.0, 0–0.5% NaCl concentration in both strains. In addition, the enzymatic activities were stable at 10–40°C for 2 h. When antifungal activity was examined against *Aspergillus flavus* and *Aspergillus niger*, the culture filtrate of GJ-Sj1 and TOP-Co8 inhibited hyphal growth of the *Aspergillus* spp. To the best of our knowledge, this is the first report of the chitinolytic and antifungal activity of marine-derived *T. bissettii*.

#### B014

# Culture-dependent and -independent Microbial Diversity Study of Bacteria Isolated from Domestic Honey Bee Gut

Eui-Sang Cho<sup>1</sup>, Hyun Jee Kim<sup>2</sup>, Jeong Hyeon Lee<sup>2</sup>, Chi Young Hwang<sup>1</sup>, Hyung Wook Kwon<sup>2,3</sup>, and Myung-Ji Seo<sup>1,4,5\*</sup> <sup>1</sup>Department of Bioengineering and Nano-Bioengineering, Incheon National University, <sup>2</sup>Convergence Research Center for Insect Vectors, Incheon National University, <sup>3</sup>Department of Life Sciences, Incheon National University, <sup>4</sup>Division of Bioengineering, Incheon National University, <sup>5</sup>Research Center for Bio Materials & Process Development, Incheon National University

We studied the honey bee gut microbiota with feeds including Feedbee, Megabee, sugar syrup 60% (w/v), YBNH, and Test A to monitor culture-dependent and culture-independent microbial diversity. In culture-dependent study, most isolates belonged to *Apilactobacillus* (24.1%), *Fructobacillus* (21.8%), and *Bacillus* (15.4%) at the genus level. On the other hand, culture-independent analysis revealed that *Bacillota* and *Pseudomonadota* were the most prevalent group (99%) in honey bee gut microbes, while other bacterial groups accounted for less than 0.1%. Although there was little change in the microorganisms according to the different feed intakes of honey bees at the phylum level, there was a significant difference compared to the control group and the sugar syrup 60%-fed group. The genus *Lactobacillus* was predominantly distributed in the Feedbee-fed group than in other diet-fed groups. These lactic acid bacteria may play an important role in honey bee health, protection from pathogens, support food processing such as carbohydrate metabolism, and contribute to the antibacterial properties of honey. The current results could provide basic information on the isolation and characterization of functional bacteria from the honey bee gut and the effect of pollen substitutes on the intestinal microbiota of honey bees.

[This work was carried out with the support of Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01575502).]

# A Colistin-degrading Protease from the Opportunistic Pathogen *Stenotrophomonas maltophilia* Confers Collective Resistance in Polymicrobial Infection Communities

Do-Hoon Lee<sup>1</sup>, Ju-Hee Cha<sup>1</sup>, Dae-Wi Kim<sup>1,2</sup>, Kihyun Lee<sup>1</sup>, Yong-Seok Kim<sup>1</sup>, Hyo-Young Oh<sup>3</sup>, You-Hee Cho<sup>3</sup>, and Chang-Jun Cha<sup>1\*</sup>

<sup>1</sup>Department of Systems Biotechnology and Center for Antibiotic Resistome, Chung-Ang University, <sup>2</sup>Division of Life Sciences, Jeonbuk National University, <sup>3</sup>Department of Pharmacy, College of Pharmacy and Institute of Pharmaceutical Sciences, CHA University

The increasing prevalence of resistance against the last-resort antibiotic colistin is a significant threat to global public health. Here, we discovered a novel colistin resistance mechanism via enzymatic inactivation of the drug and proposed its clinical importance in microbial communities during polymicrobial infections. A bacterial strain of the Gram-negative opportunistic pathogen *Stenotrophomonas maltophilia* capable of degrading colistin and exhibiting a high-level colistin resistance was isolated from the soil environment. A colistin-degrading protease (Cdp) was identified in this strain and its contribution to colistin resistance was demonstrated. Coculture and coinfection experiments revealed that *S. maltophilia* carrying *cdp* gene could inactivate colistin and protect otherwise susceptible *Pseudomonas aeruginosa*, which may seriously affect the clinical efficacy of the drug for the treatment of cystic fibrosis patients with polymicrobial infection. Our results suggest that Cdp should be recognized as a colistin resistance determinant that confers collective resistance at the microbial community level. Our study will provide vital information for successful clinical outcomes during the treatment of complex polymicrobial infections, particularly including *S. maltophilia* and other colistin-susceptible Gram-negative pathogens such as *P. aeruginosa*. [This work was supported by the Korea Ministry of Environment and the National Research Foundation of Korea.]

#### B016

# Beyond the Opportunistic Inhabitant: Bacteriobiome and Function of Core Taxa in Lichens Inhabiting Polar Regions

Seonghan Jang<sup>1</sup>, Hyun-Ju Noh<sup>2</sup>, Soon Gyu Hong<sup>1</sup>, Binu Mani Tripathi<sup>1</sup>, Mincheol Kim<sup>1</sup>, Seung Chul Shin<sup>1</sup>, Timofey Pankratov<sup>3</sup>, Jae Eun So<sup>1</sup>, Hyoungseok Lee<sup>1</sup>, and Yung Mi Lee<sup>1\*</sup>

<sup>1</sup>Korea Polar Research Institute, <sup>2</sup>Honam National Institute of Biological Resources, <sup>3</sup>Russia Academy of Science

Lichen has long been considered as a symbiotic association of lichen-forming fungi (mycobiont) and algae and/or cyanobacteria (photobiont). In spite of the view to expand lichen holobionts to include lichen-associated bacteria, the functions of bacteria remain poorly understood. Firstly, we investigated the bacterial communities of 174 lichen specimens of 8 genera collected from polar and subpolar regions. Proteobacteria (67.7 ± 18.2%) followed by Acidobacteria (18.9 ± 15.0%) were predominant across the lichens with the different proportion at the lower taxonomic level depending on the lichen genus. The primary driver to shape lichen bacteriobiome was mycobiont type with the relatively weak effects of other factors suggesting that a bacteriobiome of the polar lichens may have co-evolved with their host. A single operational taxonomic unit (OTU) belonging to the genus Lichenicoccus was detected in 163 specimens, indicating that its significance as a core bacterial taxon in polar lichens. Next, we cultivated 'Lichenicoccus sp. PAMC 29875' showing 99.0% 16S rRNA gene similarity with core OTU. Genomic information and physiological characteristics unveiled that PAMC 29875 is able to degrade methylamine, solubilize phosphate, synthesize amino acids, and vitamin B12. These results underpin that core bacteria may enable lichens to survive in extreme environments and thus validation of these functions in the context of lichen symbioses will be performed.

# Effects of Manures and Compost on the Microbiome and Resistome during the Cultivation of Lettuce and Cabbage

Yong-Seok Kim, Do-Hoon Lee, Kihyune Lee, Dae-Wi Kim, and Chang-Jun Cha<sup>\*</sup> Department of Systems Biotechnology and Center for Antibiotic Resistome, Chung-Ang University

Agricultural products such as raw vegetable are highlighted as important sources for the understanding of antibiotic resistance gene (ARG) flow in One-Health sectors. Manure and compost coming from antibiotic-treated domestic animals used for soil fertilization have been nominated as a major source of ARG transmission in the agricultural environment. In this study, we constructed test-beds for the cultivation of lettuce and cabbage to monitor the ARG transmission from three manures and one compost. Cattle, pig, and poultry manures and one compost product (poultry manure-based) were used for soil fertilization of each test-bed at the beginning of cultivation. Manures and compost (n = 8), control soil and fertilized soil (n = 22), and product (n = 10) samples were analyzed for their microbiomes and antibiotic resistomes using the Illumina sequencing platform. Beta-diversity analysis showed that ARG profiles were strongly associated with sample types. Most of the manure and compost samples were shown to have higher ARG abundance than the other samples. Proportion of ARGs against aminoglycoside, sulfonamide, and tetracycline antibiotics were maintained during the cultivation periods or increased in soils at product-harvesting times. Lettuce and cabbage samples had higher ARG amounts than soil samples. Our results would provide useful information to develop the antimicrobial resistance surveillance system from agricultural environments to products.

[Supported by grant from the RDA.]

#### B018

## Bacillus velezensis TSA32-1 as a Biological Control Agent for Plant Diseases

Jung-Ae Kim<sup>1,2</sup>, Jeong-Sup Song<sup>1</sup>, Pyoung II Kim<sup>1</sup>, Dae-Hyuk Kim<sup>1,2,3</sup>, and Yangseon Kim<sup>1\*</sup> <sup>1</sup>Department of Research and Development, Center for Industrialization of Agricultural and Livestock Microorganisms, <sup>2</sup>Department of Bioactive Material Science, Jeonbuk National University, <sup>3</sup>Departments of Molecular Biology and Bioactive Material Sciences, Institute for Molecular Biology and Genetics, Jeonbuk National University

The use of synthetic fungicides has caused major problems such as soil and water pollution and negatively affects non-target species. We isolated *B. velezensis* TSA32-1 from soil and identified its characteristics by sequencing its 16S rRNA. *Bacillus velezensis* TSA32-1 showed enzyme activity and antimicrobial effects against phytopathogenic fungi by inhibiting the growth of *Fusarium graminearum*, *F. fujikuroi*, *Alternatia alternate*, and *Diaporthe actinidiae*. Additionally, *B. velezensis* TSA32-1 protected against head blight disease on corn and pepper seeds caused by *F. graminearum* and decreased the occurrence and severity of diseases caused by *Pythium ultimum*. The complete genome of TSA32-1 was 4.05Mb with a G + C content of 46.3 mol% possessed the bacillaene biosynthesis cluster, surfactin synthesis cluster, which biosynthesizes the antibacterial substance lipopeptide. Surfactin and fengycin family compound, secondary metabolites known as key factors in biological control, were also detected *B. velezensis* TSA32-1 showed potential as a biocontrol agent for the controlling plant pathogens in agriculture. [This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, and Agriculture and Forestry (IPET) through Agricultural Machinery/Equipment Localization Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (122022-3).]

### **Animal Gut Microbiome Bank**

Seoung Woo Ryu<sup>1</sup>, Byeong Seob Oh<sup>1</sup>, Seung Yeob Yu<sup>1</sup>, Jeong Eun Bak<sup>1</sup>, Se Won Kang<sup>1</sup>, Seung-Hwan Park<sup>1</sup>, Mi-Kyung Lee<sup>1</sup>, Jiyoung Lee<sup>1</sup>, Hyunjung Jung<sup>2</sup>, Tai-Young Hur<sup>2</sup>, Hyeun Bum Kim<sup>3</sup>, Jae-Kyung Kim<sup>4</sup>, Ju-Hoon Lee<sup>5</sup>, and Ju Huck Lee<sup>1\*</sup>

<sup>1</sup>Korean Collection for Type Cultures, Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>National Institute of Animal Science, <sup>3</sup>Department of Animal Resources Science, Dankook University, <sup>4</sup>Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, <sup>5</sup>Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Seoul National University

As gut microbiota (GM) shown to play a fundamental role in human health, in animal population and diversity of the gut microbiota are responsible for their health including nutritional, physiological and immunological functions. In several studies, livestock including pig and cow microbiota were strongly correlated with host fitness and production along with different growth stages. However, dysbiosis leads to loss of gut barrier function, and gives rise to pathogenic infection. While metagenomics study contributed to discover the importance of GM in animal health, isolation of GM based on culture-dependent study has been recently realized more important because isolated gut microbiota needed for further study such as verification or characterization of the functions of GM, and development of probiotics and pharmabiotics. Therefore, we planned to collect large scale of GM from pigs and cows, and has performed Animal Gut Microbiome Bank (AGMB) project since 2019. Until now, we have isolated and preserved 8,714 strains comprising 524 species from 192 pigs, 224 Korean cows, and 76 dairy cow samples. Furthermore, from this year we have initiated the distribution of our isolated gut microbiota; the information of microbes can be checked in the AGMB homepage (https://www.kobic.re.kr/agmb/), and please contact to juhuck@kribb.re.kr for the distribution. We believe that our resources will help the research and development of animal gut microbiota.

[Project No. NRF-2019M3A9F3065226]

## B020

## Pathogenicity Assessment of Culturable Bacteria from the Arctic Regions

Woohyun Kim<sup>1</sup>, Seonghan Jang<sup>1</sup>, Jung Yong Yeh<sup>2</sup>, and Yung Mi Lee<sup>1\*</sup> <sup>1</sup>Korea Polar Research Institute, <sup>2</sup>Incheon National University

The Arctic is experiencing the most rapid warming by 1.9°C over the last 30 years and this increase is two to three times faster than global average. Permafrost where accounts 25% of the northern hemisphere is a huge repository of microorganisms. The thawing of permafrost by climate change has potential to release ancient pathogens and this in turn can be a threat to human health. To understand the risk of bacteria in rapid warming Arctic, we cultivated environmental samples from 5 Arctic countries and identified them by 16S rRNA sequencing. Total 274 bacterial strains of 41 genera of the 5 phyla were obtained. Among them, genome sequences of 9 strains of the genera of *Bacillus, Brevundimonas, Caulobacter, Cellulomonas, Chryseobacterium, Clavibacter, Cohnella, Rahnella* were obtained. In particular, PAMC 29434 showing 99.9% 16S rRNA gene sequence similarity with *Bacillus mycoides* included virulence factor genes such as *plcA, piplc, sph, hlyIII, NheA* and polysaccharide capsule which are involved in exotoxin secretion or immune modulation. In addition, PAMC 29434 harbored antimicrobial resistance genes such as SatA, BcII, FosB. As a further study, we will conduct virulence-associated phenotypes including temperature response, antibiotic resistance, and hemolytic activities to verify the virulence and their response to the temperature rise.

# Isolation and Characterization of a *Comamonas thiooxydans* N1 Strain able to Degrade the Fungicide Biphenyl of Control of Postharvest Agricultural Products

Gaeul Lee and Nari Lee<sup>\*</sup> Korea Food Research Institute

Biodegradation of biphenyl (BP) is an environmentally friendly and cost-effective method of purification, which has recently attracted attention. In this study, BP-degrading strains were isolated by culturing the contaminated soil of a farm in *Jeju* Island in minimal medium with BP. Five morphologically distinct single colonies were isolated by repeating the culturing process. As a result of analyzing their BP degradation and bacterial growth by HPLC and spread plating, respectively, N1 of five strains showed the best degrading ability and cell growth. As a result of whole genome sequencing, N1 was finally identified as *Comamonas thiooxydans*, and 43 genes of BP and benzoate/catechol degradation were divided located in 4 clusters, and their functions were confirmed, the degradation pathway of BP was established based on this. In addition, the activity of the enzyme involved in the biphenyl degradation pathway and the mRNA expression level of gene encoding each enzyme were analyzed using N1 culture incubated in the various carbon sources. As a result, it was confirmed that the gene involved in BP degradation were normally expressed and corresponded to the meta-cleavage pathway among the lower pathways of BP degradation.

[This research was supported by the Main Research Program of the Korea Food Research Institute (KFRI), funded by the Ministry of Science and ICT.]

#### B022

# Difference of Microbiota in Pharynx and Gut of Sea Urchin (*Mesocentrotus nudus*) According to Urchin Barren Severity in South Korea

Joon-Young Park, Jae-Won Jo, Yu-Jeong An, Jin-Jae Lee, and Bong-Soo Kim<sup>\*</sup> Department of Life Science, Multidisciplinary Genome Institute, Hallym University

The proliferation of barren has been reported in the coast of South Korea during decades. Algae-predator is a biological factors for barren. This study analyzed the microbiota in pharynx and gut of sea urchin, which is a major algae-predator, in urchin barren regions. Sea urchins (*Mesocentrotus nudus*) were collected from 5 mild- (n = 30) and 3 severe-barren regions (n = 19). Microbiota in sea urchin (n = 98) and their habitat samples (sand and seawater) were analyzed using Illumina MiSeq system. The microbiota and their predicted functions were significantly different between pharynx and gut as well as between sea urchin and habitat (p < 0.05). The microbiota in sea urchins was different according to regions, and this was related to seawater temperature, which caused differences of algae composition in each region (p < 0.0001). The differences of microbiota between mildand severe-barren regions were 14 and different pathways were 11 in the pharynx, and 12 genera and 11 pathways were different in the gut. In conclusion, the microbiota in pharynx and gut was different, and the difference of microbiota between mild and severe barren regions could be due to the limited feeding algae in severe regions. [Grant number-20210469 & 2021R166A1A03044501]

#### **Isolation, Identification and Characterization of Various Myxobacteria for the Production of Algicidal Agents** Jun Yeong Park and Bongsoo Lee<sup>\*</sup>

Department of Microbiology and Biotechnology, Mokwon University

Algal bloom is a common phenomenon in reservoir and lake freshwater all over the world, but these are increasingly endangering human health due to their widespread toxicity, odor, high biomass productivity and human use of freshwaters. In an effort to identify a specific algicidal agent, we isolated several myxobacteria which known as the superior producers of bioacttive compunds such as secondary metabolites and emzymes. Among 23 myxobacteria strains, we finally isolated four myxobacteria stranis which have negative effects on the growth of dominant algal blooms cyanobaceria including *Microcystis* sp., *Anabaena* sp., and *Aphanizomenon* sp. To identify myxobacteria derived algicidal agents, we next prepared myxobacteria extracts and treated to cyanobacteria through the disc diffusion assay and broth culture, repectively. The results showed that various myxobacteria have the potential algicidal effects. We are in the porcess of evaluating 25 mutant lines that disrupted the secondary metabolite genes in *M. stipitatus* in order to find which genes affect algicidal effects.

#### B024

# Influences of Rice Roots on Methanogenic and Methanotrophic Communities in Micro-scale Eun Ryul Oh and Hyo Jung Lee<sup>\*</sup>

Department of Biology, Kunsan National University

Methane (CH<sub>4</sub>) is the second-most abundant greenhouse gas. To investigate influences of rice root on methanogens and methanotrophs, nylon mesh with a pore size of 100  $\mu$ m used to separate roots from microbial habitats. The root exudates including oxygen can release through nylon mesh, but eukaryotic roots were isolated in rhizosphere. Changes of microbial communities and abundances were investigated in Wagner pots (1/2,000 size) transplanted with Korean rice cultivar Dongjinbyeo (Oryza sativa, Japonica type) using amplicon sequencing of 16S rRNA gene and quantitative PCR, respectively. Horizontal 5.4 mm soil from nylon mesh including rice roots was sampled and sectioned at 30, 50, and 100  $\mu$ m thickness using a microtome. Microbial community analysis showed that the phyla Proteobacteria, Fermicutes, Chloroflexi, Acidobacteria, and Actinobacteria were dominant in all soils. Three parts, middle, low, and high, were divided by alpha-diversity indexes, about 1 mm, 1–1.5 mm, and 1.5–5.4 mm far from roots. Relative abundances of bacteria and archaea were also clustered into the three parts. The changes of methanogen and methanotroph and their abundances will be discussed in detail.

#### The Phyllosphere Virome of Fresh Vegetables

Ji-Woo Park<sup>†</sup>, Yeo-Eun Yun<sup>†</sup>, and Min-Soo Kim<sup>\*</sup> Department of Microbiology and Molecular Biology, Chungnam National University

Fresh vegetables harbor many different types of microorganisms. Metagenomics studies consistently showed that viruses, mostly bacteriophages, are the most abundant biological entities and are important for modulating microorganisms. Due to difficulty in obtaining sufficient viral biomass in a sample, researches on the phyllosphere virome are lacking. Here, we collected 44 vegetable samples, and characterized the structure and composition of phyllosphere virome in fresh vegetables using virus-like particles (VLPs) enrichment and high-throughput Illumina sequencing. On average,  $6.5 \pm 1.4 \log$  VLPs per gram of leaf were estimated in six vegetables, and no difference in total viral abundance was observed among the vegetables. Unlike other environments where viruses outnumber bacteria, total abundance of viruses was similar to that of bacteria. Average  $267 \pm 506$  viral operational taxonomic units (vOTUs) were identified per vegetable, and were specific to each of six vegetable. The composition of the virome differed among produce types, and varied temporally within each produce type. This study expands our knowledge on viral ecology in the phyllosphere of fresh vegetables.

#### B026

# **The Temporal Variation in the Phyllosphere Fungal and Bacterial Microbiota of Retail Lettuce** Sua In and Min-Soo Kim<sup>\*</sup>

Department of Microbiology and Molecular Biology, Chungnam National University

The phyllosphere provides for a tremendous diversity of microbial populations, and the phyllosphere microbiota have the potential to influence crop production and maintenance, but it is poorly understood. Using culturing and amplicon sequencing of 16S rRNA and ITS2 region, this study investigated the composition and structure of bacterial and fungal communities present on the surfaces of green and red lettuces which were monthly obtained from three different retail stores. Bacterial community were dominated by the species of *Proteobacteria*, *Firmicutes*, and *Actinobacteria*. Fungal community was dominated by *Ascomycota* and *Basidiomycota*. Bacterial communities differed between produce types, and fungal communities temporally changed. Our results demonstrated that retail lettuces harbored diverse and abundant bacterial and fungal populations, and we are regularly exposed to different microorganisms from fresh vegetables.

#### Influence of Culture Temperature on the Isolation of Endolichenic Fungi

Yehyeon Cha, Tae Jeong Moon, Eun Woo Ryu, Chae Eun Seo, Myung Jun Son, Yunhyeok Jang, and Seung-Yoon Oh<sup>\*</sup> Department of Biology and Chemistry, Changwon National University

Lichens are symbionts of fungi and algae. Fungi that live symbiotically in lichens and do not show disease symptoms are called endolichenic fungi. It has high biological, medical, and industrial values. In East Asia including South Korea, however, there is a lack of research on the diversity and characteristics of endolichenic fungi. In this study, we isolated endolichenic fungi from lichen specimens collected in the Changwon, and compared their diversity between culture temperatures. A total of 113 species of endolichenic fungi were identified, among which *Daldinia childiae* was the most isolated. In addition, the highest number of strains were isolated at 25°C, and taxonomic compositions were different between culture temperatures. Therefore, it is recommended to use various incubation temperature conditions to isolate diverse endolichenic fungal species.

[This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT) (NRF-2021R1C1C1008045).]

#### B028

# Antibiotic Resistance-susceptibility Profiles and Genome Analysis of Acquired Resistances of *Enterococcus* spp. Isolated from the Environment and Crops

Soo Jeong Lee, Joon-Gi Kwon, Ji-Soo Lee, and Ju-Hoon Lee\*

Department of Agricultural Biotechnology, Department of Food and Animal Biotechnology, Center for Food and Bioconvergence, Research Institute of Agriculture and Life Science, Seoul National University

Antibiotic resistance is a major health problem that is rapidly spreading worldwide. *Enterococcus* spp. are Grampositive and non-spore forming bacteria which tend to acquire and disseminate antibiotic resistance genes. In this study, 27 *Enterococcus* spp. were isolated from 4 crops, 3 agricultural waters and 2 sewer near hospital. And then, eleven *Enterococcus* spp strains were selected through multidrug resistance test of 14 antibiotics. MIC tests on 16 antibiotics were conducted on 11 screening strains. *E. faecalis* EN010 isolated from cucumber showed the highest Minimum inhibitory concentrations value in 4 antibiotics (Clindamycin, Erythromycin, Minocycline, Tetracycline). Complete genome analysis of *E. faecalis* EN010 was conducted using Nanopore technique and confirmed that *ermB* (erythromycin resistance gene), *Isa(E)* (clindamycin resistance gene) and ANT(6)-Ia (aminoglycoside antibiotics resistance gene) exist between the IS6 family. After that, we downloaded all *E. faecalis* complete genome sequences from the NCBI to compare the IS6 family sequence with other strains, confirming that 15 *E. faecalis* strains include IS6 family region and 4 strains have similar IS6 family sequence with *E. faecalis* and provide the genetic information of their acquired resistance.

# Unraveling Carbohydrate Metabolic Features of *Leuconostoc mesenteroides* J18 through Metabolite and Transcriptome Analyses

Ju Hye Baek, Kyung Hyun Kim, Dong Min Han, and Che Ok Jeon<sup>\*</sup> Department of Life Science, Chung-Ang University

The fermentative metabolic features of *Leuconostoc mesenteroides* strain J18, which is mainly responsible for kimchi fermentation, on various carbon sources were investigated through metabolite and transcriptome analyses and carbon utilization tests. Fructose, xylose, sucrose, glucose, lactose, maltose, and trehalose were well utilized, but galactose, mannose, arabinose, ribose, and cellobiose were rarely utilized. Lactic acid, acetic acid, ethanol, mannitol, 2-acetoin, diacetyl, 2,3-butanediol, and riboflavin production significantly varied depending on the carbon source. Almost no ethanol was produced by strain J18 in fructose and xylose, and mannitol was produced only in fructose and sucrose. 2-Acetoin and riboflavin were most abundantly produced in xylose, and diacetyl production was significantly low in fructose and xylose. The metabolic characteristics of strain J18 on different carbon sources were verified through transcriptomic analyses of SEED functional categories and reconstructed metabolic pathways. Transcriptional expression was clearly differentiated depending on the carbon source, and uptake and the initial metabolic genes related to the carbon sources were highly upregulated. The transcriptional expression of the metabolic genes in response to the carbon sources was consistent with metabolites production. This study will provide insights into understanding the metabolic features of *Leu. mesenteroides* during fermentation.

[Supported by grants from NRF by MSIT]

#### B030

# The Effect of Wastewater Treatment Plants and Hospital Sewage for Antimicrobial Resistance in Natural Environment

Jaehong Jeong<sup>1</sup>, Jaeyong Oh<sup>1</sup>, Min-jeong Kim<sup>2</sup>, Min Young Kim<sup>2</sup>, Lan Hee Kim<sup>3</sup>, Sungpyo Kim<sup>4</sup>, Jong-Chan Chae<sup>1</sup>, and Gyu-cheol Lee<sup>2\*</sup>

<sup>1</sup>Division of Biotechnology, Jeonbuk National University, <sup>2</sup>Water Environmental Safety Management Department, Kwater, <sup>3</sup>Research Institute for Advanced Industrial Technology, Korea University, <sup>4</sup>Department of Environmental Engineering, Korea University

The antimicrobial resistance of bacteria in an aquatic ecosystem may be changed by various environmental factors. This study investigated the microbial communities and Enterobacteriaceae's antimicrobial resistance in representative locations including hospital sewage of an urban water cycle model. Sampling was conducted at the sequential points from hospital effluent to wastewater treatment plants (WWTP) and river samples as water supply sources were taken for comparison. The genetic diversity for beta-lactams, aminoglycosides, and MDRs resistance was relatively higher in influent and effluent before UV treatment of WWTP, and hospital effluent when compared to river. The genes for ESBL, PABL, and MBL were abundantly detected both in WWTP influent and hospital effluent. Similar to the above data, *Escherichia coli* and *Klebsiella pneumoniae* strains carrying carbapenem-resistant genes were also isolated from the WWTP. *E. coli* strains harboring CTX-M-14, -15, -27, and -55 genes were mainly detected from the influent of WWTP. Moreover, carbapenem-resistant *K. pneumoniae* (CRKP) ST307 strains harboring KPC-2 were also detected in the influent. The clonality of the CRKP and ESBL-producing *E. coli* strains showed high genetic similarity. This indicates that WWTP and hospital effluent possibly play a role in resources increasing antibiotic resistance in the natural environment.

### Characteristics of Respiratory Microbiome in Patients with Bronchiectasis

Chaeeun Kang<sup>1,2</sup>, Eun Young Kim<sup>3</sup>, Sungmi Choi<sup>2</sup>, Ji Ye Jung<sup>3</sup>, and Hana Yi<sup>1,2,4\*</sup>

<sup>1</sup>Integrated Biomedical and Life Science, Graduate School, Korea University, <sup>2</sup>Interdisciplinary Program in Precision Public Health, Korea University, <sup>3</sup>Division of Pulmonary and Critical Care Medicine, Severance Hospital, Department of Internal Medicine, Yonsei University College of Medicine, <sup>4</sup>School of Biosystems and Biomedical Sciences, Korea University

Bronchiectasis is a condition in which the sputum discharge function is weakened due to a permanent expansion of the bronchial tubes that have been continuously damaged by respiratory inflammation. The pathogenesis of this disease and the role of microbiome has not yet been fully studied. Our objective was to identify the sputum microbiome profiles of bronchiectasis patients. Sputum samples were collected from 47 patients. The bacterial community profiles of the patients' sputum were analyzed by amplification of the V3-V4 region of the bacterial 16S rRNA gene and sequencing using the Illumina MiSeq platform. The resultant sequencing reads were analyzed using the QIIME pipeline and the EzTaxon-e database. Microbial communities in bronchiectasis were very diverse and individual's characteristics tended to exist. The specific bacteria were found to be present in all samples, and they were *Streptococcus* (19.7%), *Prevotella* (10.8%) and *Haemophilus* (8.7%). In some samples, single bacterial genus dominated more than 50% of the total, and we called them dysbiosis. The major genera that leads to the dysbiosis were *Pseudomonas, Haemophilus* and *Staphylococcus*. With a few exceptions, dysbiosis samples were seemed to be in poor clinical condition. Additional samples will be collected and analyzed for three years of follow-up research.

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#### B032

## Isolation and Characterization of Formate-utilizing Bacteria

Hye Rin Seo<sup>1</sup>, Haesoo Shin<sup>1</sup>, Yoonyong Yang<sup>2</sup>, Sungho Choi<sup>2</sup>, Moonsuk Hur<sup>2</sup>, Byounghee Lee<sup>2</sup>, and Jong-Geol Kim<sup>1\*</sup> <sup>1</sup>Department of Biological Science, Wonkwang University, <sup>2</sup>National Institute of Biological Resources

Greenhouse gas has long been mentioned as an environmental problem. to solve this problem, various studies using greenhouse gases are being conducted. carbon dioxide and carbon monoxide, which are greenhouse gases mainly generated in the industry, can be converted into formate through chemical catalysts or biocatalysts. Formatotrophs have received attention in biotechnology in that synthetic formate can be used as nutrients for microorganisms. however, formate is only studied for hydrogen transfer and storage, and its use is very limited. So we conducted an experiment to cultivate microorganisms using greenhouse gas-derived formate. we cultured by adding only formate to the substrate using a minimum medium such as AFM (artificial fresh media). To date, 15 strains have been isolated from soil, freshwater, and ponds. two of these strains are seen as novel strains. however, since these Formatotrophs grow very slowly, further studies will be conducted to identify their optimized culture conditions. In addition, an experiment is being conducted to produce high-value-added materials using microorganisms using formate.

#### Analysis of Microbiome of Marine Algae in the East Sea of Korea

Jeong Min Kim, Baolei Jia, Kyung Hyun Kim, and Che Ok Jeon<sup>\*</sup> Department of Life Science, College of Natural Science, Chung-Ang University

Bacteria living near marine algae have various symbiotic relationships with marine algae. More than 10 marine algae were collected from the East Sea of Korea in July 2021, and their loosely and closely associated bacterial communities, along with the bacterial communities of seawater, were analyzed. Interestingly, some marine algae have also been identified that show significant differences in bacterial community structure depending on the host algae. Through LEfSe analysis, the genera *Pleurocapsa, Blastopirellula, Roseibacillus, Granulosicoccus, Hellea, Maribacter, Acaryochloris, Sulfitobacter, Croceitalea* were significantly distinguished in the closely associated bacterial communities (CABC) compared to the loosely associated bacterial communities (LABC). In the algal sphere including loosely and closely associated bacterial communities, *Vibrio, Pseudoalteromonas, Marinomonas, Psychromonas,* and *Alteromonas* were identified as the core microbiome of marine algae. Predicted functional capabilities of algal sphere bacteria using PICRUSt analysis revealed the abundance of genes related to the two-component system, secretion system, bacterial motility, biosynthesis of siderophore, bacterial chemotaxis, and cell wall degradation. Information on the core microbiome of marine algae will provide the basis for the next step of studies toward understanding the symbiotic interactions of bacteria and marine algae. [Supported by grants from KIOST of the Ministry of Oceans and Fisheries.]

#### B034

## Analysis of Microbial Community in Groundwater Affected by Pollution of Septic Tank Effluent

Mijin Kim<sup>1,2</sup>, Saem Han<sup>3</sup>, Ji-Hoon Lee<sup>4</sup>, and Man-Young Jung<sup>3,4\*</sup>

<sup>1</sup>Interdisciplinary Graduate Program in Groundwater Studies, Jeju National University, <sup>2</sup>Jeju Research Institute, <sup>3</sup>Interdisciplinary Graduate Program in Advance Convergence Technology and Science, Jeju National University, <sup>4</sup>Department of Biology Education, Jeju National University

In Jeju island, most streams flow only after rain because of the highly permeable volcanic rock structure. Given the unique geological feature of Jeju island, more than 90% of drinking water is originated from groundwater, and therefore the groundwater quality is critically important, which directly affects human health. However, despite the importance of groundwater, anthropological pollution and thoughtless development threaten groundwater quality deteriorating. Where not connected to public sewerage network systems, septic tanks are used for treating wastewater. In total, more than 10,000 septic tanks are operated, and the effluents are directly discharged into the groundwater. Therefore, septic tank effluent could significantly affect groundwater quality but has not been appropriately evaluated. This study 1) analyzed the microbial community in 10 groundwater wells located in septic tank dense areas to identify microbial contamination, 2) compared the microbial communities and biogeochemical properties in groundwater and septic tank effluent to verify the correlation of water pollution and microbial diversity, and 3) found a microbial biomarker to estimate the range of groundwater contamination originated from septic tank effluent. This study would be the first scientific result for establishing the legal standard to manage septic tanks to preserve groundwater quality.

[This study was supported by Jeju Special Self-Governing Province and Jeju Groundwater Research Center]

# Bacterial Isolation from the Gut of Domestic Dog Using In-house Transport Dilution and Culture Media

Forbes Avila<sup>1,2</sup>, Neak Muhammad<sup>1,2</sup>, Jae-Rhim Yu<sup>1</sup>, Tra Thi Huong Nguyen<sup>1,2</sup>, and Song-Gun Kim<sup>1,2\*</sup> <sup>1</sup>Biological Resource Center/Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>University of Science and Technology (UST)

*Canis lupus familiaris*, domestic dogs, have been through evolution and domestication to suit the human lifestyle for more than 30,000 years. Shifting from their ancestral diet; true carnivores evolved into omnivores with rich polysaccharides. Which causes a shift in their gut microbiota (GM). Thus, dog have unique GM and similar to human, especially their owner. Hence, studying dog GM could yield different and new insights compared to conventional studies (primates, mice, & swine). The subject of this study is a 5-year-old male Shiba Inu that is fed with dry food. We cultured the homogenized fecal sample and diluted sample in our in-house isolation medium, containing multiple oxygen scavengers to remove oxygen. It consists of multiple: simple and complex polysaccharides, proteins, growth factors, vitamins, & fatty acids. After culturing in an anaerobic chamber, the sample 16S-rRNA sequences were determined. We isolated *Firmicutes* (46) and *Fusobacteriota* (1); 5 species: *Clostridium ramosum; Glucerabacter* sp. nov.; *Fusobacterium* sp. nov.; *Rombutsia weinsteinii; & Ruminococcus gnavus. Glucerabacter* sp. has been reported to be able to degrade glucosylceramide efficiently. *Fusobacterium* sp. is a bacteria that exists in humans and mammals, some of which are highly pathogenic. The genome of both novel species have been sequenced and will be characterized in the future.

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# Pyrosequencing Analysis Reveals a Compositional Shift in the Soil Microbiome Influenced by Veterinary Antibiotics Entering a Soil System

Hyeon Ji Choe<sup>1</sup>, Song Yeob Kim<sup>1</sup>, hadjer Chohra<sup>1</sup>, Yerim Shin<sup>1</sup>, Saranya Kuppusamy<sup>2</sup>, Mi Sun Cheong<sup>3</sup>, and Yong Bok Lee<sup>3\*</sup>

<sup>1</sup>Division of Applied Life Science (BK21 Four), Gyeongsang National University, <sup>2</sup>Centre for Environmental Studies (CES), Anna University, Chennai 600 025, India, <sup>3</sup>Institute of Agriculture and Life Science, Gyeongsang National University

Veterinary antibiotics (VAs) entering the soil could potentially disturb the soil microbiome, which drives the soil quality and ecological functions. This study investigated the phylogenetic changes in soil induced by frequently detected VAs in the agricultural environment, such as chlortetracycline (CT), oxytetracycline (OT), tetracycline (TC), sulfamethoxazole (SMX), sulfathiazole (STZ), and sulfisoxazole (SXZ) through a microcosm experiment and identified the primary bacterial responders. The prime objective was to understand how veterinary antibiotics alter the soil bacterial community structure and diversity in a soil system and which taxonomic groups are majorly influenced. As a result, Lower concentration of SMX resulted in a declining effect on the soil microbiome. The soils exposed to OT and SMX presented significantly similar bacterial structures rather than CT, TC, STZ, and SXZ. The veterinary antibiotics sensitive bacterial responders belonged predominantly to the phylum Proteobacteria and class Alphaproteobacteria. Most of the taxa, including Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, with highly increased relative abundance in the VAs treated soils might be potential antibiotics resistant bacteria. These findings largely extended our understanding that the soil microbiome is greatly altered in agricultural soils where livestock manure-based fertilizers containing low to high levels of residual antibiotics are frequently treated.

#### B038

# Light Responses of *Candidatus* Puniceispirillum marinum IMCC1322 under Nutrient-augmented Conditions Hyun-Myung Oh

Pukyong National University

Strain IMCC1322 isolated from a surface water from the East Sea of Korea was proposed as *Candidatus* Puniceispirillium marinum. Growth characteristics of IMCC1322 were further evaluated based on genomic information but no light-enhanced repsonse was observed though there was a proteorhodopsin from IMCC1322, an opsin that was characterized as a functional a light-driven proton pump in the cytoplasmic membrane. PRdependent phototrophic potential of strain IMCC1322 was only observed under CO-inhibited and nutrientdepleated conditions. Here I report a light-enhanced growth response was observed under nutrient augmented conditions. Strain IMCC1322 cultivation analysis revealed enhanced ATP production under light conditions. The strain IMCC1322 may depend more on nutrient depletion than light enhanced growth responses according to cellular ATP measurement and transcriptome analysis.

[Supported by grants from Pukyong National University.]

## Influent of Wastewater Treatment Plants Reflects Antibiotic Resistome by Anthropogenic Activity

Seunggyun Han, Yongjin Kim, and Hor-Gil Hur\* Gwangju Institute of Science and Technology

Since the introduction of antibiotics into clinical environmental settings, antibiotic resistance has developed within the microbial community. Wastewater receives sewages from various sources such as households, hospitals, agricultural fields, and other sources. Influent is a habitat for the microbial community due to electron acceptors, nutrients, and other chemicals. Among the chemicals, antibiotic residues and heavy metals act as a selective pressure for the evolution of antibiotic-resistant bacteria by conferring phenotypic and genotypic resistance via horizontal gene transfer. Thus, it may have the potential to reflect antibiotic resistome of anthropogenic activity. In this study, we collected 13 influent samples from nine wastewater treatment plants, in South Korea. High throughput qPCR was applied to investigate 343 antibiotic resistance genes and 43 mobile genetic elements. The relative abundance of antibiotic resistance genes and mobile genetic elements were compared according to the capacity and the main source of wastewater treatment plants. In addition, the class 1 integron integrase gene was used as a proxy of anthropogenic activity. Thus, the correlation between the class 1 integron integrase gene and various clinically relevant antibiotic resistance genes would be evaluated to figure out the link to human- and animal-derived sources.

#### B041

**The Characteristics of Chemical Properties and Microbial Community from Upland Soils in Jeonnam Province** So Youn Lee<sup>1</sup>, Hyeon Ji Kim<sup>1</sup>, Sung Woo Kim<sup>1</sup>, Kyung Jin Kwak<sup>1</sup>, Duck Soo Choi<sup>1</sup>, Jin Woo Lee<sup>1</sup>, and Chang Muk Lee<sup>2\*</sup> <sup>1</sup>Jeonnam Agricultural Research & Extension Services, <sup>2</sup>National Institute of Agricultural Sciences, RDA

This study was conducted to analyze the microbial diversity and the characteristics of the microbial community structure in upland soil and to determine the correlation between the microbial community and the soil chemical properties. Twenty-five upland soils were selected in Jeonnam province and topsoil was collected. The soil chemistry was investigated in accordance with the Soil Chemistry Analysis Method of the Rural Development Administration (National Institute of Agricultural Sciences, 2010). Biomass C and dehydrogenase activity were analyzed in the collected soil, and DNA was extracted and the distribution of microorganisms, species abundance, and diversity were analyzed through ilumina NGS analysis. As a result of the soil chemistry analysis, pH was 6.4, the EC was 0.89 dS/m, the content of organic matter was 23.75 g/kg, the content of available phosphate was 629.04 mg/kg, and the content of K, Ca, and mg was 1.04, 7.72, and 2.02 cmolc/kg, respectively. The microbial biomass C of upland soils was 432.12 mg/kg and dehydrogenase activity was 36.9 µg TPF g-1 24 h-1. The relative aboundance of bacterial dominant phylum was in order of Proteobacteria > Acidobacteria > Bacteroidetes > Actinobacteria > Firmicutes. In many previous studies, soil microbial communities have been known to be affected by various environmental factors, and this study found that pH and Ca have a significant impact. [Supported by research grants from National Institute of Agricultural Sciences]

# *Neobacillus* spp. Strains Isolated from Rice Paddy Soil Perform DNRA Despite of Nitrate Presence Seohyun Ahn, Sua Lee, and Jeonghwan Jang<sup>\*</sup>

Jeonbuk National University

Seventeen *Neobacillus* spp. strains were isolated from soil collected at the rice paddy field in Iksan-si, Jeollabuk-do. The colorimetric screening method using the Griess reagent and vanadium (III) revealed that all of the isolates are capable of removing nitrate from the culture medium. The DNRA functional genes *nrfA* and/or *nirB* were amplified from 12 of the 17 isolates and 4 of them were shown to harbor the denitrification functional gene *nosZ* by conventional PCR with primer sets designed in this study. Based on 16S rRNA sequence identity and N cycle functional genes (*nrfA*, *nirB*, and *nosZ*) profile of the 17 strains, the 5 strains among them were selected for nitrate removal and ammonium production test in time-based manner. Interestingly, *Neobacillus* spp. strains PS3-34 and PS3-40 produced ammonium despite of nitrate remained, which is not common since dissimilatory nitrate reduction to ammonium (DNRA) is limited by presence of nitrate. Further studies such as whole genome investigation and transcript analysis for the *Neobacillus* spp. strains would help us to observe how their DNRA functional genes work under anaerobic condition with nitrate and provide insight into ecophysiology of *Neobacillus* spp. for the N cycle in rice paddy soil environment.

# **B043** *Pedobacter flavus* sp. nov. Isolated from Oil-contamination Soil Jun Jin and Jaisoo Kim<sup>\*</sup> *Kyonggi University*

## A Gram-negative, motile, rod-shaped bacterium

, VNH31<sup>T</sup>, was isolated from an oil-contamination soil sample collected from Hue, Vietnam. Colonies on R2A agar are yellow, entire, convex, and circular, size is 1–2 mm on R2A agar for 7 days at 10°C. Cells grow at 10–40°C (optimum 20–35°C), pH 6.0–9.5 (optimum pH 6.5–8.0) and tolerate NaCl up to 1.5% (w/v). The major polar lipid was phosphatidylethanolamine, and the major quinone was MK-7. Catalase and oxidase are positive. Hydrogen sulfide is not produced. Negative for urease activity. Hydrolysis of aesculin and DNA is positive but hydrolysis of casein, starch, gelatin, tyrosine, and Tween 80 is negative. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain VNH31<sup>T</sup> belongs to the genus *Pedobacter* in the family *Sphingobacteriaceae*. The isolate is closest to the *Pedobacter antarcticus* DSM 15311<sup>T</sup> (96.12% level of sequence similarity). Based on phenotypic, chemotypes, and genotypic evidence, strain VNH31 could be differentiated phylogenetically and phenotypically from the recognized species of the genus *Pedobacter*. Therefore, strain VNH31<sup>T</sup> is considered to represent a novel species, for which the name *Pedobacter flavus* sp. nov. is proposed. The type strain is VNH31<sup>T</sup>.

## Dysbiosis of Gut Microbiome Associated with IgA Nephropathy

Min-Jung Lee<sup>1</sup>, Yu Jeong An<sup>1</sup>, Ha jeong Lee<sup>2</sup>, and Bong-Soo Kim<sup>1\*</sup> <sup>1</sup>Department of Life Science, Multidisciplinary Genome Institute, Hallym University, <sup>2</sup>Department of Internal Medicine, Seoul National University Hospital

The gut microbiome plays important roles in the host health, and dysbiosis of gut microbiome can be related to disease by irregulated interactions with the host immune system. Immunoglobulin A nephropathy (IgAN) is the most prevalent primary glomerulonephritis, is characterized by the deposition of IgA in the glomerular mesangium. The dysbiosis of gut microbiome can be associated with the progression of IgAN because of the large amount of secretory IgA is produced in the gut. However, the role of gut microbiome in IgAN is not fully understood. We analyzed the differences of composition and functional genes in the gut microbiome from healthy (n = 51) and IgAN participants (n = 85) by using metagenomics sequencing. Age-dependent shifts of gut microbiome were dysregulated in adult IgAN patients compared to healthy subjects. Disordered age-dependent shifts were more clearly identified in male than female subjects. Functional genes of gut microbiome were significantly different between the HC and IgAN groups in specific ages group. This dysregulated gut ecosystem could be related to immune responses and abnormal IgA production in patients with IgAN. These results can help to understand the potential role of gut microbiome in the pathogenesis of IgAN.

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#### B045

**Isolation and Selection for the Potential Crude Oil-degrading Bacteria** Jun Jin and Jaisoo Kim<sup>\*</sup> *Kyonggi University* 

The objective of this study was to isolate and identify the biosurfactant-producing bacteria from oil-contamination soil which were collected from oil-contaminated regions of South Korea, China, and Vietnam to use in microbial enhanced oil recovery was investigated. Crude oil utilizing bacteria was isolated from soil by enrichment method on oil-MSM media at 28°C for 14 days by using six transwell plates. To confirm the ability of isolates in biosurfactant production, different screening methods including hemolysis, emulsification, penetration, and oil spreading assay were assessed. Among the 24 isolated strains with high growth rates in crude oil, 3 strains KR2-4, KR2-6, and CN1-3 show that they have the potential production of biosurfactants in testing oil spreading with a surface activity of 68–72 mm. The highest biosurfactant producer was KR2-4 which produced 9.21 g/L of the crude biosurfactant, followed by CN1-3 (8.95 g/L) and KR 2-6 (7.37 g/L). These isolated strains degraded 67%–96% of crude oil after 14 days applied, the highest oil-degrading activity was KR 2-4. The strains which degrade crude oil belonged to the members of the genera *Paenibacillus, Rhodococcus*, and *Sphingobacterium*. Further study will include their optimal degradation conditions and their roles in oil-contaminated soil for field application.

### Novel Psychrophilic Bacteria Isolated from Intestine and Stomach of Marine Shrimps

Sang-Eon Kim<sup>1,2</sup>, Hye-Jin Park<sup>1,2</sup>, and Kyoung-Ho Kim<sup>1,2\*</sup>

<sup>1</sup>Department of Microbiology, Pukyong National University, <sup>2</sup>School of Marine and Fisheries Life Science, Pukyong National University

Two different shrimp species, *Pandalopsis japonica* and *Lebbeus groenlandicus*, live in the deep cold water in the East Sea of Korea. The microorganisms present in these shrimps are generally high in various psychrophilic bacteria. Their stomach and intestine were extracted and used as samples, and each sample was diluted and spread on Marine Agar (MA). The inoculated media were incubated at 4°C for at least 5 days. During the investigation of the microbial diversity of intestine and stomach, several strains have low 16S rRNA sequence similarity with their closely related species as follows; M4, *Psychrobacter alimentarius* JG-100<sup>T</sup> (98.51%); M13, *Psychrobacter luti* NF11<sup>T</sup> (98.55%); M17, *Psychrobacter arcticus* 273-4<sup>T</sup> (98.35%); DM4, *Psychrobacter glacincola* DSM 12194<sup>T</sup> (98.75%); DM8, *Psychrobacter alimentarius* JG-100<sup>T</sup> (97.6%); DM9, *Psychrobacter celer* T-3-2<sup>T</sup> (98.02%) respectively. Physiological and biochemical tests were conducted for each strain. Genomic analysis of these psychrophilic bacteria showed their phylogenetic positions in each taxonomic level and their potential roles in their hosts.

## B047

**Microbiome Analysis of Three Species of Dokdo Shrimps with Amplicon Sequencing** Sang-Eon Kim<sup>1,2</sup>, Hye-Jin Park<sup>1,2</sup>, and Kyoung-Ho Kim<sup>1,2\*</sup> <sup>1</sup>Department of Microbiology, Pukyong National University, <sup>2</sup>School of Marine and Fisheries Life Science, Pukyong National University

The three shrimp species *Pandalopsis japonica, Lebbeus groenlandicus,* and *Pandalus hypsinotus* are taxonomically distant but have similar living environments. These shrimps live in the cold and deep waters of the East Sea of Korea and are collectively referred to as Dokdo shrimps after their local names. To compare the differences between the three species of shrimp, the microbiome of the stomach, hepatopancreas, anterior intestine, and posterior intestine, which are organs related to feeding and digestion, were analyzed. Sampling was carried out every month, and each sample was separated by size by four shrimps to obtain a total of 120 samples, thereby reducing the error between samples. To distinguish each sample, a dual barcode primer system capable of sequencing a total of 144 samples simultaneously using 12 forward primers and 12 reverse primers was selected. The primers used amplify the V3-V4 region of bacteria. Each organ microbiome was analyzed by 16S rRNA gene amplicon sequencing using the Illumina Miseq platform. Results analysis identified bacterial diversity using the QIIME2 pipeline and other applications.

# Rhodosyntase glutamiense sp. gen. nov., sp. nov., Isolated from Park Soil

Thi Tuyet Nhan Le and Jaisoo Kim<sup>\*</sup> Kyonggi University

A Gram-negative, aerobic, oxidase and catalase-positive, non-motile, non-spore-forming, spherical to ovoid, designated RN2-1<sup>T</sup>, was isolated from a park soil sample. The isolate grew in a pH range of 6–9, with pH optima of 7–8; a temperature range of 20–40°C, with temperature optima of 30–35°C, and in the presence of 0–0.5% (w/v) NaCl. The major polar lipid profile comprised diphosphatidyl glycerol, phosphatidylglycerol, phosphatidylethanolamine, and phosphatidylcholine. The major quinone was unidentified quinone 10.756 (ubiquione 10). The main fatty acids were  $C_{14:0}$ ,  $C_{16:1}\omega7c$ ,  $C_{16:1}\omega5c$ ,  $C_{16:0}$ ,  $C_{17:0}$  cyclo,  $C_{18:1}$  2OH, and  $C_{18:0}$  3OH. A phylogenetic analysis based on 16S rDNA sequences revealed that strain RN2-1<sup>T</sup> clustered in the family *Acetobacteraceae*, which includes the genera *Rhodovastum, Acidisoma, Acidisphaera*, and *Acidibrevibacterium*. The aerobic phototrophic bacterium *Rhodovastum atsumiense* G2-11<sup>T</sup> was the closest relative to RN2-1<sup>T</sup> (95.97% level of sequence similarity). The G + C content of genomic DNA is 68.68%. Genome analysis revealed the presence of genes *gdhA* and *glnA*, which are known to play essential roles in the biological control of ammonia assimilation. On the basis of these results, strain RN2-1<sup>T</sup> represents a novel species of a new genus for which the name *Rhodosyntase glutamiense* is proposed.

#### B049

# **Removal of Ammonia from Swine Manure by Microbes-surfactant Mixture** Thi Tuyet Nhan Le and Jaisoo Kim<sup>\*</sup>

Kyonggi University

In this study, we isolated and screened for indigenous microorganisms capable of effectively degrading ammonia and combining them with AOS and gelatin to create bio-foam technology wherein the surfactant foam acts as a physical barrier suppressing odors release, and microorganisms infiltrated and degraded odors in manure. The final concentration of AOS and gelatine selected on the basis of the previous study were 0.2% and 0.3%, respectively. Five strains of microorganisms were selected based on their ability to remove ammonia and the influence of carriers on their growth: *Lactobacillus* sp., *Bacillus* sp., *Saccharomyces cerevisiae*, and *Pichia* sp. were combined with ratio 1:1:1:1:1. The experiments were performed in a reactor system with a working volume of dimensions: length 25 cm; width 25 cm; height 10 cm and each chamber was filled with 1 kg ammonia-contaminated soil or swine manure, onto which the tested preparations were sprayed. The mixed culture applied for deodorization was particularly active against ammonia with the concentration degradation by 90–100% for the ammonia-contaminated soil, and 84–97.7% for the swine manure observed after 3 days. The experiments also showed that the preparation can be stored for at least 2 months at room temperatures with no decrease in microbial activity. Therefore, the application of microbes -surfactant opens up a possibility for the treatment of odor compounds within manure in swine farming facilities.

## Novel Marine Bacteria in Bacteroidota Phylum Producing Many Polysaccharide-degrading Enzymes

Tra Thi Huong Nguyen<sup>1,2</sup>, Forbes Avila<sup>1,2</sup>, Neak Muhammad<sup>1,2</sup>, Jae Rhim Yu<sup>1</sup>, and Song-Gun Kim<sup>1,2\*</sup> <sup>1</sup>Biological Resource Center, Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>University of Science and Technology (UST)

*Bacteroidota* is the primary group of marine polysaccharide-degrading bacteria, which plays a principal role in the marine carbon cycle. To explore their potential polysaccharide-degrading enzymes, the novel bacterial isolates of phylum *Bacteroidota* is essential. Here, we isolated novel *Bacteroidota* bacteria and studied their ability of polysaccharide degradation. Green algae, marine squirts, and seagrasses were collected from the East and the West Sea of Korea. By mimicking the natural condition, nine novel strains of *Bacteroidota* were isolated. The novel strains exhibited the highest 16S rRNA similarity of 87.7–95.6% to existing taxa, indicating for novel taxa of species, genus or family. The neighbour-joining tree showed the phylogenetic positions of the novel strains belonging to two classes *Bacteroidia* and *Cytophagia* in the phylum *Bacteroidota*. The whole-genome sequences of novel strains were determined by the hybrid of Nanopore and Illumina platforms. Polysaccharide-degrading action of them on agar plate were detected by Lugol's iodine solution and the production of reducing sugar from broth culture was determined by 3, 5-dinitrosalicylic acid assay. They could degrade agar, alginate, chitin, laminarin, starch, and xylan. For further uncovering the role of *Bacteroidota*, especially novel strains, in the marine ecosystem through polysaccharide degradation, the taxonomic studies, genome mining, and enzymatic characterization need to be assessed.

## B051

**Resistance Spectra of Novel Environmental β-Lactamases against β-Lactams and β-Lactamase Inhibitors** Seong-Jun Jo, Sang-Gyu Kim, and Dae-Wi Kim<sup>\*</sup> *Division of Life Sciences, Jeonbuk National University* 

The importance of the environment in the emergence of novel antibiotic resistance genes (ARGs) and their dissemination, has been emphasized regarding its role as a reservoir and a transmission route of ARGs. In a previous study, a new opportunistic pathogen, *Scandinavium* sp. strain SJ1, was isolated from the environment. Its phylogeny and ARG contents were investigated based on its complete genome sequence, revealing the presence of two novel class A and class C  $\beta$ -lactamases in the strain. Here, we analyzed resistance spectra of these  $\beta$ -lactamases against various  $\beta$ -lactams (penicillin, cephalosporin, carbapenem, and monobactam) and  $\beta$ -lactamase inhibitors (sulbactam, clavulanic acid, tazobactam, and avibactam). They exhibited extended spectrum  $\beta$ -lactamase (ESBL) and AmpC resistance spectra, respectively. They also displayed the resistance against several  $\beta$ -lactamase inhibitors. Structural modeling and ligand binding analyses will be further conducted to elucidate critical amino acid residues involved in the resistance against  $\beta$ -lactamas and  $\beta$ -lactamase inhibitors.

[Supported by a research grant from the National Research Foundation of Korea (NRF-2019R1I1A1A01059574).]

# Comparative Genomic Analysis of Two *Limnohabitans* Stains Comprising a Novel Subcluster, Isolated from an Oligotrophic Lake

Miri Park, Suhyun Kim, Ilnam Kang, and Jang-Cheon Cho<sup>\*</sup> Department of Biological Sciences and Bioengineering, Inha University

Genus *Limnohabitans* is one of major bacterial lineages in freshwater environment. Previous phylogenetic analyses of 16S rRNA gene sequences showed that the genus can be divided into five subclusters: LimA, B, C, D, and E. Nevertheless, only five valid-published species have been proposed so far. Therefore, the genus *Limnohabitans* was predicted to have high microdiversity. Here, we report the cultivation and genomic characteristics of two colony-non-forming *Limnohabitans* strains that is not affiliated with any of the previous subclusters. The IMCC26093 and IMCC26116 were isolated by dilution-to-extinction method from an oligotrophic freshwater lake in Korea. Genomic sequences of two strains were analyzed using SMRT technique, and single, circular contigs were obtained for both strains. The sizes of two genomes were 2.95 Mb and 3.37 Mb and the G + C contents were 53.3 and 59.9%, respectively. Comparing other *Limnohabitans* strains, only a novel strains IMCC26116 encoded the complete Calvin cycle that proceeds with CO2 fixation. In addition, the strain was found to harbor genes for the denitrification cycle, which is likely to be deeply involved in the nitrogen cycle in the ecosystem. In fragment recruitment analyses using > 100 various freshwater metagenomes, these novel strains showed distribution patterns distinct from other *Limnohabitans* strains. These results suggest that the genus *Limnohabitans* were highly microdiversified in their phylogenetic and genomic characteristics.

#### B053

*Aequorivita ciconicae* sp. nov., Isolated from the Feces of an Oriental Stork, *Ciconia boyciana* Jeong Eun Han<sup>1</sup> and Jin-Woo Bae<sup>1,2\*</sup>

<sup>1</sup>Department of Biology, Kyung Hee University, <sup>2</sup>Department of Life and Nanopharmaceutical Sciences, Kyung Hee University

A novel bacterial isolate, designated as strain HM23<sup>T</sup>, was isolated from the feces of an oriental stork, *Ciconia boyciana*, which was collected from the Seoul Grand Park Zoo in Seoul. Strain HM23<sup>T</sup> was non-motile, Gram-stain-negative, strictly aerobic, and rod-shaped. Optimum growth of the isolate occurred at 30°C, in the presence of 1% (w/v) NaCl, and at pH 7. The 16S rRNA gene sequence analysis showed that strain HM23<sup>T</sup> belonged to the genus *Aequorivita* in the family *Flavobacteriaceae* with 96.36% sequence similarities to *Aequorivita capsosiphonis* A71<sup>T</sup>. The major cellular fatty acids (> 10%) were Iso-C<sub>15:0</sub> (24.4%), Iso-C<sub>17:0</sub>-3OH (15.9%) and Anteiso-C<sub>15:0</sub> (13.9%). Complete genome sequence of strain HM23<sup>T</sup> comprised 3,682,614 bp with 38.25 mol% G+C contents. The polar lipid profiles of strain HM23<sup>T</sup> comprised phosphatidylcholine, phosphatidylglycerol, diphosphatydylglycerol, three unidentified aminolipid, and four unidentified lipids. The predominant respiratory quinone was menaquinone-6 (MK-6). The results of the phylogenetic, phenotypic and genotypic analyses indicated that strain HM23<sup>T</sup> represents a novel species in the genus *Aequorivita*, for which the name *Aequorivita ciconiae* is proposed. The type strain is HM23<sup>T</sup> (= KCTC 62809<sup>T</sup> = JCM 33229<sup>T</sup>).

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### Discovery of Novel Predatory Bacteria in the West Sea, Korea

Neak Muhammad<sup>1,2</sup>, Tra Thi Huong Nguyen<sup>1,2</sup>, Forbes Avila<sup>1,2</sup>, Jae-Rhim Yu<sup>1</sup>, and Song-Gun Kim<sup>1,2\*</sup> <sup>1</sup>Biological Resource Center/Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>University of Science and Technology (UST)

Studies on predatory bacteria have received much attention recently because of their potential role as biocontrol agents and in balancing the ecosystem. There are very few studies on predatory bacteria (*Bdellovibrio, Myxococcus, Lysobacter*) because of difficulties in culturing them. Second, in the era of increased antimicrobial resistance, the uses of bacteriophages and predatory bacteria are emerging.

Thus, multiple sea samples have been collected from three islands in the West Sea. Low-nutrient and prolonged incubation facilitated the isolation of novel strains among which 42% were gliding bacteria. A total of 120 isolates; 22 were novel Gram-negative belonging to *Bacteroidetes* and *Proteobacteria*; and 2 were novel *Actinobacteria*. The novel gliding bacteria were screened for predatory activities against *E. coli, P. aeruginosa, V. harveyi, V. parahaemolyticus, K. pneumonia, Erythrobacter* sp., *Streptococcus bovis, Staphylococcus aureus, B. subtilis*, etc. The predation was tested using lawn predation and cross streak method. Our preliminary results showed that 2 novel species that belonging to the phylum *Bacteroidetes* showed predatory activities against *E. coli, Erythrobacter* sp., & *K. pneumonia*. The activities were further tested using SEM analysis. Furthermore, the comparative genomics and transcriptomics, among novel predators and non-predators will be analyzed.

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#### B055

# Study on Physiological Properties and Niche Differentiation of Comammox Bacterium, *Nitrospira ionopinata* Related to Soil Acidification in Nature

Yun Ji Choi<sup>1</sup>, Saem Han<sup>1</sup>, and Man-Young Jung<sup>1,2\*</sup>

<sup>1</sup>Interdisciplinary Graduate Program in Advance Convergence Technology and Science, <sup>2</sup>Department of Biology Education, Jeju National University

Soil acidification enhanced by human activities is one of the critical drivers of the environmental factors affecting niche differentiation and community composition of nitrifying microorganisms. Many studies on N<sub>2</sub>O production by ammonia-oxidizing archaea (AOA) or bacteria (AOB) have been tested at neutral pH conditions. However, various biological and chemical reactions involved in N<sub>2</sub>O production are pH dependent. This study revealed that the comammox strain, *Nitrosospira inopinata*, tolerates a wide pH range (pH 5.5–9, optimum 7.5), and ammonia oxidation at the lowest pH 5.5 showed almost 20% compared to the optimum pH condition. The comammox strain produces NO and N<sub>2</sub>O differently under various pH conditions. In addition, less hydroxylamine (NH<sub>2</sub>OH) emission was discovered at pH 6.5 than at pH 7.5. This may explain the lower N<sub>2</sub>O production (e.g., from abiotic NH<sub>2</sub>OH decomposition) at low pH. It has also been verified that the growth of comammox strain was completely inhibited by 5  $\mu$ M chlorate (ClO<sub>3</sub><sup>-</sup>), a nitrite-oxidizing inhibitor. Therefore, the result has identified nitrite as a critical compound for comammox growth. Collectively, this study improves understanding to get needed intuitions into nitrogen compound diversions and greenhouse gas production by comammox under divergent environmental conditions.

# Effect of Dietary Mineral Supplementation on Rumen Microbiota of Holstein Calves during Environmental Temperature Change

A-Rang Son<sup>1</sup>, Seon-Ho Kim<sup>1</sup>, Ye Pyae Naing<sup>1</sup>, Michelle Miguel<sup>1</sup>, Su Kim<sup>2</sup>, and Sang-Suk Lee<sup>1\*</sup> <sup>1</sup>Department of Animal Science and Technology, College of Bio-industry Science, Sunchon National University, <sup>2</sup>Woosungvet Co., Ltd.

This study determined the effect of inorganic and organic minerals supplementation on the microbial composition of Holstein calves during environmental temperature change. Eight Holstein calves were assigned to four treatments in four periods of  $4 \times 4$  Latin square design. Calves were kept in a temperature-controlled barn and the experimental period consisted of 14-day in heat stress condition (HS), 14-day in recovery condition, and 7-day in dietary adaptation for the next period. Treatments were mineral-free (Con), inorganic minerals (IM), organic minerals (OM), and high-concentration organic minerals (HOM). Results showed at the phylum level that Firmicutes and Actinobacteria decreased while Fibrobacteres, Spirochaetes, and Tenericutes increased (p < 0.05) in the HS condition. *Treponema* increased in the HS condition (p < 0.05), while *Christensenella* was high (p < 0.05) in HOM and OM during HS and recovery conditions, respectively. In conclusion, Holstein calves supplemented with HOM altered the rumen energy metabolism where the abundance of *Christensenella* was relatively richer, suggesting that supplementation with high-concentration of organic minerals may alleviate the adverse effects of heat stress. [This research was supported by the Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ015039032022), Rural Development Administration, Korea.]

#### B057

## Beneficial Bacteria Isolated from Swine and Cattle Feces in South Korea

Eun Sol Kim<sup>1</sup>, Gi Beom Keum<sup>1</sup>, Hyunok Doo<sup>1</sup>, Jinok Kwak<sup>1</sup>, Sriniwas Pandey<sup>1</sup>, Sumin Ryu<sup>1</sup>, Yejin Choi<sup>1</sup>, Seungjin Yun<sup>1</sup>, Juyoun Kang<sup>1</sup>, Sheena Kim<sup>1</sup>, Ju-Hoon Lee<sup>2</sup>, and Hyeun Bum Kim<sup>1\*</sup>

<sup>1</sup>Department of Animal Resources Science, Dankook University, <sup>2</sup>Department of Food Animal Biotechnology, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Seoul National University

It has been well known that the gut bacteria play pivotal roles in the hosts. There are a variety of beneficial gut bacteria which help treat various metabolic disorders, exclude pathogens, and trigger immune responses. This study was focused on isolating such useful bacteria from swine and cattle feacal samples. Swine and cattle feces samples were collected and cultured in different culture medias including MRS agar, enterococcus selective agar, M17 agar, and modified RCM agar. For identification of isolated colonies, the full-length 16S rRNA genes were sequenced using Sanger sequence platform. MEGA software was used for 16S rRNA gene sequence analysis. The isolated bacteria were tested for acid and bile tolerance in the pH 3.0, 5.0, 7.0 and bile concentrations of 0%, 0.3% and 0.5%. The cell count for the acid tolerance test was obtained at an interval of every 2 h until 12 h, respectively and was spread on agar and incubated at 37°C for 24 h. A total of 100 bacteria were isolated from each swine and cattle samples. A total of 20 species were identified from cattle sample and 18 species were identified from swine sample. Among the selected bacteria, only 5 species (*B. amyloliquefaciens, B. subtilis, E. faecalis, E. faecium, L. johnsoni*) have the bile and acid tolerance. Previous studies have also shown that these species are the useful microorganisms. However, more detailed study is needed to elucidate its safety and beneficial effects inside the host.

# **Characterizing Versatile Adaptation, Defense, and Metabolic Attributes of Novel** *Brachybacterium* **sp.** Myunglip Lee, Adeel Farooq, and Man-Young Jung<sup>\*</sup>

Jeju National University

The species of the *Brachybacterium* belonging to the family *Dermabacteriaceae* within the phylum Actinomycetota are Gram-positive bacteria. In this study, we isolated a novel *Brachybacterium equifaecis* JHP9 from horse feces to uncover its kinetic properties together with biochemical, and genomic features. This study presents first report on the kinetic properties of any *Brachybacterium* species. Delineation of the genome was performed through 16S rRNA analysis, genome based phylogenetic, and similarity matrix based on nucleotide, and amino acid identity. Strain JHP9 was carrying carotenoid biosynthesis, various carbohydrate metabolism under aerobic and anaerobic conditions, antibiotic resistance, and CRISPR (clustered regularly interspaced short palindromic repeats) systems were observed in genotypic and/or phenotypic properties suggesting its flexibility and defense system in various environments. Additionally, substantial metabolic versatility and lactic acid production exhibited by the strain JHP9 could explain its adaptability and utilization across various industrial environments. Interestingly, kinetic studies disclosed that the strain JHP9 has high oxygen and substrate affinity, which may manifest niche differentiation. Our study contributes to the knowledge of the kinetics and genomic properties of *Brachybacterium equifaecis* JHP9, advocating its tolerating and thriving nature in various environments,

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#### B059

# Investigation of Cultivable Groundwater Bacteria in Shallow Aquifers Based on Dilution to Extinction Culturing and Their Gnome Analysis

Sumin Kim, Jaeho Chang, Suhyun Kim, Ilnam Kang, and Jang-Cheon Cho<sup>\*</sup> Department of Biological Sciences and Bioengineering, Inha University

Bacteria play crucial biogeochemical roles in groundwater, a water resource important for human use. Cultivation of groundwater bacteria is important to understand their ecophysiological and genomic features relevant to groundwater ecosystems. But, research on cultivation of diverse groundwater microbes has not been performed much. To expand the diversity of cultured bacterial groups in groundwater, we performed high-throughput dilution-to-extinction culturing (HTC) for water samples collected from two groundwater wells located in an agricultural area, South Korea, during four seasons. A total of 1,042 putative pure bacterial strains were successfully isolated. Phylogenetic analyses of 16S rRNA genes showed that these HTC isolates were affiliated with diverse phyla, such as Proteobacteria, Bacteroidetes, and Actinobacteria. Notably, isolates belonging to abundant but not-yet or rarely-cultured bacterial groups were obtained, including the OPB56, env.OPS\_17, SJA-28, KD4-96 and OM190 clades. Genome sequencing and analyses were performed for three isolates of the OPB56 clade (c\_Kapabacteria; GTDB). Biosynthetic pathways for quinone and heme, essential electron carriers in aerobic respiration, were lacking, implicating either auxotrophy or facultatively anaerobic lifestyle. This culture collection of diverse groundwater bacteria would be a valuable resource for better understanding of ecophysiological and genomic characteristics of groundwater microbes relevant to their habitats.

### **Characteristics of Sputum Microbiome in COPD Patient**

Min Hong Kim<sup>1,2</sup>, Sungmi Choi<sup>2</sup>, Sung Woo Moon<sup>3</sup>, Ji Ye Jung<sup>4</sup>, and Hana Yi<sup>1,2,5\*</sup>

<sup>1</sup>Integrated Biomedical and Life Science, Graduate School, Korea University, <sup>2</sup>Interdisciplinary Program in Precision Public Health, Korea University, <sup>3</sup>Division of Integrated Medicine, Department of Internal Medicine, Severance Hospital, Yonsei University College of Medicine, <sup>4</sup>Division of Pulmonology, Department of Internal Medicine, Severance Hospital, Yonsei University College of Medicine, <sup>5</sup>School of Biosystems and Biomedical Sciences, Korea University

Chronic Obstructive Pulmonary Disease (COPD) refers to continuous stenosis of the airway that occurs with emphysema, obstructive bronchitis, or their disability. Coughing accompanied by sputum is reported as a representative symptom. Severity of COPD symptoms is divided into four GOLD stages according to the degree. We aimed to study how the composition of sputum microbiome differs depending on the severity of COPD symptoms. Sputum samples were collected from a total of 80 COPD patients and were the subject of amplicon sequencing targeting the V3-V4 hypervariable region of the bacterial 16S rRNA gene. The sequencing was performed with Illumina MiSeq v3 platform and the resultant sequencing reads were analyzed using QIIME2 program. As a result of the analysis, we found that *Streptococcus, Prevotella*, and *Veillonella* were the dominant bacteria of sputum microbiome in the COPD patients. We also identified how the abundance of some minor species differs according to the GOLD stage. To elucidate the association between microbiome dysbiosis and the COPD exacerbation, the prospective longitudinal study addressing the microbiome composition is now underway.

[This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Ministry of Education (No. 2022R1A2C1007966)]

#### B061

## Microbiota Characterization of Free-living and Attached Bacterial Communities in a Biofloc-based Shrimp Aquaculture System

Rajeev Meora<sup>1</sup>, Ilsuk Jung<sup>2</sup>, Jaeho Song<sup>2</sup>, Ilnam Kang<sup>1</sup>, and Jang-Cheon Cho<sup>1\*</sup> <sup>1</sup>Department of Biological Sciences, Inha University, <sup>2</sup>Honam National Institute of Biological Resources

Bacterial communities thriving in various aquaculture components determines the host's health and water quality. Despite the growing application of biofloc technology in aquaculture, studies assessing the microbiota composition of bioflocs and comparing it to the peripheral rearing water remained scarce. Therefore, we examined the biofloc-associated (attached) and rearing water (free-living) bacterial communities of a commercial shrimp aquaculture system to ascertain how their diversity, community composition, inter-species interactions and functional potential varies. The obtained 16S rRNA gene amplicon sequencing showed that, despite sharing the same niche, the attached community harbors a higher bacterial richness and noticeably distinct community composition than the free-living community. Co-occurrence analysis showed a complex ecological network and strong inter-species interactions in the attached community. Furthermore, members of *Halieaceae* and *Rhodobacteraceae* were identified as major keystone taxa in the attached and free-living communities, respectively, highlighting their vital roles in sustaining the stability of these communities. Taken together, we proved that the attached and free-living bacterial communities served as two distinct microbial consortia, each performing different tasks, and hence might play diverse ecological roles in aquaculture.

### **Optimization of Siderophore Production by** *Bacillus velezensis* **XC1**

Ha Yeon Byun<sup>1,2</sup>, Seung Hee Ham<sup>1,3</sup>, Sang-Jun Lee<sup>1</sup>, and Jeong-Mi Park<sup>1\*</sup> <sup>1</sup>National Institute of Biological Resources, <sup>2</sup>Department of Biology and Chemistry, Changwon National University, <sup>3</sup>Department of Bio Health Science, Changwon National University

Heavy metals (HM) accumulation in the soil causes serious problems. Microorganisms which produce siderophore can act as HM chelation agents to purify soil and protect organisms from HM stress by bioremediation. In this study, we screened siderophore-producing bacteria and established the optimum culture conditions for siderophore production microbe. Bacteria were isolated from the soil in an abandoned mine area in Miryang of Korea. Among them, the strain XC1 that produces maximum siderophore was identified as *Bacillus velezensis* based on 16S ribosomal RNA sequence. For measuring siderophore production of the strain, Chrome Azurol S (CAS) liquid assay method was used with nutrient broth. The maximum amount of siderophore was produced at 24 h, pH 7, and 30°C. To increase siderophore production, additional carbon sources, nitrogen sources and inorganic salts were optimized. Based on these, optimal culture conditions for producing siderophore were 0.5% of fructose, 1.5% of yeast extract and 0.3% of potassium phosphate dibasic. All these results indicate that *Bacillus velezensis* XC1 has significant HM mitigation effects as a soil-purifying microorganism against soil pollution.

[This work had been supported by a grant from the National Institute of Biological Resources (NIBR202215102).]

### B063

# Characterization of a Bisphenol A-degrading Bacterium *Priestia aryabhattai* BA-1 Isolated from Reclaimed Land Soil in Korea

Sihyun An, Hang-Yeon Weon, Jung-Jun Kim, Dayeon Kim, Joon-Hui Chung, Jehyeong Yeon, and Jae-Hyung Ahn<sup>\*</sup> Agricultural Microbiology Division, National Institute of Agricultural Sciences

Bisphenol A (BPA) is one of the important chemicals and used as polycarbonate resin and plastic additive. It has been detected in various environments such as groundwater and soil, and even human body, in which BPA can be acted as an endocrine disruptor. A novel bacterium, *Priestia aryabhattai* BA-1, capable of degrading BPA, was isolated from reclaimed land soil in Buan, Korea. The strain could degrade approximately 52% of 20 µg/ml BPA in liquid mineral medium within 3 days at 28°C. The metabolites were identified by liquid chromatography with mass spectrometry (LC/MS) analysis. We proposed a degradation pathway of BPA by this strain in which BPA is transformed to tetracarbonylation BPA, dihydroxylated BPA, and monohydroxylated BPA. This study concluded that strain BA-1 has significant potential for the bioremediation of BPA-contaminated environments and can be applied for their treatment at an industrial scale.

[Supported by grants from Rural Development Administration (Project No. PJ015591)]

# Isolation of Bacteria Capable of Biodegrading Geosmin and 2-Methylisoborneol (MIB)

Seoung Hyeok Soung and Song-Ih Han<sup>\*</sup> Department of Microbiology and Biotechnology, Mokwon University

Odorous substances in the water supply source, are grabbing attention as an environmental problem that causes aesthetic displeasure. In this study, 151 strains using geosmin and 2-MIB as a single carbon source were isolated for the management of odorous substances. Those isolates were tested for antibacterial activity against *Streptomyces* sp. 1PDS 1-11, the *Actinomyces* that produce odorous substances, 28 isolates were selected as the bacteria inhibiting the *Actinomyces* that produce odorous substances. The 28 isolates were affiliated to six genera *Pseudomonas* (21 isolates), *Bacillus* (3 isolates), *Burkholderia* (1 isolate), *Citrobacter* (1 isolate), *Microbacterium* (1 isolate), and *Microvirgula* (1 isolate) based on 16S rRNA gene sequence analyze. Especially, *Pseudomonas* sp. 7SBR-5 and *Bacillus* sp. AE reduced geosmin and 2-MIB by about 86% during two days. Illumina MiSeq sequencing analysis was performed on the *Bacillus* sp. AE with the highest reduction rate among the strains that could reduce odorous substances, it showed 99.93% homology with *Bacillus wiedmannii*, and included 29 genes with the antibacterial activity function such as Antibiotic polyketide synthase gene and Bacteriocin synthase gene.

### B065

### Screening of Antimicrobial Activity of Essential Oils against Xanthomonas oryzae pv. oryzae

Mi Hee Kim<sup>1</sup>, Hyeonbin Kim<sup>1</sup>, Sungbeom Lee<sup>2</sup>, Moon-Soo Chung<sup>2</sup>, Chul-Ho Yun<sup>3</sup>, Young Kun Shim<sup>4</sup>, Jaejun Oh<sup>4</sup>, Ui-Lim Choi<sup>1</sup>, and Gun Woong Lee<sup>1\*</sup>

<sup>1</sup>Jeonju AgroBio-Materials Institute, Future Agriculture Team, <sup>2</sup>Korea Atomic Energy Research Institute, Radiation Research Division, <sup>3</sup>Chonnam National University, School of Biological Sciences and Technology, <sup>4</sup>Microzyme Corp., Affiliated Institute

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is well known as a pathogen that infects plant host and causes leaf blight. Few bactericides have been developed that can effectively prevent and eliminate *Xoo*-induced diseases. The purpose of this study is to select candidates of antibacterial substances, essential oils, that suppress the growth of *Xoo* through several screening methods. The antimicrobial effect of the essential oil against *Xoo* was compared and analyzed using the drop method. According to the results of the inhibitory effect of the candidate materials, carveol showed the highest clear zone against *Xoo* among the six candidates (D-limonene, L-limonene, Carveol, D-limonene + carveol, L-limonene + carveol, and D-limonene + L-limonene). In addition, the carveol was developed a prototype in three different formulating types (coating, emulsion, and microemulsion) increasing the possibility of commercialization. Results of the present study suggest that the antimicrobial effect of essential oil was confirmed against *Xoo*. Also, the essential oil can be used to develop natural control agents against plant pathogens. [This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Crop Viruses and Pests Response Industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (321102-03-1-CG000).]

# Isolation and Biodegradation of Phenol-degrading Bacteria from North-Han River Watershed

Min Hui Kim and Song-Ih Han<sup>\*</sup>

Department of Microbial Biotechnology, Mokwon University

Phenol-degrading bacteria exist widely in the environments, and they are usually isolated from the phenolcontaminated site. The purpose of this study is designed to isolate and characterize the phenol-degrading bacteria. One hundred fifty isolates were isolated from the sedimentary layers of the North-Han River, and fifty-six isolates were screened for phenol degradation through a colorimetric method. Nine strains showed the activity of phenol oxygenase, and it was confirmed that 100 mg/ml phenol degradation during 24 h. Among them, *Azotobacter, Microvirgula,* and *Pseudomonas* strains were specifically degraded chloroform, toluene, and n-hexane. Obviously, the phenol-degrading isolates may be seen as an important tool in the bioremediation of wastewater effluent, and petrochemical complex.

### B067

**Isolation of Indole-3-Acetic Acid (IAA) Producing** *Arthrobacter* **Species and Study on Their Plant Growth Effects** Da Som Kim, Ho-Young Shin, and Song-Ih Han<sup>\*</sup> *Department of Microbial Biotechnology, Mokwon University* 

Plant Growth-Promoting Bacteria (PGPB) were producing plant growth hormones, bio-composting, and biodegradation of organic substances in the ecosystem. We isolated 104 isolates that confirmed plant growth-promoting abilities (Siderophores producing, Nitrogen-fixing, IAA activation). Especially, 13 isolates showed high IAA activity. These isolates were classified into seven genera; Arthrobacter, *Streptomyces, Bacillus, Pseudomonas, Rhodococcus, Flavitalea,* and *Massilia*. Among them, three isolates belong *Arthrobacter* gave maximum IAA production (169.5 mg/L) with the optimal medium containing R2A medium containing 0.1% L-tryptophan at 28°C for 24 h. To investigate the growth-promoting effects on the crops, *Arthrobacter* species were placed in water cultures and seed pots of mung beans. In consequence, the adventitious seed germination of mung beans was 73.4% higher than the control. In conclusion, the study suggests the IAA-producing *Arthrobacter* species as efficient biofertilizer inoculants to promote plant growth.

# Plant Disease Controlling Activities and Plant Growth Promoting Effect of Pseudomonas knackmussi SH-26

Ho-Young Shin, Da Som Kim, and Song-Ih Han<sup>\*</sup> Department of Microbial Biotechnology, Mokwon University

In this study, we conducted to investigate both plant growth-promoting and plant disease-controlling activities of bacterial strains isolated from soil. All the isolated strains were able to grow at various temperatures. All the strains, except SH-26, showed antagonistic effects against various phytopathogenic fungi. The strain exhibits remarkable root colonizing efficiency and acts strongly against phytopathogen like *Fusarium solani* (75–87.0%) under *in-vitro* conditions. This antagonism can be ascribed to the production of siderophores and antibiotic substances. In addition, all the strains showed abilities such as nitrogen fixation, phosphate solubilization, and siderophore production. Therefore, this study suggests that *Pseudomonas knackmussi* SH-26, which was selected through analysis of comparative advantages for both plant growth promotion and disease-controlling activity, may be used as a biological agent.

### B069

**Isolation and Characterization of β-Glucosidase Producing Yeast from Ginseng Byproduct** Min Young Kwon and Song-Ih Han<sup>\*</sup> *Department of Microbial Biotechnology, Mokwon University* 

β-Glucosidases are observed in all domains of living organisms, it is essential for removing nonreducing terminal glucosyl residues from saccharides and glycosides, which are used in various fields. We isolated 10 strains of β-Glucosidase producing bacteria from the ginseng byproduct. Phylogenetic analysis among 10 strains from ginseng byproduct confirmed that one yeast belongs to the *Rhodotorula*, nine bacterial strains belong to *Pseudomonas* (7 strains), and *Brachybacterium* (2 strains). Especially, verifying yeast's β-glucosidase activity, GPY-1 has been selected as high perform yeast. Enzymatic characteristics of β-glucosidase produced by GPY-1, high production of β-glucosidase activity was appealed at 30°C and stability showed the range of 20–40°C. Optimum pH showed the highest activity at 5.0, and enzyme stability was maintained up to pH 5.0–8.0. β-Glucosidase produced by the *Rhodotorula* sp. GPY-1 showed activity in converting ginsenosides Rb1 into minor ginsenosides. In addition, *Rhodotorula* sp. GPY-1 demonstrated protease, cellulase activity, and antibacterial against the ginseng root rot disease by *Botrytis*. It is expected to be used for ginseng cultivation and the production of effective components from ginseng byproducts.

### Growth Inhibitory Effect by Limonene and Carveol against Xanthomonas oryzae pv. oryzae

Hyeonbin Kim<sup>1</sup>, Mi Hee Kim<sup>1</sup>, Sungbeom Lee<sup>2</sup>, Moon-Soo Chung<sup>2</sup>, Chul-Ho Yun<sup>3</sup>, Young Kun Shim<sup>4</sup>, Jaejun Oh<sup>4</sup>, Ui-Lim Choi<sup>1</sup>, and Gun Woong Lee<sup>1\*</sup>

<sup>1</sup>Jeonju AgroBio-Materials Institute, Future Agriculture Team, <sup>2</sup>Korea Atomic Energy Research Institute, Radiation Research Division, <sup>3</sup>Chonnam National University, School of Biological Sciences and Technology, <sup>4</sup>Microzyme Corp., Affiliated Institute

The antibacterial activity of limonene and its derivatives against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was tested. In broth media respectively added with carveol, D-limonene, L-limonene, and mixtures with two compounds by concentration, the growth inhibition of *Xoo* was measured and compared. In the further study, the growth inhibition of *Xoo* was measured by three formulating methods (coating, emulsion and microemulsion type) with the test compounds. As a result, the growth inhibition of carveol was better than that of D-limonene and L-limonene. The growth inhibition of the mixtures with two compounds was more effective than that of the single compounds. Then, pot experiment and detached leaves assay were performed on the emulsion type of carveol and D-limonene + L-limonene that were showing effective growth inhibition against *Xoo*. In addition, qRT-PCR was performed to confirm that the test compounds affect the expression level of the virulence gene of *Xoo*. According to the result, the antibacterial activity of limonene and carveol against *Xoo* has been demonstrated. Limonene and carveol are expected to be used as natural microbicides.

[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Crop Viruses and Pests Response Industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (321102-03-1-CG000).

### B071

# **Methodological Approach for Identification of Antibiotic Resistance in Environmental Samples** Gihan Lee<sup>1,2</sup> and Keunje Yoo<sup>1\*</sup>

<sup>1</sup>Department of Environmental Engineering, Korea Maritime and Ocean University, <sup>2</sup>Interdisciplinary Major of Ocean Renewable Energy Engineering, Korea Maritime and Ocean University

Antibiotics from the 1920s to the present have been widely used in human therapeutic and livestock settings. However, the overuse and misuse of antibiotic drugs have caused the development and dissemination of antibiotic resistance genes (ARGs) and bacteria in different environments, such as soil, wastewater, livestock, compost, lakes, oceans, and sediment. The emergence of ARGs could significantly increase the spread of antibiotic resistance, particularly via mobile genetic elements (MGEs), such as plasmids, integrons, and insertion sequences. Therefore, we suggested that the combination of molecular biotechnologies and bioinformatics tools to identify ARGs and MGE in the environment is needed to better understand ARGs' environmental behavior and evaluate the potential risks ARGs pose to human health. High-throughput qPCR and metagenomics provide details of ARG occurrences, abundance, and quantitative information regarding the ARG dissemination. This concept will also contribute to developing advanced detection technologies and equipment soon to set up environmental monitoring systems efficiently.

The Impact of Seasonal Hypoxia on Sulfur-oxidizing Bacteria in the Coastal Sediments of Jinhae Bay, South Korea Hyeonji Lee, Hyeyoun Cho, Yong Jae Baek, Sang Beom Baek, Ha Neul Kim, Ye Rim Lee, Seo Jin Lee, and Jung-Ho Hyun<sup>\*</sup>

Department of Marine Sciences and Convergent Technology, Hanyang University

Prokaryotic communities sensitively respond to environmental changes. To elucidate the impact of coastal hypoxia (oxygen-depleted water mass) development on benthic bacterial communities, we carried out 16S rRNA gene amplicon sequencing and analyzed some geochemical properties at two sites (Center of basin; CB and Dangdong; DD) in Jinhae Bay. The sulfate reduction (SR) rates at DD site (~968 nmol/cm<sup>3</sup>/d) were higher than CB site (~611 nmol/cm<sup>3</sup>/d) in surface sediments during hypoxia, which indicates that it promoted highly sulfidic conditions at DD site. In the case of bacterial communities, the difference in sulfur-oxidizing bacteria (SOB) was distinct in the surface sediments between CB and DD. *Thioprofundaceae* and *Woeceiaceae* (*y-proteobacteria* class) predominated during all times. On the other hand, major SOB groups at DD site shifted from *y-proteobacteria* to *Chlorobiaceae* (green sulfur bacteria) and *Sulfurovaceae* (*e-proteobacteria* class) along with the development of hypoxia. Since they are chemoautotrophs that use H<sub>2</sub>S and S<sup>0</sup> as energy sources, they prefer an environment in which H<sub>2</sub>S is continuously supplied. Therefore, H<sub>2</sub>S produced by SR is highly accumulated in DD site with severe hypoxia, which allowed it to appear these SOB groups within surface sediments. The above results suggest that seasonal hypoxia is an important factor in determining the ecological niche of benthic SOB in Jinhae Bay. [Supported by the NRF grant funded by the MSIT and project BK21 FOUR]

### B073

# Colonization and Surface Modification of Polyethylene Films by Thermophilic Microbial Communities Originated from Compost Samples

Joon-hui Chung, Ja-Yeon Lee, Si-Hyun An, Da-Yeon Kim, Jehyung Yeon, Jeong Jun Kim, Hang Yeon Weon, and Jae-Hyung Ahun<sup>\*</sup>

### National Institute of Agricultural Sciences

Biodegradation of plastics has been emerged against the global plastic pollution. Due to plastic wastes introduced during composting process, thermophilic compost samples have been known as one of the good resources for polyethylene (PE) biodegradable microbes. In an aseptic condition, the oxidation of PE film was observed in minimal media at 60°C after 2 weeks thus the incubation temperature was adjusted to 50°C. We collected compost samples from 24 composting facilities treating garbage and livestock manure in South Korea. PE-degrading microbes were enriched in the minimal media with PE as a sole carbon source at 50°C for 3 months. FT-IR spectra of the incubated PEs showed surface modification for the samples from Gwangyang, Goesan, and Jangsu. Microbial community analysis was conducted for the PE film and supernatant of the enrichment culture from Jangsu. PCA and NMMDS analysis of the bacterial communities showed the differences between PE films and supernatants. The relative abundances of *Thermobispora*, *Caldinitratiruptor*, and *Geobacillus* were higher on the films than in the supernatants, which are known as thermophilic bacteria. Thermophilic isolates were isolated from the enrichment culture and some of them showed the ability to use paraffin wax as a carbon source. These results suggested that the thermophilic microbes from compost samples may colonize and oxidize PE films.

[This work was supported by the National Institute of Agricultural Sciences (project no. PJ014974).]

# Ecology Role of a Novel Clade of *Thermoplasmatales* in Geothermal Springs Revealed by Culture and Genomic Analysis

Gi-Yong Jung<sup>1,2</sup>, Joo-Han Gwak<sup>2</sup>, Sung-Keun Rhee<sup>2</sup>, and So-Jeong Kim<sup>1\*</sup> <sup>1</sup>Mineral Resources Division, Korea Institute of Geoscience and Mineral Resources, <sup>2</sup>Department of Biological Sciences and Biotechnology, Chungbuk National University

Geothermal springs are a unique ecosystem and information on microorganisms inhabiting these environments are limited. Here, we isolated novel archaeal strain AK belonging to a novel clade of *Thermoplasmatales* from mud volcano sampled at Pisciarelli in Italy. Phylogenetic analysis showed that strain AK is closely related to alphabet-plasmas that were mostly detected in acid mine drainage (AMD). The strain AK grew at 50°C–60°C (optimum: 55°C) and pH 1.2–2.4 (optimum: 1.4–1.8). The results represented that strain AK is a thermoacidophilic archaeon. Genomic analyses revealed that strain AK is an aerobic heterotroph with ability to uptake and utilize organic compounds including peptides and amino acids. Lack of genes for some amino acid synthesis pathways suggested that this strain might rely on organic compounds produced by other community members in the environments. Strain AK was grown on yeast extract, beef extract, peptone, and tryptone as a sole substrate. Single organic compounds such as glucose were utilized in the presence of yeast extract (over 0.0001%). The results indicate that strain AK may function as a heterotrophic scavenger. These results of cultivation and genomic analysis expand the understandings of alphabet-plasmas of *Thermoplasmatales* in geothermal springs and AMD. [Supported by the Basic Research Project of the KIGAM (22-3412) and a grant from NRF (2022R1A2C1091457).]

### B075

The Self-bleaching Process of *Microcystis aeruginosa* is Delayed by a Symbiotic Bacterium *Pseudomonas* sp. MAE1-K and Promoted by Methionine Deficiency

Jaejoon Jung, Ju Hye Baek, and Che Ok Jeon<sup>\*</sup> Department of Life Science, Chung-Ang University

Various interactions between marine cyanobacteria and heterotrophic bacteria have been known, but the symbiotic relationships between *Microcystis* and heterotrophic bacteria remain unclear. An axenic *M. aeruginosa* culture (NIES-298) was quickly bleached after exponential growth, whereas a xenic *M. aeruginosa* culture (KW) showed a normal growth curve, suggesting that some symbiotic bacteria may delay this bleaching. The bleaching process of *M. aeruginosa* was distinguished from the phenomena of previously proposed chlorosis and programmed cell death in various characteristics. Bleached cultures of NIES-298 quickly bleached actively growing *M. aeruginosa* cultures, suggesting that *M. aeruginosa* itself produces bleach-causing compounds. *Pseudomonas* sp. MAE1-K delaying the bleaching of NIES-298 cultures was isolated from the KW culture. Bleached cultures of NIES-298 treated with strain MAE1-K lost their bleaching ability, suggesting that strain MAE1-K rescues *M. aeruginosa* from bleaching via inactivation of bleaching compounds. From Tn5 transposon mutant screening, a *metZ* mutant of strain MAE1-K (F-D3) unable to synthesize methionine, promoting the bleaching of NIES-298 cultures was promoted with the coculture of mutant F-D3 and delayed by methionine supplementation, suggesting that the bleaching process of *M. aeruginosa* is promoted by methionine deficiency

# Profiling of Microorganisms and Oxidation Enzymes in the Plastisphere of Polyethylene Using a Metagenomics Approach

Sang-Gyu Kim<sup>1</sup>, Nam-Hoon Kim<sup>1</sup>, Dong-Hwan Kim<sup>1</sup>, Seong-Jun Jo<sup>1</sup>, Jae-Hyung Ahn<sup>2</sup>, Joon-Hui Chung<sup>2</sup>, and Dae-Wi Kim<sup>1\*</sup>

<sup>1</sup>Division of Life Sciences, Jeonbuk National University, <sup>2</sup>Agricultural Microbiology Division, National Institute of Agricultural Sciences

The excessive and widespread uses of polyethylene (PE) resulted in its accumulation in the environment as among the most threatening pollutants. However, microorganisms and enzymes responsible for PE degradation have not been characterized. A metagenomics has been recognized as an appropriate tool to scrutinize cryptic metabolic ability including PE-degradation in the plastisphere. Here, to derive plastisphere-specific microorganisms and enzymes, we compared microbial community structures and oxidation enzyme contents in the plastispheres of waste PE films with their surrounding soils using assembled contigs. The phylum actinobacteria was found to be enriched in the plastispheres. All of 25 genera enriched in the plastispheres belonged to actinobacteria. Among those, *Mycolicibacterium, Gordonia*, and *Aeromicrobium* were the most distinct and abundant genera in the plastispheres and the surrounding soils, which was caused by oxidation enzymes originated from actinobacteria. Furthermore, orthologous group-based comparison and phylogenetic convergence analyses of oxidation enzymes elucidated potential PE-oxidation enzymes. The information for plastisphere-specific microorganisms and oxidation enzymes can be further applied to identify potential PE-degrading bacteria and enzymes. [Supported by a grant from Rural Development Administration (PJ014974).]

### B077

**Changes in Bacterial Community Structure in Upland Soil According to Time Period** Youngmi Lee, Jiyoung Yoon, Jung-Rai Cho, Seulgi Lee, and Sung-Jun Hong<sup>\*</sup> *Organic Agricultural Division, National Institute of Agriculture Sciences, Rural Development Administration* 

Organic farming is a sustainable agriculture system that preserve the ecosystem because pesticides and chemical fertilizers are not used. Microorganism is known to promoting plant growth and control pathogens, but soil microbial communities and functional research in organic farmland are still insufficient.

This study was conducted to investigate the changes in bacterial community in Chinese chives soil according to treatments and time period. Organic materials were treated with animal manure, microorganism, organic matter, and the cultivated soil in the first and second years were compared. The composition of bacterial communities was analysed using 16S rRNA genes. It was found that Proteobacteria, Firmicutes, Actinobacteria were dominant phylum in all treatments and time period. It is noteworthy that there is difference in Cyanobacteria, Bacteriodetes, and Chloroflexi between year of cultivation. The sequences were clustered into OTUs were higher in the second year cultivated soil. Also, difference in bacterial community was lower and the distance close. Rarefaction curve is higher in second cultivated soil. These results speculate that bacterial community were stabilized result of microorganisms settled in the soil over the years of cultivation. In conclusion, this study further support assertions that the microbial communities could be used biocontrol for plant health and defense if there's an additional study such as environmental and microbial functional research.

**Purification and Characterization of Novel Plant Growth Promoting Salt-tolerant Halophyte Rhizosphere Bacteria** Hye-Eun Choi, Min Hui KIM, Da Som Kim, Ho-Young Shin, Min Young Kwon, Hyun-Soo Roh, and Song-Ih Han<sup>\*</sup> Department of Microbial Biotechnology, Mokwon University

Halophytes are naturally salt-tolerant plants that have evolved to grow in saline soils, and have been widely studied, but little is known about their associated bacteria. We isolated bacteria using various cultural methods from indigenous halophytes rhizosphere in Coastal dunes. A total of 264 isolates were identified by 16S rRNA gene sequencing analysis. Phylogenetic analysis of 16S rRNA genes, these isolates were divided into 5 phyla:  $\alpha$ -,  $\beta$ -,  $\gamma$ -*proteobacteria, Actinobacteria, Firmicutes, Bacteriodetes,* and *Verrucomicrobia.* In particular, 26 strains were at least a 16S rRNA gene similarity value of < 96.1% and/or > 98.7% with another species, proposed to novel genus or species. These novel bacteria confirmed the plant growth promoting activity within siderophore productivity, N2-fixing, IAA production ability, and phosphorus solubilization activity. Isolates were tested for maximum salt tolerance, and some were able to grow in the presence of up to 10% NaCl. As a result, these strains can be grown under high salinity conditions and are expected to be used as halophytes growth promoters in salt soils.

### B079

Community Analysis of Lactic Acid Bacteria by Culture-dependent Method from Environmental Samples in Jeju Island

Hina Ayub<sup>1</sup> and Man Young Jung<sup>1,2\*</sup>

<sup>1</sup>Interdisciplinary Graduate Program in Advance Convergence Technology and Science, Jeju National University, <sup>2</sup>Department of Biology Education, Jeju National University

The study of lactic acid bacteria (LAB) is more and more paid attention to, not only in the food industries but also in the medical and health-related industries. Various microbial species, including bacteria, fungi, yeast, cyanobacteria, and algae, produce lactic acid using carbohydrates as the only primary carbon source. Jeju Island, created by volcanic activity, has a specific natural ecosystem, and its flora and fauna differ from mainland Korea. Therefore, an analysis of microbial diversity in Jeju Island could be worth considering. In this study, we identified the LAB diversity in different environmental samples (marine water, seaweed, and forest wetted and dried soil) in Jeju Island by the culture-dependent method using LAB-specific growth media. Genus *Bacillus* and *Lysinibacillus* dominated in dried and wetted soil, respectively. In contrast, specific bacterial strains, *Vibrio, Paracoccus, Yokenella*, and *Staphylococcus* were only identified in marine water and seaweed samples. Approximately 80 to 85% of bacteria isolated from marine water and soil samples are pathogenic, even though we used LAB-specific media. Therefore, it is worth checking the pathogenic facility and identifying the fermentation product in future studies to apply the isolated microbial strains.

# Unraveling the Ecosystem Adaptative Strategies Exhibited by a AOA Strain, *Nitrosocosmicus oleophilus* MY3 Saem Han<sup>1</sup> and Man-Young Jung<sup>1,2\*</sup>

<sup>1</sup>Interdisciplinary Graduate Program in Advance Convergence Technology and Science, Jeju National University, <sup>2</sup>Department of Biology Education, Jeju National University

Nitrification is an important process of the biogeochemical nitrogen (N) cycle. Ammonia oxidation, the first step of nitrification, is mediated by ammonia-oxidizing archaea (AOA), a key player of ammonia-oxidizing microbes. In this study, physiological and comparative genomic analyses were performed to characterize the differential adaptive features displayed by the *Nitrosocosmicus oleophilus* MY3, a representative soil AOA strain, across various environmental factors. Hydrophobic cell surface and biofilm formation ability are the attributes that enable its proliferation among different habitats. Hence, the effect of substrate on biofilm formation of strain MY3 was identified by determining the degree of biofilm formation according to the ammonia concentration. Furthermore, we observed high hydroxylamine accumulation at low pH, implicating that hydroxylamine became more stable with low pKa (~5.9) displayed during ammonia oxidation by the strain MY3. Additionally, the transcription level of the ammonia transporter (amt) gene of the strain MY3 was higher at low ammonia concentration, which is indicative of its low ammonia affinity. Taken together, this study will enhance the existing knowledge and provide deep insights into the distinctive adaptation strategies of strain MY3.

[This research was supported by National Research Foundation of Korea (NRF) funded by the Ministry of Education (2019R1A6A1A10072987), and MSIT (NRF - 2021R1C1C1008303)]

# Comparative Analyses of Kimchi Fermentation Process according to the *Leuconostoc mesenteroides* Strains as Starters

Hye Su Jung, Jeong Ui Yun, Mi-Ja Jung, Hye Seon Song, Yeon Bee Kim, Yujin Kim, Tae Woong Whon, and Se Hee Lee<sup>\*</sup> Kimchi Functionality Research Group, World Institute of Kimchi

*Leuconostoc mesenteroides* is a major lactic acid bacteria in kimchi and is used to maintain uniformly high quality during kimchi fermentation. To investigate the fermentative features according to the *L. mesenteroides* as starters, kimchi samples were prepared using the four strains (*L. mesenteroides* subsp. *mesenteroides* WiKim32, *L. mesenteroides* subsp. *jonggajipkimchii* WiKim33, *L. mesenteroides* subsp. *mesenteroides* WiKim0121, and *L. mesenteroides* subsp. *dextranicum* CBA3628) accompanied by non-starter kimchi and microbial communities, metabolites of kimchi were analyzed during the fermentation. Additionally, genomes of the four starter strain performed comparative analyzes. The genes involved in the metabolism of various carbon sources (glucose, fructose, sucrose, cellobiose, gluconate, ribose, xylulose, galactose, arabinose, and ribulose) were present in strains WiKim32, WiKim33, and WiKim0121. However, strain CBA3628 lacked genes involved in the metabolism of galactose, arabinose, and ribulose. Microbial communities analysis showed that *L. mesenteroides* ASVs were predominant only in the kimchi containing strains WiKim32, WiKim33, and WiKim0121, regardless of seasoning types. After 33 days of fermentation, the decrease rate of major carbon sources and production rate of final products were different between starter kimchi and the slowest for non-stater kimchi. The fermentation features of kimchi are determined differently depending on the starter, even for the same species.

### B083

# Interaction between Ammonia, and Methane Oxidizers on Various Copper Concentrations Modulating the Nitrification

# Min-Ju Kang<sup>1</sup>, Miye Kwon<sup>2</sup>, and Man-Young Jung<sup>1,3\*</sup>

<sup>1</sup>Interdisciplinary Graduate Program in Advance Convergence Technology and Science, Jeju National University, <sup>2</sup>Biodiversity Research Institute, Jeju Technopark, <sup>3</sup>Department of Biology Education, Jeju National University

Copper (Cu) is an essential element involved in various physiological processes, including the electron transport chain, oxygen transportation, and denitrification. However, copper concentrations above moderate levels make reactive oxygen species and become toxic to cells. Here, we determined the modulations in the nitrification process by incubating ammonia and methane oxidizers with different copper concentrations. At low copper concentrations, methanotrophs produce and release methanobactin, which is then reabsorbed in a copper-bound state for use in pMMO. In this study, the modifications in the activity of a methanotroph, *Methylosinus trichosporium* OB3b, and an ammonia oxidizer, *Nitrosomonas europaea*, were detected in a co-culture containing various Cu concentrations. Strain OB3b is known to produce methanobactin at a copper concentration in which the activity of the ammonia oxidation is inhibited. Subsequently, a change in the nitrification process is likely to have occurred. To the best of our knowledge, this is the first study exploring the link between methane and ammonia oxidation processes by each type of autotrophic oxidizer. Overall, this study will provide a deep understanding of controlling nitrification through copper, and methanotrophs, which can be used for beneficial purposes like improving the nitrification activity in the soil environment, and restoration of wastewater treatment plants with lost nitrogen removal efficiency.

#### Bambusicolous Apiospora: Investigation of Apiospora Community Variation in the Bamboo Forest

Sun Lul Kwon, Minseo Cho, Yeonjae Yoo, Sang Hyun Lee, Young Min Lee, Gyu-Hyeok Kim, and Jae-Jin Kim<sup>\*</sup> Division of Environmental Science & Ecological Engineering, College of Life Sciences & Biotechnology, Korea University

Many *Apiospora* species have been reported as plant pathogens causing black dots on the bamboo culms. However, they were also found in healthy leaves and culms of bamboo as endophytes. Although bambusicolous *Apiospora* has been studied on its pathogenicity and biological activities, the community study of *Apiospora* was not conducted. Therefore, in this study, the *Apiospora* communities in bamboo were mainly targeted according to the bamboo host, organs, and state. Based on the high-throughput sequencing analysis using ITS2 rDNA region, the *Apiospora* communities in the three different bamboo species (*Phyllostachys bambusoides, Ph. pubescens,* and *Ph. nigra* var. *henonis*) with unidentified bamboo were investigated. The bamboo materials were collected according to the three states (health, disease, and death) and six organs (culms, leaves, roots, shoots, shoot roots, and rhizome roots) with bamboo forest soil. Through this, we confirmed 1) the structures and diversity of the *Apiospora* communities in the bamboo forest were presented, and the variation of the *Apiospora* community along with the bamboo state and organs were suggested with the statistical analysis. [Supported by grants from National Research Foundation of Korea (NRF) [2021R1A2C1011894]]

### B085

# Comparative Genomic, Metabolic, and Safety Characteristics of *Enterococcus faecium* and *Enterococcus lactis* Revealed by Pangenome Analysis

Dae Gyu Choi, Dong Min Han, Ju Hye Baek, Jeong Min Kim, and Che Ok Jeon<sup>\*</sup> Department of Life Science, Chung-Ang University

Enterococcus includes both harmful and beneficial species and is ubiquitously present in natural environments. In this study, we sequenced the high-quality genomes of 15 *Enterococcus lactis* and 5 *faecium* strains and compared the genomes with publicly available genomes of *E. faecium* and *E. lactis* strains. We reassess their phylogenetic relationships based on the core genome, which revealed that *E. faecium* and *E. lactis* strains were separated into two main clades. Most of the food isolates belonged to the *E. lactis* clade, while *E. faecium* clade comprised strains mainly isolated from clinical samples. Genome analysis based on antibiotics resistance and virulence factors showed a clear separation between *E. faecium* and *E. lactis* clade. Genomic and metabolic features indicated that genes associated with carbohydrate, amino acid metabolism and membrane transport in *E. lactis* and *E. faecium* clade, respectively, were most abundant. At the tertiary level of carbohydrates metabolism, the pan- and core-reactome were mainly associated with glycolysis/gluconeogenesis, fructose and mannose metabolism, starch and sucrose metabolism, galactose metabolism. The carbohydrates reconstructed pathways showed that strains from both clades had the ability to perform homo-lactic and hetero-lactic fermentation. Our study, including genomic comparisons, and identification of metabolic characteristics, highlights the genetic and metabolic diversity between *E. lactis* and *E. faecium* clades.

# Water Depth, One of the Major Factors that Modulate Gill Microbiome of Seven Wild Fish Species

Gyeong Hak Han<sup>1,2</sup>, Jihyun Yu<sup>1,2</sup>, Min Joo Kang<sup>3,4</sup>, Choong Hwan Noh<sup>3</sup>, Yun Jae Kim<sup>1</sup>, and Kae Kyoung Kwon<sup>1,2\*</sup> <sup>1</sup>Marine Biotechnology Research Center, Korea Institute of Ocean Science and Technology, <sup>2</sup>KIOST School, University of Science and Technology, <sup>3</sup>Marine Bio-Resources Research Unit, Korea Institute of Ocean Science and Technology, <sup>4</sup>Department of Marine Biology, Pukyong National University

Fish plays important roles in aquatic environment, as nutrients recycling and vectors for microorganisms. However, the microbial ecology has been understudied for most wild fish and for fish body excluding the intestines. The gills of fish are responsible for multiple physiological functions including respiration, osmoregulation, pH regulation and mucosal immunity. Therefore, gill-associated microbiomes are predicted to play a key role in the health and physiology of fish. In this study, 16S rRNA gene amplicon sequencing was conducted against 7 wild fish species (AS, *Acanthopagrus schlegelii*; PP, *Parajulis poecilepterus*; HA, *Hexagrammos agrammus*; SI, *Sebastes inermis*; SQ, *Seriola quinqueradiata*; SM, *Sebasticus marmoratus*; CS, *Chelidonichthys spinosus*) purchased from port market of Tongyeong city (Samdeok port market) to understand the factors that affect their gill microbiome. We found that, in the gill microbiome of all fish, the most abundant phylum was *Proteobacteria*. At the genus level, *Vibrio* and *Phaeobacter* were observed to be antagonistic as well as dominant. Beta-diversity analysis showed significant divergence between AS, HA, SQ, and CS with Bray-Curtis distance metrics. Additionally, AS and HA showed a clear difference in species diversity. Considering the ecological characteristics of the fish used in the analysis, the composition of the gill microbiota of wild fish appears to be greatly affected by water depth.

### B087

# A Pilot Study on the Alteration of Soil Microbial Community by Wild Plant Species

Wanro Kim<sup>1</sup>, GyuDae Lee<sup>2</sup>, Raoul Colince Kuate<sup>3</sup>, Subeen Lee<sup>2</sup>, and Jae-Ho Shin<sup>1,2,4\*</sup> <sup>1</sup>Department of Integrative Biology, Kyungpook National University, <sup>2</sup>Department of Applied Biosciences, Kyungpook National University, <sup>3</sup>Department of Food Security and Agricultural Development, Kyungppok National University, <sup>4</sup>NGS Core Facility, Kyungpook National University

Plants and environments interact constantly and dynamically construct ecosystems. In particular, the area where roots and soil face each other is a window through which plants and environments communicate directly. Thereat characteristic microbiota associated with plant species is formed.

We sampled on the campus of Kyungpook National University located in 80, Daehak-ro, Buk-gu, Daegu, Republic of Korea. At one sampling site, the roots and bulk soils of four species of plants, *Artemisia princeps, Erigeron canadensis, Erigeron annuus, and Ehrharta erecta,* were collected, and a total of three sampling sites were selected. After pretreatment, sequencing of roots and bulk soils was performed with MiSeq using 16S rRNA V4-V5 region.

We conducted microbiome analysis through MicrobiomAanalyst. As the result, there is a significant difference in the diversity of the microbial community according to plant species even in the same location. In our study, we were able to observe changes in microbial communities induced through plant interactions in addition to environmental factors.

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# Growth Prediction of Burkholderia spp. Depending on Temperature

Chang Og Geum<sup>1</sup>, Sang-Min Kim<sup>1</sup>, Hyunjung Chung<sup>1</sup>, Tae Hwan Noh<sup>1</sup>, Jun-Yeol Choi<sup>1</sup>, Bong Choon Lee<sup>2</sup>, and Soo Yeon Choi<sup>1\*</sup>

<sup>1</sup>Crop Foundation Research Division, National Institute of Crop Science, Rural Development Administration <sup>2</sup>Crop Protection Division, National Institute of Agricultural Sciences, Rural Development Administration

Bacterial grain rot (BGR) caused by *Burkholderia* species is one of the important diseases in rice (*Oryza* sativa L.). Infection of the *Brukholderia* spp. in the rice panicles reduces the rice yield due to discoloration and poor ripening. Recently, incidence of BGR has been increasing because of climate change. In this study, two experiments were conducted to predict the occurrence of BGR by temperature in preparation for climate change. In the first experiment, to compare the growth of *Burkhoderia* spp. by temperature (25, 30, 35°C), the medium was inoculated with a bacterial concentration of 10<sup>5</sup> CFU/ml and observed for 3 days. As a result, it was confirmed that the pathogens grew the fastest at 35°C until 12 h, the early stage of growth. In the second experiment, the motility of *Burkholderia* spp. was measured depending on temperature for 3 days. As a result, it was observed that the mobility of strains 4 and 10 increased significantly as the temperature increased. This experiment suggests that the higher the temperature, the faster the growth and motility of pathogens are affected.

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# B089

# Seasonal Variations of Airborne Microbial Assemblages of Seoul Metropolitan Rapid Transit Subway SMRT, South Korea

Zohaib Ul Hasan<sup>1,2,3</sup>, Hana Cho<sup>4</sup>, Changwoo Park<sup>1,2,5</sup>, Yong-Hyeon Yim<sup>3,4</sup>, and Seil Kim<sup>1,2,3\*</sup> <sup>1</sup>Group for Biometrology, Korea Research Institute of Standards and Science (KRISS), <sup>2</sup>Convergent Research Center for Emerging Virus Infection, Korea Research Institute of Chemical Technology (KRICT), <sup>3</sup>Department of Bio-Analytical Science, University of Science & Technology (UST), <sup>4</sup>Inorganic Metrology Group, Division of Chemical and Biological Metrology, Korea Research Institute of Standards and Science, <sup>5</sup>Department of Agricultural Biotechnology, Seoul National University (SNU)

SMRT has developed to become one of the world's greatest public transportation networks. The average number of passengers traveling daily is 7.2 million, and the total number of people traveling annually is 2.6 billion. We used 16S rRNA and ITS sequencing to estimate the seasonal airborne microbial diversity profiles at SMRT sites in this work. Particulate matter samples were taken from air purifiers installed at SMRT subway station platform areas. Three stations, including the busiest, were chosen for sampling. The sampling was carried out each season during 2019. After extracting the total DNA from all seasonal samples, PCR were performed with Illumina overhang adapter primers for the V3-V4 region of 16S rRNA gene and ITS2 region of the ITS gene. Sequencing was carried with the Illumina Miseq Sequencing system (Illumina). The SMRT microbiome had a high level of taxonomic diversity, with the most prevalent bacterial species related with skin. Overall, stations included in this study harbored different phylogenetic communities based on  $\alpha$ -diversity and  $\beta$ -diversity comparisons. Microbial assemblages also varied depending upon the season sampled, as well as the station. This study is the first extensive microbiome study at one of the world's largest mass transit subway systems. The study shows that the microbial composition of the SMRT subway stations comes from a diverse combination of environmental, human sources, seasons and the lifestyle of commuters.

# Evolution of Lactobacillus delbrueckii Subspecies

Donghoon Shin<sup>1,2</sup>, Min-gyung Baek<sup>2</sup>, and Hana Yi<sup>1,2,3\*</sup> <sup>1</sup>Integrated Biomedical and Life Science, Graduate School, Korea University, <sup>2</sup>Interdisciplinary Program in Precision Public Health, Korea University, <sup>3</sup>School of Biosystems and Biomedical Sciences, Korea University

Lactobacillus delbreuckii comprises six subspecies (bulgaricus, lactis, jakobsenii, delbreuckii, sunkii, and indicus). According to previous genomic analysis, the two major subspecies bulgaricus and lactis, adapt to different environments and have evolved with different characteristics in glucose metabolism. Therefore, this study aims to identify the evolutionary trend in the *L. delbreuckii* group through a comparative genomic analysis on its six subspecies and examine a genomic characteristic that can be used to distinguish between the subspecies. A total of 31 genomes, including 6 type strain, showed that the overall evolutionary trend in *L. delbreuckii* was an increase in the number of genes due to mobile elements and gene fragmentation. Phylogenomic analyses confirmed that *L. delbrueckii* divided into three broad lineages. The repertoire of subspecies-specific gene generation through gene gain and loss differentiated from other subspecies a long time ago and independently underwent convergent evolution. The *lactis-jakobsenii-delbrueckii-sunkii* (LIDS) group, which includes the *lactis* subspecies, was a heterogeneous group that comprised four subspecies and showed an evolutionary trend of increasing genetic variability with increasing genome size. The *indicus* lineage showed an independent evolutionary trend. The results of this study are significant as they explain the causes of differing habitat and nutritional requirements for different subspecies of *L. delbrueckii* using the genomic evolutionary trends.

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# B091

**Short-chain Alkanes Consumption in** *Mycobacterium* **sp. SM1 Isolated from an Acidic Geothermal Environment** Samuel Imisi Awala, Joo-Han Gwak, Chanmee Seo, Lorraine A. Bellosillo, Yong-Man Kim, Ok-Ja Si, and Sung-Keun Rhee<sup>\*</sup>

Department of Biological Sciences and Biotechnology, Chungbuk National University

Thermoacidophilic verrucomicrobial methanotrophs are the main biological sink of methane, a potent greenhouse gas, from acidic geothermal environments. However, little is known about the microbial sink of climate-active gases such as short chain alkanes (SCAs), e.g., ethane and propane, released from these environments. Here, we describe the isolation of an extremely acidophilic gaseous-alkane-oxidizing bacterium, *Mycobacterium* sp. strain SM1, that grows at pH 1-6 (optimal 2-4). The pure isolate was recovered from a methane+propane-utilizing enrichment culture from a geothermal sample and utilized C<sub>2</sub>-C<sub>4</sub> alkanes and a variety of multi-carbon compounds. Analysis of the draft genome of strain SM1 revealed the presence of the *hmoCAB*, a type of the CuMMO family. Gene clusters of a group I (*tmoABCDEF*) and II (*dmpLMNOQBP* and *dmpHFGEIC*) soluble di-iron monooxygenases (SDIMO) involved in toluene and phenol degradation but not gaseous alkanes were also found. Complete inhibition of SM1 growth on C<sub>2</sub>-C<sub>4</sub> alkanes is revealed by physiological experiments in the presence of ATU but not glucose, indicating *hmoCAB* is responsible for oxidation of C<sub>2</sub>-C<sub>4</sub> alkanes. This is the first report of a CuMMO-containing acidophilic bacterium excluding the verrucomicrobial methanotrophs in acidic geothermal fields, capable of oxidizing SCAs. Finally, It expands the substrate spectrum of CuMMO-containing microbes in acidic geothermal thermal ecosystems and their contribution to global SCAs cycles.

# **Resistance to H**<sub>2</sub>**O**<sub>2</sub> is a Key Factor in Community Development of Ammonia-oxidizing Archaea in Rhizosphere Ui-Ju Lee, Joo-Han Gwak, Seung-Yeon Choi, Ok-Ja Si, and Sung-Keun Rhee<sup>\*</sup> Department of Biological Sciences and Biotechnology, Chungbuk National University

The rhizosphere microbiome has a profound influence on plant growth and development. There has been relatively limited study focusing on root archaeal microbiome and its interactions with plants. In this study, we analyzed the ammonia-oxidizing archaea (AOA) community associated with the roots of pepper plants. A distinct AOA community was developed in the rhizosphere of pepper plants from bulk soils. A group I.1b AOA clade, "*Ca*. Nitrosocosmicus", prominently contributed to the clustering of the rhizosphere archaeal communities distinct from those of bulk soils. A key genomic trait of the clade "*Ca*. Nitrosocosmicus" is the possession of a manganese catalase (Mn catalase) and the activity of this enzyme is proposed to be crucial in AOA selection and enrichment from pepper rhizosphere. The demonstration of catalase activity of a representative strain "*Ca*. Nitrocosmicus" oleophilus" MY3 and the increased copy numbers of thaumarchaeal Mn catalase gene in ratio to archaeal *amoA* gene in soil slurries amended with H<sub>2</sub>O<sub>2</sub> supported this hypothesis. A distinct AOA community attributed to "*Ca*. Nitrosocosmicus" clade predominance in the 2-, 4-, and 6-year-old ginseng plant rhizosphere cultivated in a different geographical region was also observed. This study suggests a key AOA clade for investigating plant–AOA interactions which is crucial for sustaining agricultural productivity while diminishing environmental pollution.

### B093

### The Effect of Cold Temperature on the Growth and Diversity of Bacteria Isolates from Antarctica

Ju Hong Kang<sup>1</sup>, Ji Hyeon Lee<sup>1</sup>, Rye Gyeong Park<sup>1</sup>, Ji Eun Lee<sup>1</sup>, Jae Won Lee<sup>1</sup>, Won Ho Jeong<sup>1</sup>, Sung Ju Cho<sup>2</sup>, Hyun Cheol Oh<sup>3</sup>, and Jae Hak Sohn<sup>1,2\*</sup>

<sup>1</sup>Maior in Food Science and Culinary Arts, College of Health and Welfare, Silla University, <sup>2</sup>Seafood Research Center, IACF, Silla University, Advanced Seafood Processing Complex, <sup>3</sup>Institute of Pharmaceutical Research and Development, College of Pharmacy, Wonkwang University

The purpose of this study is to investigate the diversity and cold adaptability of bacterial strains isolated from samples collected in Antarctica (Southern Chile, King George Island, Jangbogo Antarctic Research Station, King Sejong Antarctic Research Station, and Ross Sea). Sample diluted by 10 fold dilution method after homogenization, were inoculated on NA, 0.1% NA, and R2A agar plate and then incubated at  $15^{\circ}$ C for 20 days. The 404 bacterial strain were isolated by morphological properties of the grown group and preserved in 15% (v/v) glycerol solution. For the growth of bacterial strain in different temperatures, bacterial strain were inoculated in NA and then cultured at  $5^{\circ}$ C,  $10^{\circ}$ C and  $25^{\circ}$ C for 20 days. Among the strains to be tested, 79 (19%) bacterial strains were psychrophiles, 269 (64%) were pychrotolerants, and 71 (17%) were mesophiles. As a result of phylogenetic analysis based on the 16S rRNA gene sequence, bacteria consisted 202 taxa from 36 genera.

### Growth of Ammonia Oxidizing Archaea on Floating Filter

Christiana Abiola, Joo-Han Gwak, Ui-Ju Lee, Olabisi Adebisi, Ok-Ja Si, and Sung-Keun Rhee<sup>\*</sup> Department of Biological Sciences and Biotechnology, Chungbuk National University

Nitrososphaerales affiliated with the group I.1b of the phylum Thaumarchaeota represent is a key ammoniaoxidizing archaea (AOA) in terrestrial environments. So far, there is no feasible technique to isolate colonies of AOA. In this study, we report growth of strain *Nitrososphaera viennensis* (EN76), a representative AOA of group I.1b, on floating filter. At the same time, it was compared with those in liquid medium. After 1 mM ammonia oxidation in liquid cultures, cell density of about 10<sup>7</sup> cells/ml of EN76 culture was diluted ten-fold giving varying cell density in each dilution. One milliliter from the diluted cultures was inoculated on floating filters with a range of 10<sup>7</sup> cells/filter to 10<sup>3</sup> cells/filter as inoculum cell density on 10 ml liquid media. The same dilutions were inoculated in the liquid medium as controls. Cell growth of strain EN76 were observed as nitrite concentration was increased in liquid media below floating filter. We look further to isolating colonies of strain EN76 on the floating filters, as well as comparing with the growth of strain "*Ca.* Nitrosoarchaeum koreensis" (MY2), a representative AOA of group I.1a, on floating filters. These findings will expand our knowledge on developing new isolation techniques for novel clades of AOA from terrestrial environments.

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### B095

### Adaptation and Tolerance to Hypotonicity, a Niche Differentiating Factor for Nitrifiers

Olabisi Adebisi, Ui-Ju Lee, Joo-Han Gwak, Christiana Abiola, Ok-Ja Si, and Sung-Keun Rhee<sup>\*</sup> Department of Biological Sciences and Biotechnology, Chungbuk National University

Nitrification, the sequential aerobic oxidation of ammonia to nitrate via nitrite, is considered a central part of the global biogeochemical nitrogen cycle. This process is microbially executed by three different groups of ammonia-oxidizing microorganisms, i.e., ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and complete ammonia oxidizer (comammox). The major factors that determine the population kinetics and nitrifying activities of these microorganisms and their possible inclusion in the production of nitrous oxide (a greenhouse gas) are not fully understood. Here, we report the effect of low salinity with conductivity ranging from 71 µS/cm to 2700 µS/cm of soil pore water media (SPWM) on the nitrification activity of AOA groups 1.1a (*Nitrosotenuis chungbukensis, Nitrosoarchaeaum koreensis*) and 1.1b (*Nitrososphaera viennensis*). Low salinity negatively affects archaea's growth and nitrifying activities; however, the effect is lesser in group 1.1b compared to group 1.1a AOA. Besides, low salinity had no significant effect on AOB and comammox. This variation might be due to their differences in cell structure and morphology, and particularities in adaptation to environmental osmolarity. Furthermore, the kinetics analysis indicates that each of these groups of nitrifiers has varying tolerances to low salinity. Findings from this study have enlightened us on the niche segregation attribute of low salinity on nitrifiers in terrestrial environments.

# Confirmation of the Potential of CRISPR Array as an MST Marker Using Oxford Nanopore Amplicon Sequencing Analysis

Hyejun Jo<sup>1</sup> and Tatsuya Unno<sup>1,2\*</sup>

<sup>1</sup>Faculty of Biotechnology, School of life sciences, SARI, Jeju National University, <sup>2</sup>Subtropical/Tropical Organism Gene Bank, Jeju National University

Environmental contamination by feces matter poses a threat in terms of antibiotic resistance genes as well as pathogenic bacteria. Microbial source tracking (MST) using 16S rRNA sequencing has been reported as one of the methods for analyzing contaminants, but there are limitations in using it as a high-resolution marker. Thus, a new marker is required for the identification of the contaminants. Clustered regularly interspaced short palindromic repeats (CRISPR) are hypervariable loci widely distributed in prokaryotes that provide acquired immunity against foreign genetic elements. If we use CRISPR array with MST, it will be possible to identify more specific contaminants than analyzing microbial community. In this study, we sequenced the CRISPR array using Oxford nanopore sequencing to validate its potential as an MST marker of the CRISPR gene, by amplifying the *E. coli* CRISPR gene from the fecal sample and analyzing the CRISPR array. CRISPR array reads ranging from a 52 bp to a 3087 bp were produced after the analysis. The result shows a deviation in length due to the number of spacers each bacterium has. Although, there was no significant difference but the distribution among pig, cow, horse, and human groups was confirmed. This study reveals that the CRISPR gene can be representative of each sample, suggesting the possibility of using it as a more specific discriminative marker by analyzing the pattern of the spacer gene.

#### B097

# Metagenomics Uncovers the Microbial Communities and Antimicrobial Resistance Genes in Mass Transit Systems in Seoul, South Korea

Robin Guevarra<sup>1</sup>, Juchan Hwang<sup>1</sup>, Hyunjung Lee<sup>1</sup>, Hyung Jun Kim<sup>1</sup>, Yunmi Lee<sup>1</sup>, David Danko<sup>2</sup>, Krista Ryong<sup>2,3</sup>, Christopher Mason<sup>2,3,4</sup>, and Soojin Jang<sup>1\*</sup>

<sup>1</sup>Antibacterial Resistance Laboratory, Institut Pasteur Korea, <sup>2</sup>Weill Cornell Medicine, New York, NY, USA, <sup>3</sup>The Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, New York, NY, USA, <sup>4</sup>The WorldQuant Initiative for Quantitative Prediction, Weill Cornell Medicine, New York, NY, USA

Mass transit systems, including subways and buses, are useful environments for studying the urban microbiome, as the vast majority of populations in urban areas use public transportation. In this study, we used shotgun metagenomic sequencing to profile microbial communities sampled from various surfaces found in subway stations and bus stops within the Seoul mass transit system. We uncovered 598 bacterial species in 76 samples collected from various surfaces within the Seoul mass transit system. *Salmonella enterica* (40%) and the human skin bacterium *Cutibacterium acnes* (19%). Significantly abundant biomarkers detected in subway station samples were associated with bacteria typically found in the human oral cavity and respiratory tract, whereas biomarkers detected in bus stop samples were associated with bacteria commonly found in soil, water, and plants. In total, 41 unique ARG subtypes were identified, associated with single-drug or multidrug resistance to clinically important and extensively used antibiotics, including aminoglycosides, carbapenem, glycopeptide, and sulfonamides. We revealed that Seoul subway stations and bus stops possess unique microbiomes containing potential human pathogens and ARGs. These findings provide insights for refining location-specific responses to reduce exposure to potentially causative agents of infectious diseases, improving public health.

## Peptide X Exerts an Anti-obesity Effect by Influencing the *Firmicutes/Bacteroidetes* Ratio in the Gut

Ga Hyeon Baek<sup>1</sup>, Ki Myeong Yoo<sup>2</sup>, Seon-Young Kim<sup>2</sup>, Da Hee Lee<sup>2</sup>, Suk-Chae Jung<sup>3</sup>, Sung-Kyun Park<sup>2</sup>, and Jun-Seob Kim<sup>1\*</sup>

<sup>1</sup>Department of Nano-Bioengineering, Incheon National University, <sup>2</sup>Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>3</sup>Sempio Fermentation Research Center, Sempio Foods Company

Today, numerous individuals use bioproducts such as probiotics and prebiotics to prevent an imbalance of the gut microbiota and raise the number of beneficial bacteria. Six types of peptides produced from dietary proteins were evaluated for their ability to induce significant alterations in the gut microbiome of mice in this study. We attempted to evaluate the anti-obesity activity of chosen peptide X in a mouse model fed a high-fat diet because an abnormal increase in the F/B ratio is strongly associated with obesity. Consequently, peptide X significantly reduced the increase in the F/B ratio generated by a high-fat diet in a mouse model, and the number of bacteria such as Clostridium sensu stricto 1, Muribaculaceae, Faecalibaculum, Escherichia-Shigella, Bacteroides, and Streptococcus increased. In addition, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) and Kyoto Encyclopedia of Genes and Genomes (KEGG) revealed that all the signs of obesity caused by a high-fat diet, including weight gain and abnormal fat accumulation, were diminished. Therefore, when ingested jointly, peptide X can produce changes in the gut flora and is anticipated to be employed as an adjuvant treatment for obesity-related disorders.

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# Effects of pH and Antimicrobial Peptides in the Staphylococcus Intraspecies Co-culture Approach

Seon Mi Lee, Su Hyeon Han, and Woo Jun Sul<sup>\*</sup> Department of systems biotechnology, Chung-Ang University

Among the various bacteria that inhabit the skin, *Staphylococcus* occupies a predominant area. *Staphylococcus aureus* is the causative agent of numerous skin infections. By controlling *S. aureus*, which acts as a pathogen on the skin, the effect of alleviating skin diseases can be expected. To control *S. aureus*, we chose intraspecies interactions of *S. aureus* with other bacteria that inhabit the skin. This study was conducted in an environment between pH4 and pH7 similar to that of human skin. We observed bacterial interactions under various pH environmental conditions. A total of 40 strains of *Staphylococcus* spp. (including 8 strains of *S. hominis*) were extracted from human skin via swab. We compared and observed growth patterns of 40 *Staphylococcus* strains alone and co-cultured with *S. aureus* USA 300. They were sorted using red fluorescent protein expression pHC48 electroporated into *S. aureus* USA300. Among the staphylococcal species showing the effect of inhibiting the growth of *S. aureus* USA300, *S. hominis* Staphy 38 showed the strongest growth inhibitory activity. By incubating Staphy 38 with *S. aureus* 10 spp., Staphy 38 effectively reduced the production of S. aureus PSM at pH 6 and pH 7. A common factor is that all isolated *S. hominis* have an Agr system, but this is not the key to inhibiting the growth of *S. aureus*. Twelve candidate genes found only in Staphy 38 suggest that bacteriocins, including BacSp222, may be key factors inhibiting *S. aureus* biofilm formation.

# B101

# Effects of the Pre-treatments on the Biodegradaton of Polyethylene Films

Jae-Hyung Ahn<sup>1</sup>, Younggun Yoon<sup>2</sup>, Joon-hui Chung<sup>1</sup>, Si-Hyun An<sup>1</sup>, Da-Yeon Kim<sup>1</sup>, Jehyung Yeon<sup>1</sup>, Jeong Jun Kim<sup>1</sup>, and Hang Yeon Weon<sup>1\*</sup>

<sup>1</sup>Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration, <sup>2</sup>Division of Biotechnology, College of Environmental and Bioresource Sciences, Jeonbuk National University

Polyethylene (PE), with polypropylene (PP), is considered to be the most recalcitrant to biodegradation among the petroleum-based plastics due to its absence of oxygen atoms and hydrolyzable functional groups. In this study three pre-treatments, heat, ozone, and Fenton reaction, were tested to examine whether they could facilitate the biodegradation of PE films. Heat treatment at  $60^{\circ}$ C in air for 12 weeks had no effects on the Fourier transform infrared (FTIR) spectra of the PE films irrespective of the presence/absence of plastic additives but heat treatment at  $60^{\circ}$ C in liquid for 12 weeks caused the formation of carbonyl peak (C=O). The PE films with no additives showed higher carbonyl indices (CI, Area<sub>1850-1650 cm-1</sub>/Area<sub>1500-1420 cm-1</sub>) (1.71 ± 0.04) than the PE films with additives (0.96 ± 0.07), suggesting that the plastic additives might inhibit the oxidation of PE. Ozone treatment in gas phase also caused the formation of carbonyl peak in the FTIR spectra of PE with no additives (CI =  $0.94 \pm 0.10$ ). Fenton reaction, which was performed at 28°C or  $60^{\circ}$ C, had no effect on the FTIR spectra of the PE films. The PE films treated with heat or ozone were inoculated with *Nocardia* sp. PE-7, which had been isolated from the soil enrichment of PE for 2 years and found to be more abundant on PE films than in the surrounding soils. At present the changes in the amount of biofilm and the physico-chemical properties of PE films according the incubation time are being examined.

# **Cosmetic Application of Novel Bioactive Carotinoid from Marine Bacterium** *Erythrobacter* sp. SDW2 Strain Jiyeong Lim<sup>1</sup>, Yong Jun Choi<sup>2</sup>, and Jeoungjin Ryu<sup>1\*</sup>

<sup>1</sup>Department of Research & Innovation Center, COSMAX BTI, <sup>2</sup>School of Environmental Engineering, University of Seoul

Carotenoid, a type of pigment containing carotene, has a high efficacy of antioxidant effect by inhibiting free radicals. Antioxidant activity influences various functions of the skin from photo-aging to producing of melanin. Here we report the identification and cosmetic application of a homo xanthophyll pigment-producing marine bacterium *Erythrobacter* sp. SDW2 strain, from coastal seawater. The isolated *Erythrobacter* sp. SDW2 strain was able to produce  $263 \pm 12.9 \text{ mg/L}$  ( $89.7 \pm 5.4 \text{ mg/g}$  dry cell weight) of yellow xanthophyll pigment. Moreover, the xanthophyll pigment produced by the SDW2 strain exhibits remarkable ROS scavenging and antioxidative activity [DPPH ( $73.4 \pm 1.4\%$ ) and ABTS ( $84.9 \pm 0.7\%$ ). Also, this novel carotenoid from the SDW2 strain stimulates the expression of SOD2 known as antioxidant enzyme and reduces inflammatory cytokine level. These results suggest that the yellow xanthophyll pigment-producing *Erythrobacter* sp. SDW2 strain could be a promising industrial microorganism for the production of a novel bioactive compound to be used as for cosmetics and pharmaceuticals.

### B103

**Community Analysis of Lichen-associated Bacteria from** *Ramalina* **Species of South West Islands in South Korea** Hyun-Ju Noh, Ji Hye Jeong, Seong Hwan Kim, Yihyun Jeon, and Jong Won Jo<sup>\*</sup> *Division of Microbiology, Honam National Institute of Biological Resources* 

Lichens are symbiotic organisms that are majorly composed of fungi (mycobiont) and algae and/or cyanobacteria. Additionally, lichen-associated bacteria as an additional and essential component in lichen symbiosis has been revealed that it has the genetic potential to support the symbiotic system, including nutrient supply for growth of fungi and algae, support of photosynthesis, hormone production, resistance against abiotic factors and pathogen defense. In order to confirm the diversity and role of bacteria in lichens inhabiting coastal and forest areas in Korea, we investigated bacterial compositions of eight *Ramalina* species from Jindo, Wando, and Jaeun islands by illumina sequencing of 16S rRNA gene amplicons. *Alphaproteobacteria* was mainly represented by *Lichenibacterium*, and the relative abundance of ASVs belonging to this group was confirmed in more than 40% of seven *Ramalina* samples. To obtain strains belonging to *Lichenibacterium*, we cultivated bacterial strains in lichens collected from Jindo, Wando, Jaeun, and Goha islands. A total of 194 strains were obtained, and 22 strains belonged to *Lichenibacterium*. Among them, 11 strains with 100% 16S sequence similarity with the top four ASVs representing more than 5% relative abundance were obtained. This study will be used as basic data to identify the role of bacteria inhabiting lichens and to confirm their contribution to the symbiotic relationship of lichens in Korea.

# *Thermococcus onnuriiensis* sp. nov., an Arginine Biosynthesis Hyperthermophilic Archaeon Isolated from the Central Indian Ocean Ridge

Yeong-Jun Park<sup>1,2</sup>, Jae Kyu Lim<sup>1,2</sup>, Yun Jae Kim<sup>1,2</sup>, Sung-Hyun Yang<sup>1</sup>, Hyun Sook Lee<sup>1,2</sup>, Sung Gyun Kang<sup>1,2</sup>, Jung-Hyun Lee<sup>1,2</sup>, Youngik Yang<sup>3</sup>, and Kae Kyoung Kwon<sup>1,2\*</sup>

<sup>1</sup>Korea Institute of Ocean Science and Technology, <sup>2</sup>KIOST School, University of Science and Technology, <sup>3</sup>National Marine Biodiversity Institute of Korea

A strictly anaerobic hyperthermophilic archaeon, designated strain  $IOH2^{T}$ , was isolated from a deep-sea hydrothermal vent (Onnuri Vent Field) area in the Central Indian Ocean ridge. Strain  $IOH2^{T}$  showed high 16S rRNA gene sequence similarity with *Thermococcus sibiricus* MM 739<sup>T</sup> (99.42%), *T. alcaliphilus* AEDII12<sup>T</sup> (99.28%), *T. aegaeus* P5<sup>T</sup> (99.21%), *T. litoralis* DSM 5473<sup>T</sup> (99.13%), *T. bergensis* T7324<sup>T</sup> (99.13%), and *T. aggregans* TY<sup>T</sup> (98.92%), but all others showed lower than 98% similarity. The ANI and *in silico* DNA-DNA hybridization (*is*DDH) values were highest between strain  $IOH2^{T}$  and *T. sibiricus* MM 739<sup>T</sup> and were 79.33% and 15.00%, respectively. The shape of strain  $IOH2^{T}$  cells are coccoid with 1.0–1.2 µm in diameter and have no flagella. Growth ranges are 60–85°C (optimum at 80°C), pH 4.5–8.5 (optimum at pH 6.3), and 2.0–6.0% (optimum at 4.0%) NaCl. Growth of the strain  $IOH2^{T}$  was enhanced by starch, glucose, maltodextrin, and pyruvate as a carbon source and elemental sulfur as an electron acceptor. Arginine biosynthesis-related genes in the strain  $IOH2^{T}$  were predicted by genome analysis, and the cell growth was confirmed under an arginine deficient medium. The genome of strain  $IOH2^{T}$  was assembled as a circular chromosome of 1946249 bp and predicted 2096 genes. The DNA G + C content was 39.44 mol%. Based on physiological properties and phylogenetic analysis, *Thermococcus onnurilensis* sp. nov. is proposed with type strain  $IOH2^{T}$  (=MCCC 4K00089<sup>T</sup> =KCTC 25190<sup>T</sup>).

### B105

# Complete Genome Sequence of *Janibacter terrae* Strain COS04-44, Polycaprolactone Degrading Bacterium Isolated from Crude Oil Contaminated Tidal Flat

Sihyun An, Hyojung Park, Chang Muk Lee, Jae-Hyung Ahn, Dayeon Kim, Jehyeong Yeon, and Hang-Yeon Weon<sup>\*</sup> Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration (RDA)

Janibacter terrae strain COS04-44, polycaprolactone (PCL) degrading bacterium, was isolated from crude oil contaminated tidal flat in Taean, Republic of Korea. Its genome shares 98.8% similarity with *J. terrae* NBRC 107853<sup>T</sup> and consists of a circular chromosome with 3,394,906 bp. The genome includes 3,299 protein-coding genes, including 5 rRNAs and 51 tRNA, and its genomic G+C content was 71.9%. [Supported by grants from Rural Development Administration (Project No. PJ015299)]

# Neutralization of the Toxic Effects of a Fungicide Difenoconazole in Soil by Sphingomonas histidinilytica C8-2

Jehyeong Yeon<sup>1</sup>, Joon-hui Chung<sup>1</sup>, Kyongmi Chon<sup>2</sup>, Jae-Cheol Lee<sup>3</sup>, Ki-Deok Park<sup>4</sup>, Da-Yeon Kim<sup>1</sup>, Si-Hyun An<sup>1</sup>, Younggun Yoon<sup>5</sup>, Jae-Hyung Ahn<sup>1</sup>, Jeong Jun Kim<sup>1</sup>, and Hang-Yeon Weon<sup>1\*</sup>

<sup>1</sup>Division of Agricultural Microbiology, National Institute of Agricultural Sciences, Rural Development Administration (RDA), <sup>2</sup>Toxicity and Risk Assessment Division, National Institute of Agricultural Sciences, Rural Development Administration (RDA), <sup>3</sup>Division of Advanced Biology, Honam National Institute of Biological Resources (HNIBR), <sup>4</sup>Gwangju Center, Korea Basic Science Institute, <sup>5</sup>College of Environmental and Bioresource Sciences, Chonbuk National University

In the present study the chemical structure of the non-toxic metabolite of a fungicide difenoconazole produced by a difenoconazole-degrading bacterium, *Sphingomonas histidinilytica* C8-2, was identified and it was found that strain C8-2 detoxifies difenoconazole by segregating the chlorobenzene group and attaching a hydroxyl group to the cleavage site. And then it was investigated whether strain C8-2 can restore earthworm reproduction and microbial community structure disturbed by the fungicide. Difenoconazole (4 mg/kg soil) did not affect the number or bodyweight of adult earthworms in soil, but it reduced the average number of newly hatched juveniles (by 71.9%) and cocoons (by 30.0%) when compared to uncontaminated soils. Difenoconazole (20 mg/kg soil) did not affect bacterial and archaeal activities in soil after 27 days of incubation, but reduced fungal activity to 4% of uncontaminated soil. It also reduced the relative activities of the bacterial genus *Arthrobacter* (8.2% to 6.7%) and the fungal genus *Humicola* (32.4% to 0.0%). These results showed that the metabolite of difenoconazole produced by strain C8-2 had no toxic effects on these important soil organisms, suggesting that the presence or intentional application of pesticide-detoxifying microorganisms can restore soil ecosystems disturbed by pesticides.

# B107

# Diversity and Dynamics of Marine Arenicolous Fungi in Three Seasides of Korean Peninsula

Junwon Lee, Wonjun Lee, Ji Seon Kim, Chang Wan Seo, Ki Hyeong Park, Yoonhee Cho, and Young Woon Lim<sup>\*</sup> School of Biological Sciences and Institution of Microbiology, Seoul National University

Various arenicolous fungal species were detected in the sea sand from the coastal area, but little has been revealed about their distribution and dynamics. To investigate the overall diversity of marine arenicolous fungi (MAF) in Korean sea-sides and whether the composition of MAF is affected by ocean currents, we isolated fungi and analyzed the fungal community from the western seaside, southern seaside, and eastern seaside of the Korean Peninsula. A total of 603 strains were isolated and all strains were identified as 259 species with appropriate sequence regions for each genus (ITS, *BenA*, *CaM*, *tef1*, *act*). The composition of MAF showed differences at each seaside. Our results indicate that many MAF inhabited the sea sand on Korean Peninsula, and the composition of MAF is also affected by ocean currents flowing along each coast.

# Mycorrhizal Fungal Diversity Associated with Six Understudied Ectomycorrhizal Trees

Ki Hyeong Park<sup>1</sup>, Chang Sun Kim<sup>2</sup>, and Young Woon Lim<sup>1\*</sup>

<sup>1</sup>School of Biological Sciences, Institute of Microbiology, Seoul National University, <sup>2</sup>Forest Biodiversity Division, Korea National Arboretum

Mycorrhizal fungi are key components of forest ecosystems and play essential roles in health of their hosts. However, their specificity and compatibility vary between the identity of their host trees. While the composition of mycorrhizal fungi is largely revealed in the most dominant tree species, it is still remained unknown in less dominant cousins. In this study, we collected soil samples from habitat of six minor ectomycorrhizal trees in preserved natural park during four seasons, and analyzed the composition of mycorrhizal fungi in each tree species using metabarcoding approach. Our results showed that each host tree species harbored unique mycorrhizal fungal communities though their habitat was closely located each other. Most of mycorrhizal taxa were belonged to ectomycorrhizal fungi, but small amount of ericoid mycorrhizal fungi and arbuscular mycorrhizal fungi were also detected. While the taxonomic identity of mycorrhizal fungi was commonly shared between host plants in genus or higher taxonomic level, we found high host specificity in species/OTU level in both frequency and network analysis. Contrary to our expectations, neither host phylogeny nor sampling site affected the mycorrhizal communities' diversity and their response to environmental factors. In conclusion, this study presents the unique diversity of mycorrhizal fungi associated with less studied tree species and their specificity.

[Supported by grants from Korea National Arboretum]

### B109

# A Metagenome Data-enabled Machine Learning Modeling Approach Provides Diagnostic Understanding of Groundwater Pollution

Jonathan Wijaya<sup>1</sup>, Tien Hoang Du<sup>1</sup>, Woosik Jung<sup>2</sup>, Joonhong Park<sup>2</sup>, and Seungdae Oh<sup>1\*</sup> <sup>1</sup>Department of Civil Engineering, Kyung Hee University, <sup>2</sup>Department of Civil and Environmental Engineering, Yonsei University

Groundwater (GW) quality monitoring is crucial for sustainable management of water resources in maintaining both public health and ecosystem functioning. Our previous machine learning (ML) modeling study using 16S rRNA gene sequence data has successfully identified microbial indicators predicting GW pollution. The present study aimed to establish a ML modeling framework using combined GW quality and metagenomic datasets as input variables. The ML modeling framework significantly increased the prediction accuracy in categorizing oil-polluted GW samples, when using metagenomic datasets compared to those of GW quality or marker gene datasets. Feature importance analyses with the ML models could reveal particular taxa and catabolic pathways highly indicative of the ecological niche associated with petroleum contamination. The ML modeling results with the known ecophysiology of the specific taxa and further metagenomic characterization collectively provided clues into the ecological roles and interplay of the organisms with the petroleum-contaminated GW. Overall, the metagenomic data-driven ML pipeline significantly would advance the current practice for GW pollution monitoring and have practical significance in devising a molecular data-enabled ML monitoring technology for early diagnosis of various target contaminants in GW environments.

[This study was supported by the Korea Ministry of Environment as part of the SEM (Subsurface Environment Management) projects (2020002480003).]

### Role of DNA Methylation in Antibiotics-driven Perturbation on the Gut Microbiome

Han Na Oh, Hoon Je Soeng, Taeyune Kim, Hye Lim Keum, and Woo Jun Sul<sup>\*</sup> Systems Biotechnology, Chung-Ang University

Antibiotics are the main factor causing microbiome perturbation and bacteria have evolved a range of mechanisms of antibiotic resistance such as a modification of the target of antibiotics. In Bacteria, DNA methylation regulates bacterial cellular processes including methylation-based transcriptional activation. To investigate the role of DNA methylation in the gut microbiome as another key strategy for antibiotic resistance, we treated clindamycin and water as a control for rats for 20 days, collected feces and cecum samples from day 0, and conducted shotgun metagenome sequencing including Oxford Nanopore Technologies for methylome analysis. Clindamycin treatment resulted in a profound shift in gut microbiome community composition and decreased diversity on day 1 right after treatment. Using day 0 and day 20 samples, shotgun sequencing was performed using short and long-read sequencing, and a total of 723 metagenome-assembled genomes (MAGs) were reconstructed from the hybrid assembly. MAGs assigned to *Escherichia coli*, and *Clostridium M bolteae* showed a higher abundance in treatment. In the *C. bolteae* MAGs, DNA methylation-related genes, including *Hhal*, *YdhJ*, and *M. EfaBMDam* were found close to the *cfrC* encoding 23S rRNA methyltransferase providing antibiotic resistance to clindamycin, and DNA methylation frequency was the highest in the treatment. We suggest this differential DNA methylation status under antibiotic stress could determine antibiotic resistance of bacteria.

### B112

# Comparison of Microbial Communities in Agricultural Soils Applied with Biodegradable Mulch Films and Conventional (Polyethylene) Mulch Films

Jae-Hyung Ahn, Joon-hui Chung, Si-Hyun An, Da-Yeon Kim, Jehyung Yeon, Jeong Jun Kim, and Hang Yeon Weon<sup>\*</sup> Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration

As an alternative for conventional (polyethylene), non-biodegradable mulch film (NBM) biodegradable mulch film (BDM) has been increasingly applied to agricultural soils. However the effects of BDM on soil microbial communities is one of those which should be elucidated before their generous use. In this study the bacterial, archaeal, and fungal communities in the soils of the agricultural fields applied with NBM and BDM were compared using both the DNA- and RNA-based approaches. An agricultural soil applied with no mulch film was also included in this study. It was found that the different treatments had no significant effects on the soil physico-chemical properties, and the diversities and overall structures of the microbial communities in the soils. However several microbial groups showed significant differences between the treatments. For *Bacteria* the relative abundance of *Actinobacteria* was lower with BDM than with NDM, and that of *Bacteroidetes* was in reverse. For *Archaea* that of *Thermoplasmatota* was much lower with BDM than with NDM. For *Fungi* that of *Basidomycota* was much higher with BDM than with no mulch film were similar to those in the soils with BDM. These results suggest that the application of BDM has lower effects on soil microbial communities than does that of NBM but more extensive study is needed to support this hypothesis.

### Microbial Degradation of Residual Pesticides in Soils at Low Concentrations

Jae-Hyung Ahn, Joon-hui Chung, Myoung-Hwa Jung, Si-Hyun An, Da-Yeon Kim, Jehyung Yeon, Jeong Jun Kim, and Hang Yeon Weon<sup>\*</sup>

Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration

Most studies on microbial degradation of pesticides have used unrealistic amounts of pesticides in their experiments but only trace amounts of residual pesticides (< 1 mg/kg of soil) were generally found in agricultural fields. These low amounts of pesticides, however, can be transferred to crops and livestock, or hinder the certification of organic farming. In this study microcosm studies were performed to examine whether the trace amounts of three pesticides could be removed by the previously isolated pesticide-degrading bacterial strains. The soil concentrations of a fungicide carbendazim and an insecticide cadusafos decreased from the initial 5 mg/kg of soil to < 0.01 by *Rhodococcus qingshengii* 3-2, and to 0.025 by *Sphingobium* sp. Cam5-1 for 12 days, respectively, while that of a fungicide difenoconazole decreased to 0.33 by *Sphingomonas histidinilytica* C8-2 for 14 days, and only to 0.28 for additional 21 days. This result was supposed to be correlated to the difference in the octanol-water partition coefficients of the three pesticides. To facilitate the biodegradation of the trace amounts of difenoconazole the amount of the inoculated bacterium, synthetic or bio-surfactants, soil water content, and additional carbon sources were examined and it was found that the adjustment of soil water content was the most effective to remove the low concentrations of difenoconazole, which decreased to 0.07 for 26 days.

### B114

# Synthesis and Engineering Applications of Environment-benign Adsorbents for Sustainable Management of Urban Water and Aquatic Microbiome

Thilini Gunawardhana, Hong Anh Nguyen, Geonhee Yeo, Tien Hoang Du, and Seungdae Oh<sup>\*</sup> Department of Civil Engineering, Kyung Hee University

Adsorption processes can be often used in a variety of water and wastewater treatment plants, due to their simplicity and flexibility with a wide range of adsorbent feedstock resources. The adsorption processes can provide an effective control not only on the fate of various organic pollutants but also on their potential environmental consequences on the ecosystem functioning and stability of aquatic microbiomes. This study thus aimed to synthesize an environment benign adsorbent, which can produce less amount of greenhouse gases and require less energy during the synthesis process. Rice husk, coconut husk, oak wood, and pine bark were used as adsorbent feedstock resources in this study. This study then systematically assessed the effects of feedstock resources, pyrolysis conditions (temperature and residence time), modification methods, and physical properties of the synthesized adsorbents on the adsorption characteristics, kinetics, and isotherm for organic contaminants. To provide a mechanistic understanding of the adsorption performance, physicochemical surface properties such as surface area and chemical interaction were characterized. We are currently exploring the beneficial effects of the adsorbents in reducing the toxic consequences of organic contaminants on the structure and diversity of aquatic microbiomes.

[This study was supported and grant funded by the Korea Environmental Industry & Technology Institute (No. G232022017151).]

# Identification of Microbial Community Characteristics of Skin Chronic Scratching Lesions Due to Skin Itching Hye Lim Keum<sup>1</sup>, Woo Jun Sul<sup>1</sup>, and Hei Sung Kim<sup>2\*</sup>

<sup>1</sup>Department Systems Biotechnology, Chung-Ang University, <sup>2</sup>Department of Dermatology, Incheon St. Mary's Hospital, The Catholic University of Korea

People with itchy skin who feel uncomfortable and irritating sensation often scratch their skin. Depending on the cause of your itchiness, your skin may appear normal, red, rough, or bumpy. Many people try to reduce this discomfort with their own care methods, such as using moisturizers, using mild skin care products and cleansers, and washing with moderate-temperature water. Despite these efforts, patients with severe itching continue to scratch the lesion area and wounds may occur. We aimed to identify the microbiome characteristics of scratching lesions caused by itchy skin and to find the differences from normal skin. Through the 16S rRNA gene full-length sequencing of the skin microbiome in the lesion and non-lesion site of a total of 17 subjects, we confirmed significant differences in the abundance of *Staphylococcus*, *Cutibacterium, Corynebacterium, Chryseobacterium*, and *Micrococcus*, the main genus of the skin. In particular, *Staphylococcus* showed different patterns in the lesion and non-lesion sites depending on which species it is. Accordingly, the metagenomic function of each *Staphylococcus* spp. was inferred through PICRUSt2. We identified microbes that characterize the lesion site when compared to the non-lesion site and confirmed the relationship among the species.

### B116

# Isolation and Genome Analysis of Novel Bacteriophages Infecting Problematic Pathogenic *Vibrio* spp. in Fishery Industry

Kira Moon\*, Seung Hui Song, Jae-Cheol Lee, Jae Hyoung Joo, Nakyeong Lee, Yun Ji Kim, and Sung Moon Lee\* Division of Environmental materials, Honam National Institute of Biological Resources

*Vibrio* species has been one of the major threats to the seafood industry, especially to inland aquacultures. Pathogenic *Vibrio* strains constantly re-emerge, causing fish death *en masse*. To prevent the spread of pathogens among fish population, aquaculture farmers use both antibiotic-supplemented pellet foods and formaldehyde bath. Most inland aquaculture farms release rearing water to the environment without any particular treatment. In this process, antibiotic remnants are released to the environment, causing disturbance. Hence, environment-friendly antibiotic replacement that can specifically target pathogenic bacteria is needed. Bacteriophages (phages) are viruses that infect bacteria, hence often utilized as phage therapy as an antibiotic replacement. Therefore, in this study, lytic phages were isolated to screen for phage therapy candidates to treat pathogenic *Vibrio* strains. From rearing waters of the inland shrimp aquafarm located in Jindo, South Korea, 24 phages were isolated. The *Siphoviriade* family with long contractile tails (3,366 nm in average). The whole genome analysis revealed that isolated phages were able to be categorized into three groups based on nucleotide similarity, each group representing novel phage genomes. These results indicate that novel phages isolated in this study have the potential to be utilized to treat pathogenic *Vibrio* species.

# Inhibition of CD82 Improves Colitis via Increasing of Deubiquitination in NLRP3 by BRCC3

Euni Cho<sup>1,2</sup> and Chul-Su Yang<sup>2,3\*</sup>

<sup>1</sup>Department of Bio-nano Technology, Hanyang University, <sup>2</sup>Center for Bionano Intelligence Education and Research, <sup>3</sup>Department of Molecular and Life Science, Hanyang University

CD82, also known as KAI1, is a member of the tetraspanin superfamily, consisting of four transmembrane domains and is a suppressor gene against cancer metastasis. Recently, a variety of studies have suggested that CD82 is involved with inducing the immune system and especially seems to be associated with the activation of NLRP3 inflammasome in several diseases. We here demonstrate that CD82 is necessary for optimal NLRP3-ASC complex formation, ASC oligomerization, caspase-1 activation, and IL-1 $\beta$  and IL-18 production upon treatment with NLRP3 ligands after the priming step, suggesting that efficient NLRP3 activation requires CD82. Moreover, we report that CD82 deficiency results in a remarkable attenuation in the severity of NLRP3-associated inflammatory diseases, including LPS-induced colitis in mice with increasing the level of IL-1 $\beta$  and IL-18. Furthermore, CD82 had two binding partners, including BRCC3 and NLRP3, which are interacting with 152-158 amino acids region in CD82. Interestingly, CD82 showed a higher affinity with BRCC3, and this binding enhanced the degradation of NLRP3 by inhibiting BRCC3-dependent K63-specific deubiquitination. Our findings reveal an CD82-mediated signaling system that regulates the activation of the NLRP3 inflammasome and suggest novel potential targets for treating NLRP3related diseases.

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# C002

**Longitudinal Evaluation of Fecal Microbiota Transplantation for Ameliorating Calf Diarrhea** Hyun Sik Kim<sup>1</sup>, Tae Woong Whon<sup>2</sup>, and Jin-Woo Bae<sup>1\*</sup> <sup>1</sup>Department of Biology, Kyung Hee University, <sup>2</sup>World Institute of Kimchi

Neonatal calf diarrhea is a common disease leading to a major economic loss for cattle producers worldwide. Several infectious and noninfectious factors are implicated in calf diarrhea, but disease control remains problematic because of the multifactorial etiology of the disease. Here, we describe the ability of a fecal microbiota transplantation (FMT), to ameliorate diarrhea and restore gut microbial composition in 57 growing calves. We conduct multi-omics analysis of 450 longitudinally collected fecal samples and find that FMT-induced alterations in the gut microbiota (an increase in the family *Porphyromonadaceae*) and metabolomic profile (a reduction in fecal amino acid concentration) strongly correlate with the remission of diarrhea. During the continuous follow-up study over 24 months, we find that FMT improves the growth performance of the cattle. The presented findings suggest that gut inflammation followed by a prolonged expansion of nontoxigenic autochthonous *Enterobacteriaceae* contributes to the onset of diarrhea in pre-weaning animals. This first FMT trial in ruminants suggest that FMT is capable of ameliorating diarrhea in pre-weaning calves with alterations in their gut microbiota, and that FMT may have potential role of improvement of growth performance.

[This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1C1C2008556)]

# Application of High-performance Liquid Chromatography to Replace Sucrose Density Gradient Ultracentrifugation for the Quantification of Foot-and-Mouth Disease Vaccine Antigens

Young-Joon Ko\*, Ah-Young Kim, Sun Young Park, Sang Hyun Park, and Jong-Hyeon Park Animal and Plant Quarantine Agency

Foot-and-mouth disease (FMD) is a highly contagious disease, caused by FMD virus (FMDV). The content of intact virus particles (146S antigens) is crucial to induce neutralizing antibodies and protect susceptible livestock after immunization. Sucrose density gradient fractionation (SDG) has been known for the gold standard as a quantification method of 146S antigens. However, it has several drawbacks in practice due to the ultracentrifugation step. Recently, Size-exclusion high performance liquid chromatography (SE-HPLC) was introduced to replace the SDG. This study aimed to adjust the difference between the two methods by employing a standard material for the quantification of 146S antigens. Whereas the SE-HPLC displayed all the virus particles in the peak fraction by SDS-PAGE and Western blotting, the SDG exhibited widely dispersed pattern of virus particles in multiple fractions. The quantity of vaccine antigen, measured by the SE-HPLC could be adjusted to the estimated value by the SDG using bovine enterovirus (BEV) as a standard material because the BEV has similarities in density and size to FMDV, while it displayed far more better stability than FMDV.

[This work was supported by the Animal and Plant Quarantine Agency (grant number B-1543386-2022-24-04).]

### C004

### Administration of Lactobacillus fermentum KBL674 Alleviate Candida albicans Infection in Mice

Sung Jae Jang<sup>1,2</sup>, Eun Jung Jo<sup>2</sup>, Woon-Ki Kim<sup>1,3</sup>, Yun Jeong Shin<sup>4</sup>, Jun Soo Song<sup>4</sup>, Nanhee Lee<sup>5</sup>, Hyungjin Lee<sup>5</sup>, GwangPyo Ko<sup>1,2,5</sup>, and SungJun Park<sup>2,5,6\*</sup>

<sup>1</sup>Graduate School of Public Health, Seoul National University, <sup>2</sup>KoBioLabs, Inc., <sup>3</sup>Institute of Health and Environment, Seoul National University, <sup>4</sup>The Food Industry Promotional Agency of Korea, <sup>5</sup>weBiom Inc., <sup>6</sup>N-Bio, Seoul National University

*Candida albicans* (*C. albicans*) is the major infectious microorganism which cause vaginal infection. *Lactobacillus* species, which are the most dominant microorganisms in vagina, are known as their inhibitory effects for vulvovaginitis. Therefore, in this study, we investigate the effects of *Lactobacillus fermentum* (*L. fermentum*) KBL674 administration in *in vivo C. albicans* infection model. 2 × 10<sup>9</sup> colony-forming unit (CFU) of *L. fermentum* KBL674 were orally administered daily to six-week-old female C57BL/6J mice with *C. albicans* infection (5 × 10<sup>6</sup> CFU). *Lactobacillus fermentum* KBL674 treatment significantly reduced *C. albicans* concentration in vaginal fluid and improved vaginal epithelium. Moreover, *L. fermentum* KBL674-treated mice showed lower levels of inflammation markers in vaginal tissues. Abundance of Genus *Lactobacillus* was increased in *L. fermentum* KBL674-treated mice. Therefore, we suggest that *L. fermentum* KBL674 holds great promise for improving vaginal *C. albicans* burden via direct inhibition, and modulation of host immune system and microbial composition.

[This research was supported by 2022 Collaborative R&BD Program of The Food Industry Promotional Agency of Korea and weBiom Inc., Republic of Korea]

# Antibacterial Effects of Lactic Acid Bacteria Isolated from Piglet Feces on Diarrhea Pathogen in Piglet

Yoonjeong Yoo<sup>1</sup>, Gayoung Lee<sup>1</sup>, Jinho Cho<sup>2</sup>, and Yohan Yoon<sup>1,3\*</sup> <sup>1</sup>Department of Food and Nutrition, Sookmyung Women's University, <sup>2</sup>Department of Animal Sciences, Chungbuk National University, <sup>3</sup>Risk Analysis Research Center, Sookmyung Women's University

Lactating piglets may have useful lactic acid bacteria (LAB) during lactation. In recent, the use of LAB is recommended to control pathogens rather than using antimicrobials, because the antimicrobials may increase the resistance of the pathogens. Therefore, the objective of this study was to isolate LAB from piglet feces to control diarrhea pathogens such as *Escherichia coli* and *Salmonella*. LAB was isolated from 118 piglet fecal samples. The LAB isolates were examined for hemolysis, gelatinase activity, and the resistance for acid, bile salt and pancreatin. The selected LAB isolates were challenged to *E. coli* (KVCC-BA2000145, BA2000146, BA2000147, BA2000148, BA2000149, BA2000150, BA2000151, BA2000152, BA2000153, and BA2000154) or *Salmonella* (KVCC-BA2000155, BA2000156, BA2000157, BA2000158, BA2000159 BA2000160, and BA2000161) in the De Man, Rogosa and Sharpe agar. After incubating the plates at 37°C for 24 h, the size of the inhibition zone was measured. One hundred sixty four isolates were obtained from 118 piglet fecal samples. Thirteen LAB isolates were selected after the analysis for hemolysis, gelatinase activity, and tolerance to acid, bile salt and pancreatin. The antibacterial effect of the 13 LAB isolates was examined against *E. coli* and *Salmonella* strains, and *Lactobacillus reuteri* SMFM2021-PF30 then showed the highest antibacterial effect. *L. reuteri* SMFM2021-PF30 isolated from piglet feces could be useful in controlling the diarrhea pathogens in piglet.

# C006

# Culture-dependent and -independent Investigations on Effect of Jogi Addition on Bacterial Communities of Kimchi

Junghyun Park, Sojeong Heo, Hong-Eun Na, and Do-Won Jeong<sup>\*</sup> Department of Food and Nutrition, Dongduk Women's University

To determine the effect of the addition of jogi (croaker; *Micropogonias undulatus*) on the bacterial community of kimchi, bacterial communities were identified using culture-dependent and culture-independent techniques (pyrosequencing of total DNA). In culture-independent analysis, *Lactilactobacillus sakei* and *Leuconostoc mesenteroides* were dominant as fermentation progressed, and in culture-dependent analysis, *Leuconostoc mesenteroides* were found to be more dominant than *Lactilactobacillus sakei*. However, the addition of jogi did not induced changes in the bacterial communities as fermentation progressed, although *Carnobacterium inhibens* originated from fish in the early stages of fermentation were discovered.

[This work was supported by Ministry of Agriculture, Food and Rural Affairs (No. 322014051HD02020982085990000)]

# Integrated Genomics and Phenotype Microarray Analysis of *Saccharomyces cerevisiae* Industrial Strains for Rice Wine Fermentation and Recombinant Protein Production

Ye Ji Son<sup>1</sup>, Min-Seung Jeon<sup>1</sup>, Hye Yun Moon<sup>1</sup>, Jiwon Kang<sup>1</sup>, Ha Jeong Kim<sup>1</sup>, Dong Wook Lee<sup>1</sup>, Jae Ho Kim<sup>2</sup>, Jeong-Ah Seo<sup>3</sup>, Seong-il Eyun<sup>1</sup>, and Hyun Ah Kang<sup>1\*</sup>

<sup>1</sup>Department of Life Science, Chung-Ang University, <sup>2</sup>Korea Food Research Institute, <sup>3</sup>School of Systems Biomedical Science, Soongsil University

The industrial potential of *S. cerevisiae* has extended beyond its traditional use in food fermentation into various healthcare sectors, such as in the production of recombinant proteins. In this study, comparative genomics analysis was carried out with three industrial strains of *S. cerevisiae*, Y98-5, KSD-YC, and Y2805. Notably, the genomes of Y98-5 and KSD-YC, the starter strains for commercial rice wine production in Korea, were revealed as heterozygous diploid, whereas that of Y2805, a host for recombinant protein production, was haploid. Genome-based phylogenetic analysis indicated that Y2805 was closely associated with the reference strain S288C, whereas KSD-YC and Y98-5 were grouped with Asian and European wine strains, respectively. A single nucleotide polymorphism (SNP) in *FDC1*, involved in biosynthesis of 4-vinylguaiacol (4-VG), a phenolic compound with clove-like aroma, was found in KSD-YC, in consistent with its lack of 4-VG production. Phenotype microarray (PM) analysis showed that KSD-YC and Y98-5 displayed broader substrate utilization than S288C and Y2805. By integrating with the genomic data, SNPs were mapped in the genes responsible for the observed differences in the PM data. Our integrated genomics and PM analysis data elucidated the evolutionary history and genetic diversity of industrial *S. cerevisiae* strains, which establishes an important foundation to improve fermentation processes and genetic manipulation of host cells.

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### C008

# Transcriptomic Analysis of *Staphylococcus equorum* KM1031, Isolated from the High-salt Fermented Seafood Jeotgal, under Salt Stress

# Sojeong Heo<sup>1</sup>, Jong-Hoon Lee<sup>2</sup>, and Do-Won Jeong<sup>1\*</sup>

<sup>1</sup>Department of Food and Nutrition, Dongduk Women's University, <sup>2</sup>Department of Food Science and Biotechnology, Kyonggi University

Staphylococcus equorum is a potential starter for Korean high-salt fermented foods because of its salt-tolerance and enzymatic activities that contribute to enhanced sensory properties of the food products. However, the mechanisms of salt tolerance of *S. equorum* are not fully understood. Here, RNA sequencing was performed on *S. equorum* strain KM1031 exposed to 7% NaCl (w/v) for 2 and 4 h to determine global gene expression changes. Salt pressure for 2 and 4 h resulted in significant differential expression of 4.8% (106/2,209) and 6.1% (134/2,209) of *S. equorum* KM1031 genes, respectively. Twenty-five core genes were differentially expressed on salt-treatment for both 2 and 4 h, seven of which were related to osmoprotectant uptake and synthesis. We analyzed the genome of strain KM1031 and identified osmoprotectant uptake (Opu) systems, potassium importers, sodium exporters, and the glycine betaine synthesis system. The RNA sequencing results indicated that the OpuD system and glycine betaine synthesis might play the main roles in the salt-tolerance of strain KM1031. Finally, the results of RNA sequencing were validated by quantitative real-time PCR of likely salt stress-related genes. This transcriptomic analysis provides evidence regarding the osmotic stress responses of *S. equorum* strain KM1031. [This work was supported by the National Research Foundation of Korea (NRF) [NRF-2019R1A2C1003639]]

# Characterization of Escherichia coli Bacteriophages and Their Biocontrol Application in Fresh Food

Eojin Kim, Min Ki Choi, and Jeong-A Lim<sup>\*</sup> Food Safety and Distribution Research Group, Korea Food Research Institute

*Escherichia coli*, a Gram-negative bacterium, is one of the most common causes of food outbreaks of fresh food. The aim of this study was to isolate lytic bacteriophages infecting *E. coli* and examine their efficacies for the biocontrol of *E. coli* on fresh food. Many bacteriophages infecting *E. coli* strains were isolated from sewage treatment facility in Korea. They formed large clear plaques on the lawn of its host strain of *E. coli*. The phage morphologies were observed under the Transmission electron microscope. All the phages showed rapid and strong lytic activity against their host bacteria and most of the *E. coli* strains which were tested were sensitive to the phages. The lifestyles of the phages were predicted as virulent and their novelty was confirmed by bioinformatic analysis. To increase their applicability, some phages were selected based on each antimicrobial activity and the effects of the phage cocktail were confirmed. As expected, it showed a broader host range and E. coli strains contaminating fresh food were controlled by the phage mixture. These results demonstrated that novel phage and its cocktail is a promising candidate for controlling *E. coli* contamination in foods owing to its safety and effectiveness.

# C010

# Comparison of the Four Multilocus Sequence Typing Schemes and Amino Acid Biosynthesis Based on Genomic Analysis for *Bacillus subtilis*

Gawon Lee, Sojeong Heo, Tao Kim, Hong-Eun Na, and Do-Won Jeong<sup>\*</sup> Department of Food and Nutrition, Dongduk Women's University

*Bacillus subtilis,* a valuable industrial microorganism used in starter cultures in soybean fermentation, is a species of bacteria with interspecies diversity. Here, four multilocus sequence typing (MLST) schemes developed to assess the diversity of *B. subtilis* or *Bacillus* spp. were applied and compared to confirm the interspecies diversity of *B. subtilis*. In addition, we analyzed correlations between amino acid biosynthesis genes and sequence types (STs); this is important because amino acids are key taste components in fermented foods. On applying the four MLST methods to 38 strains and the type strain of B. subtilis, 30 to 32 STs were identified. The discriminatory power was 0.362–0.964 for the genes used in the MLST methods; the larger the gene, the greater the number of alleles and polymorphic sites. All four MLST methods showed a correlation between STs and strains that do not possess the hutHUIG operon (which contains genes required for the production of glutamate from histidine). This correlation was verified using 168 further genome-sequence strains.

[This work was supported by the National Research Foundation of Korea (NRF) [NRF-2019R1A2C1003639]]

# Metabolic Engineering of Sphingolipid Biosynthesis Pathway in *Yarrowia lipolytica* for Cell Surface-associated Production of Sphinganine, Sphingosine, and Human-type Ceramide

Seo Hyeon Shin, Hye Yun Moon, Hae Eun Park, Jong Boem Kim, and Hyun Ah Kang<sup>\*</sup> Department of Life Science, Chung-Ang University

The biosynthesis of sphingolipid begins with the condensation of L-serine and palmitoyl-CoA to yield the C18 carbon unit 3-ketosphinganine, which is reduced to yield sphinganine. In this study, to block the fungal specific phytosphingosine-based sphingolipid biosynthesis pathway in the oleaginous yeast *Yarrowia lipolytica*, the *SUR2* gene, encoding sphinganine C4-hydroxylase responsible for conversion of sphinganine to phytosphingosine, was disrupted. The resultant *Y. lipolytica sur2* null mutants (*Ylsur2*Δ) exhibited retarded growth with increased pseudohyphal formation and displayed increased sensitivity to high temperature, osmotic, and cell wall stresses compared to the wild-type strain. Notably, the *Ylsur2*Δ mutant showed increased production of sphinganine, which was mostly secreted to the cell surface or extracellular medium even without acetylation. Additional introduction of mouse ceramidase and subsequent disruption of the *SLD1* genes, encoding fungal specific Δ8 sphingolipid desaturase, in the *Ylsur2*Δ strain further increased production of sphingosine or human-type glucosylceramide. Moreover, the *SLD1* deletion partly rescued the growth defect of *Ylsur2*Δ strains for the secretory production of sphingosine, and human-type ceramide, which are useful ingredients for cosmeceutical or nutraceutical formulations.

[Supported by MOTIE (20008739)]

# C012

# Effects of Probiotics on Motor Functions and Anxiety Levels in a Chronic Stress Mouse Model

Jae Gwang Song<sup>1</sup>, Bomi Lee<sup>1</sup>, Daye Mun<sup>2</sup>, Younghun Kim<sup>2</sup>, Minjee Lee<sup>3</sup>, Jungwoo Yang<sup>3</sup>, and Hyung Wook Kim<sup>1\*</sup> <sup>1</sup>College of Life Sciences, Sejong University, <sup>2</sup>Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University, <sup>3</sup>Ildong Bioscience

Growing evidence indicates a crucial role of gut microbiota in physiological functions. The gut-brain axis imbalance has also been associated with neuropsychiatric and neurodegenerative disorders. Studies suggested that probiotics regulate stress response and alleviate mood-related symptoms. In this study, we investigate the effects of probiotic lactobacilli on behavioral response and feces metabolite content in an unpredictable chronic mild stress mouse model. Our study shows that chronic stress to mice for three weeks resulted in significant changes in behavior, including lower locomotor activity, higher levels of anxiety, and depressive-like symptoms compared to the control group. These changes were accompanied by alteration of feces metabolite in the gut microbiota. Oral administration of the *Lactobacillus* strain ameliorated the observed changes and improved the behavioral alterations along with feces metabolites, suggesting a neuroprotective role of probiotics. [This study was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET-321033-3).]

*In Vitro* Functional Characteristics of Probiotic *Limosilactobacillus reuteri* Strains Isolated from Livestock Feces Soyeon Park<sup>1</sup>, Jun-hyeong Seo<sup>1</sup>, Dajung Lim<sup>1</sup>, Hyun-Joon Jang<sup>1</sup>, Dae-Hyuk Kim<sup>1,2</sup>, and Yangseon Kim<sup>1\*</sup> <sup>1</sup>Department of Research and Development, Center for Industrialization of Agricultural and Livestock Microorganism, <sup>2</sup>Departments of Molecular Biology and Bioactive Material Sciences, Institute for Molecular Biology and Genetics, Jeonbuk National University

*Limosilactobacillus (L.) reuteri* (known as *Lactobacillus reutei*) is one of the most studied probiotics that plays an important role in the microbial balance and maintaining the intestinal health of the host. The objective of the present study was to evaluate the probiotic properties of *L. reuteri* strains isolated from cattle and swine feces. Eight isolates of *L. reuteri* were identified by 16S rRNA sequencing, and evaluated characterization including acid and bile tolerance, antimicrobial activity, antibiotic susceptibility, and adhesion ability to the HT29 cell line. Our result showed that all the isolated *L. reuteri* were evaluated strong tolerance to 0.3% bile salt. Most of *L. reuteri* strains were showed highly survival rate of 96 – 99% in the MRS medium of pH 2.5. Especially, the CACC593, CACC607 and CACC712 strains were displayed higher adhesion capacity on intestinal cells comparable to that of the other *L. reuteri* strains. Among these isolates, CACC607 and CACC605 exhibited broad-spectrum antibiotic susceptibility as well as good acid/bile salt tolerance and cell-adhesion ability. Taken together, these results may contribute to the development of multi-functional probiotic starter such as feed additive. [This study was supported by INNOPOLIS FOUNDATION through Science and Technology Project Opens the Future

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### C014

# Genome-wide Identification and Functional Analysis of Alcohol Acyltransferases for Aroma Ester Formation in *Wickerhamomyces subpelliculosus*

Hye Yun Moon, Hyeon Jin Kim, Ha Jeong Kim, Su Jin Yoo, Yong Uk Cho, and Hyun Ah Kang<sup>\*</sup> Department of Life Science, Chung-Ang University

Aroma ester components, produced via alcohol acyltransferase (AAT)-catalyzed reactions, are responsible for the flavor character of fermented foods. In this study we performed *de novo* sequencing of 16 Mb haploid genome of *Wickerhamomyces subpelliculous* CBS5767<sup>T</sup> and identified five homologs of *ATF* encoding alcohol *O*-acetyltransferases with AATase Pfam domain, along with homologs of *EHT1* and *EAT1* encoding AATase carrying  $\alpha/\beta$  hydrolase fold domain and the Ser-Asp/Glu-His catalytic triad. Whereas the aroma volatile esters levels were not increased in the *S. cerevisiae* strain expressing the *WsATF* genes, the *S. cerevisiae* strains expressing *WsEHT1* and *WsEAT1* exhibited increased levels of ethyl decanoate and ethyl acetate, respectively. Notably, WsAtf6p, closely grouped with *S. cerevisiae* and *Wickerhamomyces ciferrii* Sli1 proteins rather than other Atf proteins in phylogenetic tree analysis, showed acetyltransferase activity towards myriocin, as reported with *S. cerevisiae* Sli1p. Subcellular localization analysis using green fluorescence protein fusion showed that WsEat1p localized at mitochondria, WsEht1p at endoplasmic reticulum and lipid droplets (LDs), and WsAtf6p at LDs, respectively. Our results based on genomic information and validation by functional analysis would provide an in-depth knowledge on formation of volatile aroma esters by *W. subpelliculosus*, a potential flavor-formers in the development of new products for future market expansion. [Supported by RDA (PJ01710102)]

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# Biochemical Characterization of Two Transcriptional Regulators CmmRI and CmmRII within the Chromomycin A3 Gene Cluster in *Streptomyces* sp. SJ1-7

Umji Choi and Chang-Ro Lee<sup>\*</sup> Department of Biological Sciences, Myongji University

Chromomycin A3 (CMA) is an anthraquinone glycoside-mithramycin A analog with the antitumor activity which is produced by *Streptomyces* strains. A novel *Streptomyces* strain, *Streptomyces* sp. SJ1-7, which produces CMA, was previously identified. In this study, we analyzed biochemical characteristics of two putative transcriptional regulators CmmRI and CmmRII within the CMA gene cluster in SJ1-7. CmmRI was predicted as an AfsR/SARP family transcriptional activator, whereas CmmRII was predicted as a PadR family transcriptional repressor. To purify two proteins, we cloned the *cmmRI* and *cmmRII* genes into a pET28a expression plasmid. CmmRII was easily purified using Talon metal affinity resins, but CmmRI was not purified due to its low solubility. Although various trials were attempted such as protein induction at low temperature, construction of a C-terminally His-tagged protein, and protein refolding using 8M urea, the purification of CmmRI was failed. Purified CmmRII specifically interacted with several promoters within the CMA gene cluster. These interactions were not influenced by the presence of CMA. Gel filtration chromatography showed that CmmRII existed as a monomer. Through these experiments, we demonstrated that CmmRII may act as a transcriptional regulator of the CMA gene cluster in SJ1-7. [Supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202124101)]

# Immunomodulatory Activity of Lactiplantibacillus plantarum GCWB1001 In Vitro

Hui Jin<sup>1</sup>, So Hyun Jeon<sup>1</sup>, Min Jung Jang<sup>1</sup>, Gyeong Eun Hong<sup>1</sup>, and Yong Pil Hwang<sup>2,3\*</sup> <sup>1</sup>Department of Research, GREEN CROSS Wellbeing Co., Ltd., <sup>2</sup>Department of Pharmaceutical Engineering, International University of Korea, <sup>3</sup>Fisheries Promotion Division, Mokpo City

Probiotics are commonly used in dietary supplement products. The benefit of probiotics in improving immune status in respiratory tract infections has aroused great interest in the last few years. Our previous study has shown that the administration of *Lactiplantibacillus plantarum* GCWB1001 ameliorated lung inflammation in mice with asthma. In this study, we investigated the immunomodulatory effects of GCWB1001 isolated from kimchi (Korean fermented food) in vitro model. To estimate the immune-enhancing effects of GCWB1001 we analyzed cytotoxicity and phagocytic effects in RAW264.7 cell lines treated with various concentrations of GCWB1001. And cytokines of Th1/Th2 have an important role in modulating the immune system. So IL-12(Th1), IL-4(Th2), and the ratio between them were measured both in RAW264.7 cells and splenocytes. Results show that GCWB1001 did not affect the viability of RAW264.7 cells and has no cytotoxicity to RAW264.7 cells. Also, GCWB1001 increased IL-4 and IL-12 in a concentration-dependent manner and also increased the ratio of IL-4 and IL-12. Taken together, these results mean that GCWB1001 has a potential capacity for immunomodulation. GCWB1001 may be effective in asthma. [Supported by grants from GREEN CROSS Wellbeing.]

# C018

**Optimization of Mouse Model Experiments for Development of Functional Verification Platform Technology** Min-Young Kim<sup>1</sup>, Keunwook Lee<sup>2</sup>, Nam Joo Kang<sup>3</sup>, and Bong-Soo Kim<sup>1\*</sup>

<sup>1</sup>Department of Life Science, Multidisciplinary Genome Institute, Hallym University, <sup>2</sup>Department of Biomedical Science and Research Institute for Bioscience and Biotechnology, Hallym University, <sup>3</sup>School of Food Science and Biotechnology, Kyungpook National University

The understanding interactions between host and microbes is important in microbiome studies. Several in vitro and in vivo system have been used to analyze these interactions. However, most in vivo studies have used the mouse models without validation of treated bacteria colonization. In this study, we analyzed the colonization of treated bacteria in mouse model after administration of antibiotics as a process of develop systems. We extracted metagenomic DNAs from 72 fecal samples of 12 mice (8-9 weeks age; 24 non-treated, 24 Amoxicillin treated, and 24 antibiotics-cocktail treated mice) and 138 fecal samples of 30 mice (8 weeks age; 25 non-antibiotics, 25 non-antibiotics+*Bacteroides fragilis*, 25 Amoxicillin treated, 25 Amoxicillin+*B.fragilis* treated, 18 antibiotics-cocktail treated and 20 antibiotics-cocktail+*B.fragilis* treated mice). The bacterial amounts and diversity were decreased after antibiotics treatment, whereas and then they were recovered after 14 days of stop antibiotics treatment. The amount of *B. fragilis* up to 14 days after treatment was similar between Amoxicillin and cocktail groups. The shift of microbiota was also analyzed and compared among groups. Results in this study will apply to develop the functional verification platform technologies for holobiome research. [grant number of 2021M3A9I4023974]

## **Five Bacteriophages Shown Effective against** *Erwinia amylovora* and *E. pyrifoliae* in South Korea Su Jin Jo<sup>1</sup>, Sang Guen Kim<sup>1,2</sup>, and Se Chang Park<sup>1\*</sup>

<sup>1</sup>Laboratory of Aquatic Biomedicine, College of Veterinary Medicine, Seoul National University, <sup>2</sup>Department of Pharmacy, College of Pharmacy and Institute of Pharmaceutical Sciences, CHA University

*Erwinia amylovora* (Ea) and *E. pyrifoliae* (Ep) cause a fatal blight on pome fruit. As the pathogens are highly contagious and cannot be eliminated, prompt and accurate control is required. We examined the therapeutic potential of five bacteriophages as an effective method to simultaneously control Ea and Ep which are co-outbreaking in South Korea. Morphological analysis showed that the phage pEp\_SNUABM\_03, 04, 11, and 12, and pEp\_08 were classified as *podoviridae*, and *sipoviridae*, respectively. The host range analysis was performed against 116 *Erwinia* strains, including 92 Ea and 24 Ep. Cocktail phage solution showed sensitivity to both Ea (98.91%) and Ep (100.00%) strains. In stability test, all phages were stable under general orchard conditions, however, the pEp\_04, 11, and 12 were unstable at abnormal temperature (i.e. 50°C), pEp\_03 was unstable at high pH condition (i.e. pH 9). The antibacterial effect of phages was determined in the short and long term at different MOIs (5, 1, 0.1). While the phage cocktail showed a rapid antibacterial effect against Ep, it exerts the growth inhibitory effect in long term against Ea. In conclusion, we suggest the phage cocktail can be a candidate for the treatment of the *Erwinia*-originated blight disease and the safety of phages would be confirmed with genome analysis in a future study.

[This research was funded by the Rural Development Administration, Republic of Korea (PJ014965022022).]

#### C020

# Phylogenetic and Genomic Analysis of Indole Acetic Acid (IAA)-producing *Stenotrophomonas* sp. nov. FBCC-B152 from Fresh Water

Ji Young Jung, Ye-Ji Hwang, Ju Hyung Jeon, Jun Sung Kim, and Hyangmi Kim<sup>\*</sup> Microbial Research Department, Nakdonggang National Institue of Biological Resources (NNIBR)

Microorganisms produce indole-3-acetic acid (IAA), that is plant hormone of the auxin class and regulates plant growth and development. The members of genus *Stenotrophomonas* are of increasing interest due to potential as bioinoculants for plant growth stimulation. In this study, *Stenotrophomonas* sp. FBCC-B152, which is capable to IAA production, isolated from fresh water was characterized by phylogenetic and genome analysis. Phylogenetic analyses based on 16S rRNA gene revealed that strain FBCC-B152 belong to the genus *Stenotrophomonas*. The 16S rRNA gene exhibited sequence similarity to *S. rhizophila* DSM 14405T (99.66%) and *S. bentonitica* BII-R7T (99.42%). However, average nucleotide identity (ANI) values were 86.21 and 88.23%, respectively, suggests that strain FBCC-B152 belong to a novel species of the genus *Stenotrophomonas*. In the genome of strain FBCC-B152, two genes encoding for amidase and aldehyde dehydrogenase related to final step in indole-3-acetamide (IAM) and indole-3pyruvate (IPA) pathways for IAA synthesis were found. And Indole-pyruvate ferredoxin oxidoreductase gene related to the direct conversion from IPA to IAA in IPA pathway was found. Moreover, the genome contained genes encoding for acid and alkaline phosphatases and a phytase related to phosphorus solubilization were found. These results demonstrated the phylogenetic position of strain FBCC-B152 and its genetic basis to promote plant growth. [Supported by KEITI through the project funded by Korea MOE.]

### Optimization of In Vitro Co-culture Conditions for SCFAs Cross-feeders Isolated from Faeces

Seol-A Jang<sup>1</sup>, Keunwook Lee<sup>2</sup>, Nam Joo Kang<sup>2</sup>, and Bong-Soo Kim<sup>1\*</sup> <sup>1</sup>Department of Life Science, Multidisciplinary Genome Institute, Hallym University, <sup>2</sup>Department of Biomedical Science and Research Institute for Bioscience and Biotechnology, Hallym University

Cross-feeding is an important metabolic interaction between microbes in their habitats. Short-chain fatty acids (SCFAs) are important for maintaining host cell metabolism and the immune system. SCFAs are produced by SCFAs cross-feeders in the gut, and their interactions are critical to maintain SCFAs concentration. However, the co-culture conditions for SCFAs cross-feeders are still limited. In this study, we evaluated previously reported co-culture conditions. *In vitro* co-culture for three acetate-producing bacteria, *Bifidobacterium adolescentis* (KCTC 5756), *Bifidobacterium longum* (MHL\_0002) and *Bifidobacterium bifidum* and three butyrate-producing bacteria by using acetate, *Faecalibacterium prausnitzii* (KGMB 08719), *Roseburia intestinalis* (KCTC 15746) and *Anaerostipes caccae* (KCTC 15019) were performed to study interspecies interactions. Six different media (MRS, TSB, RCM, RCMB, YBHI, PYG-modified) were used to evaluate co-culture conditions. Single- and co-culture were conducted in an anaerobic condition for 24 hours at 37°C. The growth of strains was assessed by OD<sub>600</sub> absorbance, quantitative real-time PCR using specific primers, CFU measurement, and the concentration of producing SCFAs were measured. We found the better media for co-culture of these strains. Results in this study will be used to analyze the interactions between SCFAs cross-feeders and host cells.

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#### C022

#### The Influence of Microbiome in Adenoid and Gut on Otitis Media with Effusion

Jae Won Jo<sup>1</sup>, Seok-Min Hong<sup>2</sup>, and Bong-Soo Kim<sup>1\*</sup>

<sup>1</sup>Department of Life Science, Multidisciplinary Genome Institute, Hallym University, <sup>2</sup>Department of Otorhinolaryngology—Head and Neck Surgery, Dongtan Sacred Heart Hospital, Hallym University College of Medicine

Otitis media with effusion (OME) is defined as the presence of middle ear effusion without acute inflammation. The microbiome in adenoid can be one of the factors in the pathogenesis of OME. However, the influence of adenoid microbiome in OME is still unclear. We analyzed the microbiome in adenoid and gut of patient group with OME compared to healthy control group. We analyzed microbiome in adenoid and feces collected from childhood (2-12 years old) with OME (adenoid n=15, feces n=14) or healthy control group (adenoid n=18, feces n=18). Whole metagenome sequences obtained from Illumina NovaSeq (250bp paired-end) were analyzed using HuManN3 pipeline. The taxonomic and functional feature of microbiome in adenoid was affected by phenotype (control, patient) and middle ear fluid (serous, mucoid), whereas these differences did not detect in gut microbiome. The diversity of adenoid microbiome in mucoid group was lower than those in control group and serous group (p<0.05). Significantly different species according to middle ear fluid were identified after adjusting age using MaAsLin2. The correlation between species was analyzed by interspecies network analysis (FastSpar), and significantly different species in network. Eighteen metabolic pathways differed between healthy control group and patients (p<0.01). Results in this study can extend understanding about the influence of microbiome in the pathogenesis of OME.

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# Anti-periodontal Inflammatory Effects of Bioactive Compounds Derived from Bioconversion of Milk by *Lactobacillus plantarum* Containing *Artemisia herba-alba* Extract

Sangeun Park<sup>1</sup>, Jiyeon Baek<sup>1</sup>, Soyeon Kim<sup>1</sup>, Yewon Lee<sup>1</sup>, Yohan Yoon<sup>1,2</sup>, and Kyoung-Hee Choi<sup>3\*</sup> <sup>1</sup>Department of Food and Nutrition, Sookmyung Women's University, <sup>2</sup>Risk Analysis Research Center, Sookmyung Women's University, <sup>3</sup>Department of Oral Microbiology, College of Dentistry, Wonkwang University

Many studies have been reported the bioactive compounds produced by bioconversion of medicinal foods such as *Artemisia herba-alba*. However, the effects on periodontal inflammation have not been studied up to date. Thus, this study aimed to investigate antiperiodontal-inflammatory effects of bioactive compounds produced by *Lactobacillus plantarum*-mediated bioconversion of *A. herba-alba* extracts. Milk was fermented by *L. plantarum* as a control (BM1), and *A. herba-alba* extracts were added to milk followed by *L. plantarum* fermentation (BM2). Then, HPLC analyses were conducted for the bioconversion products of BM1 and BM2 in order to compare their compounds. As a result, proline was significantly increased in BM2 (p<0.05) compared to BM1. Then, the effect of proline on periodontal inflammation was examined. The proline (30–500 µg/ml) was treated on RAW 264.7 cells stimulated by *Porphyromonas gingivalis*-LPS (PG-LPS). After 24–48 h, the amount of nitric oxide (NO) and the expression levels of inflammatory cytokines were significantly decreased (p<0.05) compared to compared to compared to control. These findings suggested that proline can be derived from BM2 and may have an alleviation effect on periodontal inflammation.

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#### C024

## Detection of *Weissella* Species Targeting Novel Genetic Markers Based on Comparative Genomics Using Real-Time PCR

Seung-Min Yang, Eiseul Kim, and Hae-Yeong Kim<sup>\*</sup> Department of Food Science and Biotechnology, Kyung Hee University

The genus *Weissella* is ubiquitous microorganisms that can be isolated from everywhere. Some species are involved in the fermentation of foods, whereas some species are known as opportunistic pathogens. Therefore, detection method for *Weissella* species is essential to control opportunistic pathogenic strains in human infection and to produce useful fermented foods. The objective of this study was to develop a rapid and accurate method to detect 11 *Weissella* species in food samples. With 50 genomes representing 11 *Weissella* species, novel genetic markers were mined based on their 100% presence in the genomes of the target species and absence in the genomes of non-target species. Primers based on genetic markers showed positive results for the corresponding species, whereas 79 non-target strains showed negative results. Standard curves exhibited good linearity with a high coefficient of determination ( $R^2 > 0.998$ ) in the range of  $10^3-10^8$  colony-forming units per reaction. The real-time PCR assay developed in this study was used to evaluate 74 strains isolated from food samples to demonstrate that the genetic markers provided a viable alternative to the 16S rRNA gene sequence. In addition, this assay was successfully applied to monitor fermented foods to detect *Weissella* communities. Our real-time PCR assay based on novel genetic markers can be used for accurate and rapid detection for the presence of *Weissella* species in foods.

#### Marine-derived Fungi Producing L-Asparaginase with Low Glutaminase Activity

Woon-Jong Yu, Dawoon Chung, Ji-Young Lim, and Grace Choi<sup>\*</sup> National Marine Biodiversity Institute of Korea

Fungal enzymes are regarded as efficient and compatible resources for industrial applications. L-asparaginase is an enzyme used to treat acute lymphoblastic leukemia. The adverse effects of asparaginase are related to its glutaminase activity and bacterial origin. Therefore, it is necessary to discover new resources for L-asparaginase production with low glutaminase activity. National Marine Biodiversity Institute of Korea (MABIK) collects, preserves, and studies marine-derived fungi in Korea. While screening for extracellular L-asparaginase producing fungi from Marine Microbial BioBank (MMBB) culture collections of MABIK for enzyme activities, we found twenty-eight strains showing extracellular L-asparaginase activity with low glutaminase activity. These isolates were screened for enzyme production using the plate assay method. Among them, there are 3 strains of the genus *Cladosporium*, 3 strains of the genus *Paraconiothrium*, 2 strains of the genus *Paraphaeosphaeria*, 2 strains of the genus *Peroneutypa*, etc. The discovery of L-asparaginase producers of eukaryotic origin such as fungi with low glutaminase activity from marine sources could be alternatives due to their potential of compatibility with human immune system.

[Supported by a grant from National Marine Biodiversity Institute of Korea (MABIK, 2022M00600).]

#### C026

# Development of Real-time Polymerase Chain Reaction Method for Rapid Detection and Quantification of Probiotics Based on Pan-genome Analysis

Ju-Hee Park, Ri-Ha Kim, Yoo-Kyung Kang, Ga-Yeon Chung, Jeong-Eun Kwak, and Ju-Hoon Lee<sup>\*</sup> Department of Agricultural Biotechnology, Department of Food and Animal Biotechnology, Center for Food and Bioconvergence, Research Institute of Agriculture and Life Science, Seoul National University

Probiotics are live microorganisms that survive in the human gut and have beneficial effects on health. But potential risks of probiotics are reported in several clinical trials and some commercial probiotic products provide inaccurate information about species type because the identity among species in each genus is high. So it is important to distinguish and quantify each species in probiotics products. Traditional method to quantify probiotics is time-consuming, labor-intensive, and cannot distinguish species. To solve these problems we used pan-genome analysis to designate the specific gene of each probiotics and the real-time polymerase chain reaction (PCR) method to rapidly identify and quantify probiotics.

In this study, we aimed to rapidly detect and quantify the probiotics designated by the Ministry of Food and Drug Safety with highly accurate primer and probe sets. To select the target gene of each bacteria, pan-genome analysis was performed with a total of 2,170 complete genome sequences and 95% cutoff identity. Selected genes were validated across other bacterial genus using BLASTN. After designing primer sets with the genes, they were validated by conventional PCR. After that, primer and probe sets were designed and validated by real-time PCR in triplicates.

With this novel primer and probe sets, we can accurately detect and quantify each probiotics strains. Thus, our method contributes to detecting accurate strain and establishing safety evaluation standards.

#### Growth Increase of Herbaceous Centella asiatica by Plant Growth Promoting Rhizobacteria

Kyeongmo Lim<sup>1</sup>, HyungWoo Jo<sup>2,3</sup>, Dong-Geol Lee<sup>2</sup>, and Jae-Ho Shin<sup>1,4\*</sup>

<sup>1</sup>Department of Applied Biosciences, Kyungpook National University, <sup>2</sup>COSMAX BTI, R&I center, <sup>3</sup>Department of Microbiology, Dankook University, <sup>4</sup>NGS Core Facility, Kyungpook National University

*Centella asiatica*, also known as Gotu cola, is a herbaceous and native to tropical climate. Numerous clinical studies reported about it's effect on extensive diseases and effectivity as cosmetic raw material recently. According to references, such effects came from *C. asiatica*'s secondary metabolites including madecassoside and those are commonly known to be in roots. For these reasons, it has been considered important to increase root biomass quantity. In this study, we were aimed at plant growth promoting rhizobacteria (PGPR) as biomass increasing promoter and *Bacillus megaterium* was selected as treat PGPR. To see the effectiveness of PGPR, we conducted field test. *C. asiatica* were grown with separated in 2 groups, PGPR treated and non-treated, for 4 months. As a result of the field experiment, PGPR treated group showed increased root length and fresh weight than non-treated group. Rhizosphere microbiome diversity was also higher in treated group. As a result, PGPR treatment increased *C. asiatica*'s root biomass and also affected their root rhizosphere microbiome.

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## C028

# The Effects of *Sphingomonas olei* Isolated from Healthy Skin on Epidermal Regeneration, Barrier Function, and Anti-inflammation in Human Keratinocytes

Minji Park, Wonduk Kim, Yung Jae Kim, and Wonwoo Choi<sup>\*</sup> *CutisBio Co., Ltd.* 

In the skincare industry, traditional approaches focused on eradicating skin microflora are no longer applicable. Current studies of the complex interplay between the skin microbiome, the barrier function, and the immune system indicate that the microbiome, like the gut microbiome, plays a beneficial role. To understand what constitutes a healthy skin microbiome, we have established a skin microbiome bank by collecting specimens from healthy Korean skin (cheek and arm) and isolating and identifying microorganisms. More than 1,700 bacterial and fungal strains were obtained from a total of 60 healthy volunteers during a year, and strains isolated from healthy skin are being continuously obtained and stored at the microbiome bank. We conducted *in vitro* experiments on epidermal regeneration, barrier function, and anti-inflammatory effects in human keratinocytes (HaCaT) for about 50 strains among our microbial resources and found that some strains showed positive results. Herein, the efficacy of one of them, *Sphingomonas olei* CBN003 is introduced. Furthermore, we are developing high-throughput screening assay methods to discover microbes that have beneficial functions on the skin, such as anti-aging, whitening, moisturizing, and anti-inflammation using our own automated facility 'CUTISBIOfoundry'. Therefore, this automated system is expected to more efficiently discover microbes of the skin microbiome that would be utilized to improve skin health.

#### Synercig Effects of L. plantarum HAC03 and Flavonoids in a DSS-induced Colitis Mouse Model

Sungji Jung<sup>1</sup>, Seong-Hwa Kim<sup>1</sup>, Sang-Hun Oh<sup>1</sup>, Youn-Goo Kang<sup>2</sup>, Ah-Ram Kim<sup>2</sup>, and Jin-Hwan Kwak<sup>1,2\*</sup> <sup>1</sup>HDS bio, <sup>2</sup>Department of Life Science, Handong Global University

*Lactobacillus plantarum* HAC03 is a lactic acid bacterium developed by the Department of Life Sciences, Handong Global University, and has excellent anti-inflammatory effects and inflammatory control ability. In addition, this strain has a gene expressing an enzyme essential for hydrolysis of rutin which can increase the bioavailability of flavonoids. In this study, after oral administration of *L. plantarum* HAC03 and rutin for one week, DSS was provided with drinking water to induce intestinal inflammation. Afterwards, the mice were euthanized to determine the length of the colon and the cytokine expression level of the colon tissue. Expression levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), anti-inflammatory cytokine (IL-10), mucin production-related cytokine (Muc2), Tight Junction-related cytokine (Zo-1, Occludin) were determined by real-time PCR. Compared with the group administered *L. plantarum* HAC03 or rutin, respectively, it was shown that the group treated with *L. plantarum* HAC03 and rutin in combination decreased the expression levels of TNF-α, IL-1β, and IL-6. On the other hand, the expression levels of IL-10, Muc2, Zo-1, and Occludin were increased. Co-administration of *L. plantarum* HAC03 and rutin showed a synergistic effect. This results showed that *L. plantarum* HAC03 and rutin could be developed as a good candidate for the prevention and relief of intestinal inflammation. [Supported by grants from Korea INNOPOLIS Foundation]

#### C030

# Functional Characterization of YIYdc1p, a Ceramidase Involved in Sphingolipid Metabolism of the Oleaginous Yeast *Yarrowia lipolytica*

Eun-Joo Jeon, Seo Hyeon Shin, Hye Yun Moon, and Hyun Ah Kang<sup>\*</sup> Department of Life Science, Chung-Ang University

Ceramidase plays an important role in regulating ceramide levels by hydrolyzing ceramide, a central molecule in the pathway of sphingolipid metabolism. Here, we performed functional analysis of *Yarrowia lipolytica YDC1*, encoding a predicted protein with 36% and 35% identity to *Saccharomyces cerevisiae* ceramidases Ypc1p and Ydc1p, respectively. *Y. lipolytica* Ydc1 protein (YIYdc1p), composed of 320 amino acids, has a ceramidase domain and seven transmembrane segments. Despite the absence of an ER retention sequence in YIYdc1p, the cellular localization analysis using GFP fusion showed that YIYdc1p is localized to the ER membrane. We overexpressed *YIYDC1* in the *S. cerevisiae* wild-type (WT) and *ypc1D ydc1D* double mutant strains and investigated the profile change of sphingoid long-chain bases, the breakdown products of ceramides, by TLC analysis. Notably, the *S. cerevisiae* overexpressing *YIYDC1* showed the profiles, both in WT and *ypc1D ydc1D* background, that were more similar to those of the *S. cerevisiae* overexpressing *ScYPC1* than those of the *S. cerevisiae* overexpression strongly indicated that YIYdc1p can cleavage both dihydroceramide and phytoceramide without substrate preference. Our results present YIYdc1p as a manipulation target to increase sphingoid bases in the oleaginous yeast cells with high industrial potential. [Supported by the MOTIE (20008739)]

#### Dysbiosis of Scalp Microbiome in Androgenetic Alopecia

Da-Ryung Jung<sup>1</sup>, Hye-Young Yoo<sup>2</sup>, Min-Ji Kim<sup>1</sup>, Vineet Singh<sup>1</sup>, Minsoo Jeong<sup>1</sup>, Sung-Ha Park<sup>2</sup>, Byoung-Jun Park<sup>2</sup>, and Jae-Ho Shin<sup>1,3\*</sup>

<sup>1</sup>Department of Applied Biosciences, Kyungpook National University, <sup>2</sup>Skin & Natural products Lab, Kolmar Korea Co., Ltd., <sup>3</sup>NGS Core Facility, Kyungpook National University

Androgenetic alopecia (AGA) is one of the most common types of hair loss represented in both women and men. Genetic predisposition and sex hormones are the main causes of AGA development, but various exogenous factors also exist. Therefore, by comparing the scalp microbiome of healthy controls and AGA subjects, we suggest microorganisms as a potential factor in AGA. We collected scalp microbial samples from 141 Koreans (46 healthy controls; CON and 95 AGA subjects; AGA+) and performed 16S rRNA gene sequencing using Illumina MiSeq The scalp of AGA+ had a higher water content than healthy controls. In the AGA+ group, the portion of total *Cutibacterium* and *Staphylococcus* decreased, and the rest of bacteria commonly increased compared to the CON group. This results was also observed with alpha diversity. The network of scalp microbiome in the AGA+ group was more complex and had a higher percentage of unique associations than the CON group. The observation of dysbiosis of scalp microbiome in AGA can be a basic study for the development of cosmetics and therapeutics using microorganisms.

[This research was supported by a project to train professional personnel in biological materials by the Ministry of Environment and the Commercializations Promotion Agency for R&D Outcomes (COMPA) grant funded by the Korean Government (Miinistery of Science and ICT)" (2022). Sequencing was performed at the KNU NGS core facility.]

## C032

## Soil Microbial Diversity is Related to Plant Health Following Biofumigation

Dokyung Lee<sup>1</sup>, Setu Bazie Tegele<sup>2</sup>, Flory Tino Bashizi<sup>2</sup>, Raoul Colince Kuate<sup>2</sup>, Yeon-Kyeong Lee<sup>1</sup>, and Jae-Ho Shin<sup>1,2,3\*</sup> <sup>1</sup>Department of Integrative Biology, Kyungpook National University, <sup>2</sup>Department of Applied biosciences, Kyungpook National University, <sup>3</sup>NGS Core Facility, Kyungpook National University

Biofumigation is a general method for improving soil properties in agriculture and it has a positive effect on alleviating monocropping related constraints including the buildup of soil-borne pathogens. Plant growth improvement following biofumigation is not only attributed to the change in the soil chemical properties but also through modifications of soil microbial community assembly. In this study, we determined the effect of three biofumigants on soil chemical properties, functional genes profile of soil microbiome, weed emergence, and cucumber (Cucumis sativus) yield. Furthermore, we analyzed the correlation of soil pathogen, microbiome, soil property, and plant growth following biofumigation to determine the effect of three kinds of biofumigant. The results showed that cucumber plant height, chlorophyll content, and fruit yield was remarkably improved by all tested biofumigants. Weed emergence was substantially reduced by biofumigants, and red mustard showed the highest reduction. Moreover, whole metagenome data revealed a positive correlation between microbial diversity and plant growth performance.

[This work was supported by the regional researcher program through the National Research Foundation (NRF), funded by the Ministry of Education (Grant NRF-2020R1I1A307452212), a project to train professional personnel in biological materials by the Ministry of Environment and Sequencing was performed at the KNU NGS core facility.]

## **Use of Resazurin to Rapidly Enumerate Bdellovibrio and Like Organisms and Evaluate Their Activities** Seong Yeol Choi, Hyochan Jang, Wonsik Mun, and Robert J. Mitchell<sup>\*</sup>

Ulsan National Institute of Science and Technology

A method to rapidly quantify predatory bacterial cell populations using resazurin reduction to resorufin and its resulting fluorescence kinetics (dF/dt) are described. The reliability of this method to measure the predatory populations was demonstrated with the type strain, *Bdellovibrio bacteriovorus* HD100, as well as *B. bacteriovorus* 109J and two natural isolates, *Halobacteriovorax* strains JA-1 and JA-3, with clear correlation when densities were between 10<sup>7</sup> and 10<sup>9</sup> PFU/ml. Resazurin was also used to evaluate how *B. bacteriovorus* HD100 and *Halobacteriovorax* strain JA-1 respond to harmful conditions, i.e., exposure to sodium dodecyl sulfate (SDS), with both the dF/dt and PFU/ml indicating *Halobacteriovorax* strain JA-1 is more sensitive to this surfactant. Tests were also performed using media of different osmolalities, with the dF/dt values matching the 24-h predatory activities reasonably well. Finally, this method was successfully applied in near real-time analyses of predator-prey dynamics and, when coupled with SDS, was capable of differentiating between the predatory and prey populations. All of these tests serve to prove this method is (i) very rapid, needing only 15 min from start to finish; (ii) very reliable with different predatory bacterial species; and (iii) very versatile as it can be easily adapted to measure predatory numbers and activities in a range of experiments.

## C034

**Understanding How Recombinant Endolysin LNT113 Inactivates Gram-negative Bacteria** Jeongik Cho<sup>1</sup>, Kyungah Park<sup>1</sup>, Eunsuk Kim<sup>1</sup>, and Hyunjin Yoon<sup>1,2\*</sup> <sup>1</sup>Department of Molecular Science Technology, Ajou University, <sup>2</sup>Department of Applied Chemistry and Biological Engineering, Ajou University

Endolysins are hydrolases required for bacteriophages to lyse bacterial cell walls and allow the release of progenies at the end of their infection cycle. Endolysin LNT113 is a derivative of mtEC340M endolysin where cecropin A, an antimicrobial peptide, was fused at its N-terminus. This study investigated its mechanism of action against Gramnegative bacteria. Using diverse biochemical and microscopic experiments, we observed that cecropin A significantly increased the membrane permeability, both the outer membrane and inner membrane, validating that engineering with cecropin A could improve the killing efficacy of endolysins against Gram-negative bacteria. Although LNT113 did not impair the structural integrity in bacterial lipopolysaccharide, *Escherichia coli* mutant strains possessing structural alternations in core and lipid A moieties of lipopolysaccharide, especially core and lipid A, is crucial for Gram-negative bacteria to resist the penetration of a cecropin A-conjugated endolysin across the membrane. This study sheds light on the potential of LNT113 as an alternative to antibiotics against Gram-negative bacteria.

## Characterization of Biogenic Vanadium Dioxide Nanoparticles Synthesized by Shewanella sp. Strain HN-41

Yongseok Ko, Changhu Kang, Youri Yang, Seunghyeon Kim, Sanghyeok Lee, and Hor-Gil Hur<sup>\*</sup> School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology (GIST)

Vanadium dioxide (VO<sub>2</sub>) is promising material due to its unique characteristics such as metal-insulator transition, and change in the transmittance of infrared ray caused by reversible phase transition. Therefore, various synthetic methods have been reported for synthesizing VO<sub>2</sub> through hydrothermal methods, vapor transport methods, and sol-gel methods. However, those synthesizing methods required toxic reducing agents and energy consumption. In our study, we first reported the biotic synthesizing method for VO<sub>2</sub> nanoparticles using metal-reducing bacteria, *Shewanella* sp. strain HN-41 at ambient temperature. Synthesized VO<sub>2</sub> nanoparticles were characterized to M2 phase VO<sub>2</sub> by X-ray diffraction. The average size of the VO<sub>2</sub> particles was 4.3 nm with a circular shape. Thinsectioned TEM analysis revealed that *Shewanella* sp. strain C1 secreted VO<sub>2</sub> nanoparticles through membrane vehicles from the cytoplasm. Our VO<sub>2</sub> synthetic method is eco-friendly because of its low energy consumption and absence of reducing agents.

[Supported by grants from RDA (PJ0162642022) and NRF (2021R1A5A1028138)].

## C036

# Metagenomic Analysis Revealed That the Use of Chemical Mouthwashes for Oral Health Causes an Imbalance in Oral Microbiota and Negatively Affects the Recovery of Normal Microbiota

HyunWoo Son, Sihyun Park, Yu-Jin Hyun, Seung-Eun Lee, Flory Tino Bashizi, and Jae-Ho Shin<sup>\*</sup> Department of Applied Biosciences, Kyungpook National University

One of the many factors affecting oral health is the symbiotic community of microbes in the oral cavity called oral microbiota. Some of these strains, such as *Streptococcus mutans* and *Porphyromonas gingivalis*, are known to have negative effects on teeth such as tooth decay. However, in fact, most oral microbes are beneficial to human health, protect teeth and gums, refresh breathing and facilitate the digestive process. Currently, many people are unaware of this fact and use mouthwash simply to remove harmful bacteria and bad breath in the mouth. However, not much research has been done on how the use of these mouthwashes affects the oral microbiome. Therefore, we tried to determine how mouthwashes affect the human oral microbiome through 16s rRNA gene ampliseq. A total of 29 subjects were tested for a total of 7 days. As a result, there was a significant difference in the weighted UniFrac distance between the group using mouthwash and the control group. Significant differences were identified in *Streptococcus, Capnocytophago, and Fusobacterium* genus, confirming that the use of mouthwashes resulted in an imbalance in the oral microbiome. In addition, when the recovery to the normal state was confirmed, a significant difference was confirmed in the Shannon index and bray-Curtis dissimilarity.

[This research was supported by a project to train professional personnel in biological materials by the Ministry of Environment, Sequencing was performed at the KNU NGS core facility.]

# Characterization of Broad-host-range Bacteriophage vB\_EcoM-pEE20 Infecting Shiga-toxin Producing *Escherichia* coli

Eun Jeong Park<sup>1</sup>, Seon Young Park<sup>2</sup>, Hyemin Kwon<sup>3</sup>, Keeman Lee<sup>1</sup>, Yebin Kim<sup>1</sup>, and Ji Hyung Kim<sup>1\*</sup> <sup>1</sup>Department of Food Science and Biotechnology, College of BioNano Technology, Gachon University, <sup>2</sup>Division of Animal and Dairy Sciences, College of Agriculture and Life Science, Chungnam National University, <sup>3</sup>Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University

Shiga toxin-producing *Escherichia coli* (STEC) is one of the most important food-borne pathogens to date. Recently, the emergence of multi-drug resistant STEC in food sources has arisen as a global concern and developing new alternatives to antibiotics is essential to control the bacteria. In this study, we report a new bacteriophage (phage) that infects STEC with biotechnological potential. The *Myoviridae* STEC phage vB\_EcoM pEE20 was isolated from wastewater collected from a sewage treatment plant in Korea. Interestingly, the phage could infect several species of *Escherichia* as well as STEC. The biological characteristics of the phage were analyzed by adsorption and one-step growth assay and its thermal and pH stability were also determined. The sequenced genome of the phage consisted of linear dsDNA with 166,422 bp (40.44% G+C content) and genome-based phylogeny revealed that it belonged to the genus krischvirus. Its bacteriolytic activity was evaluated, and the phage efficiently inhibited the growth of STEC. Based on these findings, the newly isolated phage could be classified as a new member of krischvirus and could be considered as alternative potential biocontrol agent against STEC in food sources.

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## C038

## Mathematical Modeling for the Growth of *Listeria monocytogenes* in Meatball at Fluctuating Temperatures Jin Hwa Park<sup>1</sup> and Hyun Jung Kim<sup>1,2\*</sup>

<sup>1</sup>Korea Food Research Institute, <sup>2</sup>Department of Food Biotechnology, University of Science and Technology

Meatball is one of the popular processed ready-to-eat (RTE) meat products in Korea. However, they have a potential risk of food poisoning due to undercooking or cross-contamination. In order to estimate the risk of *Listeria monocytogenes* (LM) from the consumption of meatball, predictive mathematical model for behavior of LM in meatball was developed under isothermal and dynamically changing temperature conditions. A cocktail of LM was inoculated onto meatball and stored at 5, 10, 20, 30, 37°C. The primary models were developed using the Baranyi model with a high degree of goodness-of fit ( $R^2 \ge 0.98$ ). As the temperature increased, lag phase duration (LPD) was decreased from 69.07 to 1.63 h and maximum specific growth rate ( $\mu_{max}$ ) was increased from 0.02 to 0.52 log CFU/g/h. The obtained LPD and  $\mu_{max}$  were further described in a secondary model using a Davey model and Square root model, respectively ( $R^2 > 0.98$ ). The developed models were 0.98-1.03 and 1.058-1.144, and RMSE was 0.03-0.35 log CFU/g. Furthermore, dynamic models were validated, with corresponding RMSE values lower than 0.3 log CFU/mL, and ASZ (acceptable simulation zone) values greater than 89%. As the results of validation, the developed dynamic model can be successfully used to predict LM growth in meatball when those are exposed to various temperature and time conditions along the food chain.

## **Comparative Genomics of Three** *Listeria monocytogenes* **Strains Originating from South Korea** Sangryeol Ryu<sup>1</sup> and Sangmi Lee<sup>2\*</sup>

<sup>1</sup>Food Science and Biotechnology Major, College of Agriculture and Life Sciences, Seoul National University, <sup>2</sup>Department of Food and Nutrition, College of Human Ecology, Chungbuk National University

The foodborne pathogen *Listeria monocytogenes* has been intensively studied in North America and Europe but has attracted little attention in South Korea; however, there is an urgent need for examining the ecology and genetic features of *L. monocytogenes* strains from South Korea given the first listeriosis outbreak in South Korea in 2018 and the 2019 USA outbreak caused by enoki mushrooms imported from South Korea. In this study, we compared the whole genome sequences of three strains (SNU7, SNU8, and SNU27) that represent dominant pulsotypes in 150 *L. monocytogenes* isolates obtained in 2004 from chicken carcasses in Seoul, South Korea. SNU7 and SNU8 (CC9 ST9) differed mainly in prophage regions and only SNU7 harbored a plasmid. SNU27 (CC24 ST224) possessed *Listeria* pathogenicity island 3 that increases virulence by modulating gut microbiota. All three strains harbored arsenic resistance genes including those in the plasmid of SNU7 and in *Listeria* genomic island 2 of SNU8. Our findings indicate that *L. monocytogenes* population in South Korea possesses various accessory genes implicated in virulence and environmental fitness, some of which might have been regionally adapted. [Supported by the National Research Foundation of Korea (NRF-2020R1F1A1064262)]

#### C040

#### A Lytic Staphylococcus ureilyticus Bacteriophage Having Anti-biofilm Activity

Hyemin Kwon<sup>1</sup>, Seon Young Park<sup>2</sup>, and Ji Hyung Kim<sup>3\*</sup>

<sup>1</sup>Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, <sup>2</sup>Division of Animal and Dairy Sciences, College of Agriculture and Life Science, Chungnam National University, <sup>3</sup>Department of Food science and Biotechnology, College of BioNano Technology, Gachon University

In response to increasing nosocomial infection caused by antibiotic-resistant coagulase-negative *staphylococci* (CoNS), bacteriophage (phage) has arisen as an alternative to antibiotics. *S. ureilyticus*, one of the species including CoNS, emerge as a pathogen of nosocomial infection that causes bloodstream infection. Herein, we isolated and characterized a lytic *S. ureilyticus* phage, named vB\_SurP-PSU3. The host range of vB\_SurP-PSU3 demonstrated that the phage could infect also *S. warneri*. Moreover, the phage vB\_SurP-PSU3 was efficient in reducing planktonic bacteria and bacterial biofilm against *S. ureilyticus*. Morphological characteristics and genome-based phylogenic analysis revealed that the phage vB\_SurP-PSU3 belonged to the genus *Andhravirus*, and its overall genomic arrangement and contents were similar to other members of the *Andhravirus*. However, the comparative genome analysis showed that the predicted endolysin of the vB\_SurP-PSU3 has distinctly differed. Based on these findings, an isolated *S. ureilyticus* phage vB\_SurP-PSU3 could be used as an efficient biological control agent against infections caused by *S. ureilyticus* and its biofilm.

[This research was supported by the National Research Foundation of Korea (NRF-2020R1I1A2068827) and the Development of technology for biomaterialization of marine fisheries by-products of Korea Institute of Marine Science & Technology Promotion (KIMST) (KIMST-20220128).]

#### Exploring Endolysins as a Therapeutic Agent against Clostridioides difficile Infection

Youngjin Cho<sup>1</sup>, Kyungah Park<sup>1</sup>, Hyeryeong Kim<sup>1</sup>, Jina Seo<sup>1</sup>, Daechan Park<sup>1,3</sup>, and Hyunjin Yoon<sup>1,2\*</sup> <sup>1</sup>Department of Molecular Science Technology, Ajou University, <sup>2</sup>Department of Applied Chemistry and Biological Engineering, Ajou University, <sup>3</sup>Department of Biological Science, Ajou University

*Clostridioides difficile* is a Gram-positive, spore-forming anaerobe that can cause toxin-mediated colitis in humans. Gut microbial dysbiosis by antibiotics treatment has been known as the major cause of *C. difficile* infection (CDI). To cope with the drawback of antibiotics, we aimed to develop an endolysin-based therapeutic agent against CDI. Endolysin is a phage-encoded peptidoglycan hydrolase and has a broader host-range activity than cognate phages, leaving commensal bacteria unaltered. A plate screening system harnessing a *Salmonella* phage SPN1S lysis cassette enabled to screen the potency of multiple endolysins without protein purification. Of 36 endolysins tested, we selected five endolysins, which lysed specifically *C. difficile* peptidoglycans but not other seven bacterial species. In parallel, an *in vitro* CDI model was established using human gut microbiota. Repetitive clindamycin treatments reduced the overall diversity of gut microbiota species with changes in bacterial abundance, while decreasing the two phyla, Firmicutes and Bacteroidetes, and increasing the proportion of Proteobacteria. In spite the clindamycin treatments, the presence of taurocholic acid stimulated *C. difficile* spores to germinate, leading to bacterial proliferation at the later stages of CDI model. The established *in vitro* CDI model can be further employed as a platform to evaluate the efficacy of native endolysins and their derivatives as a therapeutic agent against CDI.

## C042

## Microbiome Differences in Characteristics of Microbiota and Disease Groups between Liver Disease and Diabetes Yu Jeong An<sup>1</sup>, Ki Tae Suk<sup>2</sup>, and Bong-Soo Kim<sup>3\*</sup>

<sup>1</sup>Department of Life Science, Multidisciplinary Genome Institute, Hallym University, <sup>2</sup>Institute for Liver and Digestive Disease, Hallym University, <sup>3</sup>Department of Life Science, Multidicsiplinary Genome Institute, Hallym University

Liver disease and diabetes are common diseases around the world, and several studies reported the association of gut microbiome with these diseases. In this study, we analyzed the gut microbiome in healthy control (n=18), disease control (n=6), and patients (n=55) by using whole metagenome sequences. The patient group was divided into liver disease and diabetes groups. The significantly different clinical factors were analyzed among groups. The diversity of gut microbiota was significantly decreased in liver cancer group and liver cirrhosis, and those in diabetic group was decreased compared to healthy control, but there was no significant difference. The microbiota in disease groups was different to those in healthy control. The significantly different species were identified according to disease subgroup. The functional genes of gut microbiome in disease group was significantly different to healthy control. Results in this study help to understand the role of gut microbiome in liver disease and diabetes, and it can apply to develop treatment of diseases.

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# Co-application of Predatory Bacteria with Antimicrobial Peptide to Completely Eradicate a Colistin Resistant *Escherichia coli* Strain

Sumudu Upatissa and Robert J. Mitchell\* Ulsan National Institute of Science and Technology

In a period where the extensive use of antibiotics has led to the development of multidrug resistance, studies are being done to identify alternative and effective treatment options. Due to their highly specific nature, and their narrow spectrum, colicins have been identified as effective antimicrobial peptides against many pathogenic *Escherichia coli* strains. Within this study, the effectiveness of two colicins possessing divergent mechanisms were evaluated against multidrug resistant *E. coli* strains when used alone, in a mixture with one another or in co-applications with *Bdellovibrio bacteriovorus*. Pore-forming colicin B, which belongs to colicin group B, and tRNase colicin E5 of colicin group A were studied here. While mildly effective when used alone, mixtures of colicin B and/or E5 along with *B. bacteriovorus* HD100 were capable of completely killing colistin-resistant *E. coli* 7004, *i.e.*, less than 100 viable pathogens per ml after 24 hours of incubation. This is the first study to demonstrate the synergistic application of colicins and bacterial predators against a high priority drug-resistant *E. coli* pathogenic strain. Depending on these data the study is expanding to check the potential of this co-application against other multidrug resistant *E. coli*.

#### C044

# *Lactobacillus acidophilus* Protects Irradiation-induced Intestinal Damage by Enhancing Intestinal Epithelial Cell Function

Panida Sittipo<sup>1</sup>, Jin-Young Yang<sup>2</sup>, Jiyoung Kim<sup>3</sup>, Seong-Jun Cho<sup>3</sup>, and Yun Kyung Lee<sup>1\*</sup> <sup>1</sup>Department of Integrated Biomedical Science, Soonchunhyang Institute of Medi-Bio Science, Soonchunhyang University, <sup>2</sup>Department of Biological Sciences, Pusan National University, <sup>3</sup>Radiation Effects Research Section, Radiation Health Institute, Korea Hydro & Nuclear Power CO., LTD

Intestinal epithelial cells (IECs) can respond to signals generated by intestinal microbiota, promoting intestinal function. Irradiation exposure is known to alter intestinal microbiota composition and cause intestinal damage. Therefore, the discovery of beneficial intestinal microbiota in enhancing IEC function is needed to protect or recover intestinal damage from irradiation. We have previously reported an increased intestinal bacteria *Lactobacillus acidophilus* following irradiation exposure and demonstrated its role in intestinal recovery. In this study, we discovered the role of *L. acidophilus* and its cultured supernatant in the protection of irradiation-induced intestinal damage using *in vitro* and mouse model. The expression levels of IEC lineage markers were downregulated in the cells exposed to irradiation. However, these expression levels were reversed when the cells were cultured in the presence of cultured supernatant prior irradiation exposure. Moreover, the expression levels of G protein-coupled receptors and toll-like receptors were increased in cultured supernatant-pretreated cells. Finally, *in vivo* results showed that *L. acidophilus* mitigated symptoms of irradiation-induced intestinal damage in terms of reduced histological score and increased mucin2 expression. Altogether, these findings suggest the protective effect of *L. acidophilus* on irradiation-induced damage, especially by enhancing IEC function. [Supported by a research grant from KHNP(#A21IP13)]

## Unveiling the Mechanism of Bactericidal Activity by an Engineered Endolysin LNT103

Kyungah Park<sup>1</sup>, Jeongik Cho<sup>1</sup>, and Hyunjin Yoon<sup>1,2\*</sup>

<sup>1</sup>Department of Molecular Science Technology, Ajou University, <sup>2</sup>Department of Applied Chemistry and Biological Engineering, Ajou University

Bacteriophages encode endolysins at the end of their lytic cycle to degrade the peptidoglycan layer of the host bacterium, leading to release of progeny phages. In virtue of the bacteriolytic activity, endolysins have been explored as an alternative antibacterial agent. However, the outer membrane present in Gram-negative bacteria obstructs the access of exogenous endolysins to the peptidoglycan lying beneath the outermost membranous structure. In order to overcome the restriction of intrinsic endolysins, we engineered an endolysin to improve its ability to penetrate the membranous structures in Gram-negative bacteria. An endolysin encoded by *Pseudomonas aeruginosa* phage PBPA90 was engineered by substituting the 15 amino acids (mtPA90) and further by fusing the antimicrobial peptide cecropin A to its N-terminus (LNT103). Lipopolysaccharides (LPS) destabilization, bactericidal activity, membrane permeability and LPS neutralization were compared between mtPA90 and LNT103. Cecropin A of LNT103 improved the interaction with LPS and accelerated the disruption of bacterial membrane, leading to faster killing of Gram-negative bacteria. An LPS mutant strain with an altered lipid A structure was more susceptible to both endolysins, suggesting that the integrity of lipid A is important to dampen endolysin penetration into bacterial membrane. This study defined the molecular mechanism of action in destructing Gram-negative pathogens by LNT103.

## **C04**6

**Physicochemical Properties of Cabbage Fermentation Using Saccharomyces cerevisiae** Ahhyeon Chun, Sujeong Park, and Soo Rin Kim<sup>\*</sup> School of Food Science and Biotechnology, Kyungpook National University

In this study, cabbage was fermented using *Saccharomyces cerevisiae* and the physicochemical properties according to fermentation time were investigated. *S. cerevisiae* is a typical eukaryote and is a GRAS (generally recognized as safe) microorganism well known as wine or baker's yeast, so it is suitable for use in food. In addition, *S. cerevisiae* is widely used as a food additive as a source of vitamins, minerals and amino acids. Cabbage (*Brassiaca oleracea* var. *capitate*) is widely consumed around the world and is rich in vitamin C, vitamin U and soluble fiber and contains phytochemicals. It is intended to make a fermented product with amylase and protease activity through the fermentation of cabbage. Through this study, it will be possible to select a strain optimized for cabbage fermentation and suggest the possibility of developing it as a physiologically active functional food through the fermented cabbage hydrolysate.

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# Anti-biofilm Activity of the Compounds Originated from the Jellyfish-derived Fungus *Penicillium chermesinum* M42

Dan-Dan Li, Ying Wang, Wen-xin Niu, Jee Hyung Jung, and Joon-Hee Lee<sup>\*</sup> Department of Pharmacy, College of Pharmacy, Pusan National University

Peroxisome proliferator-activated receptor gamma (PPAR-γ) was known as a potential adjuvant therapy target to treat bacterial pneumonia due to the inflammation inhibition and bacterial clearance ability of PPAR-γ agonists. Under PPAR-γ-guided isolation, we obtained a new analogue (1) of the rare sesquiterpene chermesiterpenoid B (2) from jellyfish-derived fungus *Penicillium chermesinum* M42. The structures were defined by NMR, HRESIMS spectra, and electronic circular dichroism (ECD) data. Chermesiterpenoid B (2) acts as a inhibitor against human and aquatic pathogenic bacteria. In this study, compounds 1 and 2 were identified as PPAR-γ partial agonists based on luciferase reporter assay and docking simulation. In addition, 1 and 2 were found to exhibit anti-inflammation ability through inhibiting NO production in LPS-activated RAW 264.7 macrophages. Therefore, it is worth to detect the anti-biofilm formation and anti-virulence of new compound 1 and chermesiterpenoid B (2) against *Pseudomonas aeruginosa* which is a common cause of hospital-acquired pneumonia.

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#### C048

Development of *Cronobacter sakazakii*-specific Bioluminescent Reporter Phage and Signal Stabilization through Holin Expression Controls

Doyeon Kim and Minsik Kim<sup>\*</sup> Department of Food and Nutrition, Brain Korea 21 FOUR, College of Human Ecology, Yonsei University

Opportunistic foodborne pathogen *Cronobacter sakazakii* frequently contaminates dried foods and causes severe diseases. Here, we developed reporter phages to rapidly detect viable *C. sakazakii*. A bioluminescent reporter operon *luxCDABE* was inserted into the genome of lytic phage  $\Phi$ C01 after the deletion of non-essential phage genes. The resultant phage  $\Phi$ C01\_lux could specifically detect viable *C. sakazakii* within 3 h up to 10<sup>4</sup> CFU/ml. Because the bioluminescent signal was, however, readily decreased due to the superior lytic activity of the  $\Phi$ C01, the lytic activity was attenuated by controlling the expression of phage holin. When the holin promoter was substituted with a weak one or the holin expression was blocked by in-frame deletion, the duration of bioluminescent signal was stabled. Using the holin-controlled reporter phages with 5 h pre-enrichment, at least 1.25 and 4.42 CFU/ml of *C. sakazakii* in artificially contaminated powered infant formular and sun-sik, respectively, were detectable within 2 h. Desiccated *C. sakazakii* on materials of food utensils were also detected at a minimum of 11.1 CFU/ml. The rapid, sensitive, and specific detection of viable *C. sakazakii* and restrict the outbreaks caused by them.

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### Probiotics Isolated from the Korea Traditional Fermentum Foods

Jinok Kwak<sup>1</sup>, Eun Sol Kim<sup>1</sup>, Gi Beom Keum<sup>1</sup>, Hyunok Doo<sup>1</sup>, Sriniwas Pandey<sup>1</sup>, Sumin Ryu<sup>1</sup>, Yejin Choi<sup>1</sup>, Seungjin Yun<sup>1</sup>, Juyoun Kang<sup>1</sup>, Sheena Kim<sup>1</sup>, Ju-Hoon Lee<sup>2</sup>, and Hyeun Bum Kim<sup>1\*</sup>

<sup>1</sup>Department of Animal Resources Science, Dankook University, <sup>2</sup>Department of Food Animal Biotechnology, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Seoul National University

Probiotics are beneficial live microorganisms that are advantageous to the human health when consumed in an appropriate amount. This study was focused on isolating probiotics from the fermented foods. The fermented food samples (Kimchi, Soybean paste, and Cheonggukjang) were collected from the local markets. The total DNA was extracted using the phenol-chloroform DNA extraction method. Specific primer PCR were performed to identify the 19 different species of Probiotics, as mentioned by the Korea Ministry of Food and Drug Safety guidelines. The samples were cultured in different selective mediums (MRS agar, modified RCM agar, M17 agar, and Enterococcosel agar). The isolated colonies were then identified using specific primers of the 19 species, as enlisted by Korea Ministry of Food and Drug Safety, and then the full-length 16S rRNA genes were sequenced using Sanger sequencing. Molecular Evolutionary Genetics Analysis (MEGA) software were used for 16S rRNA gene sequence analysis. A total of 268 bacterial colonies were obtained from the samples and 39 of them were identified as *Enterococcus faecium*. Species belonging to *Enterococcus* genus level produce a wide variety of bacteriocins often called enterocins that are effective against Gram-positive foodborne pathogens, such as *L. monocytogenes*. This study gives a prospective to use the isolated probiotic bacteria and develop it as new potential probiotics.

## C050

# Fermented Seaweed (*Saccharina japonica*) By-products Combined with Probiotics Altered the Fecal Microbiota of Korean Native Goats (*Capra hircus coreanae*)

Eun-Jeong Ko<sup>1</sup>, Seon-Ho Kim<sup>1</sup>, Lovelia Mamuad<sup>1</sup>, Young-Sun Choi<sup>2</sup>, Min-Jung Ku<sup>2</sup>, and Sang-Suk Lee<sup>1\*</sup> <sup>1</sup>Department of Animal Science and Technology, Sunchon National University, <sup>2</sup>Jeollanamdo Agricultural Research & Extension Service

The study aimed to investigate the effect of fermented seaweed by-products (SBP) combined with probiotics on the fecal microbiota of Korean native goats (KNG). SBP was anaerobically fermented for three days with 1% of either *Saccharomyces cerevisiae* KACC30008 (SC) or *Lactobacillus plantarum* ATCC14917 (LP). Fermented SBP (0.5%) was mixed with the total mixed ration (TMR), and fermented for another three days. Sixty breeding KNG (2.2 parities) were randomly selected, divided, and fed continuously after 14 days of adaptation with four treatments. The treatments used were: CON: TMR, T1: TMR+SBP, T2: TMR+SBP+ SC, T3: TMR+SBP+LP. Fecal samples (32) were immediately collected after kidding and sent to Macrogen for metagenomic sequencing. Metataxonomic analysis revealed that Firmicutes and Bacteroidetes were the most abundant bacterial phyla in all treatment groups. The relative abundance of Firmicutes significantly increased (p<0.05) in both fermented SBP and fermented SBP with probiotics. At the genus level, *Phocaeicola* was the most dominant, followed by *Akkermansia, Acetivibrio,* and *Alistipes*. In addition, *Ruminococcus* was increased (p<0.05) in fermented SBP combined with probiotics. Overall, feeding the KNG with fermented SBP combined with probiotics. Overall, feeding the KNG with fermented SBP combined with probiotics altered its fecal microbiota. [Supported by the Jeollanamdo Agricultural Research & Extension Service (Project No.B0080418002694).]

### Compositional Changes of Human Gut Microbiota by Hemo Protein

Seohyeon Lee<sup>1</sup>, Mihye Kim<sup>1</sup>, and Hyunjin Yoon<sup>1,2\*</sup> <sup>1</sup>Department of Molecular Science Technology, Ajou University, <sup>2</sup>Department of Applied Chemistry and Biological Engineering, Ajou University

Iron is an essential molecule in all living organisms. A variety of bacteria utilize iron in diverse biochemical reactions, mainly in the respiratory chain system as a cofactor of several enzymes, thereby influencing bacterial growth. An increasing amount of evidence suggests pivotal roles of gut microbiota in human health concerned with immune system and metabolism disorder such as obesity, diabetes, antibiotic resistance, and inflammatory bowel disease. In this study, we investigated whether iron could alter the structural composition of gut microbiota. Human fecal microbiota were cultivated in a modified medium broth supplemented with hemin or single-cell hemoprotein (heme-SCP) and subjected to 16S rRNA sequencing. In comparison with the fecal microbiota inoculum, the *in vitro* cultivation with iron, regardless of hemin or heme-SCP, reduced the overall microbial diversity with changes in bacterial abundance. Despite the limitation of *in vitro* model, bacterial response to iron supplements was clearly differentiated between bacterial species. Hemin or heme-SCP tended to increase the abundance of Bacteriotedes and Proteobacteria phyla, while moderately decreasing the proportion of Firmicutes. However, species compositions were markedly different between hemin and heme-SCP treatments, which was probably because heme-SCP was dried biomass of hemoprotein-rich bacterial cells. This study demonstrated that heme compounds could shape the gut microbiota.

## C052

Efficacy of a Porcine Reproductive and Respiratory Syndrome Viruses 1 (PRRSV-1) Attenuated Vaccine by Codon Pair Deoptimization (CPD) against Heterologous Challenge in Weaned Piglets Hyang-Sim Lee, Min-A Lee, Su-Hwa You, Bang-Hun Hyun, and Hyang-Sim Lee<sup>\*</sup>

Viral Disease Division, Animal and Plant Quarantine Agency

Porcine respiratory and reproductive syndrome virus (PRRSV) is one of the most challenge infectious disease of pig population causing devastating economic loss to swine industry. PRRSV-1 isolated from PRRS-affected swine farms were attenuated by deoptimization of codon pair bias in ORF7. 3-week-old PRRSV-free piglets were allocated into four group. Vaccinated challenge group (G1, n=4) received  $10^4$  TCID50/ml via intradermal route. The next vaccinated challenge group (G2, n=4) was immunized with the  $10^4$  TCID50/ml by intramuscular route and the remaining 6 animals did not received any vaccine. Finally, all the vaccinated and 4 unvaccinated animals were challenged with heterologous strain via intranasal route, leaving the two animals as unvaccinated unchallenged animal group. After challenge, vaccinated piglets developed a PRRSV-specific IFN- $\gamma$  response to PRRSV stimulation. More importantly, vaccinated challenge piglets exhibited significant reduction in respiratory scores, viremia, viral load, microscopic lesion score. Our findings confirm the effectiveness of PRRS vaccine by attenuated CPD against virulent wild PRRSV-1.

[This research was supported by QIA (Animal and Plant Quarantine Agency; Grant No. M-15430803-2020-23), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.]

# Identification of an Insecticidal Metabolite Produced by *Paenibacillus* sp. GA015 and Their Insecticidal Activity against Fall Armyworm

Haeran Lee and Keunhwan Lim<sup>\*</sup> GAIAGRO

The fall armyworm (FAW, *Spodoptera frugiperda*) is one of the most destructive insect pests of several crops worldwide. In our search for new alternatives to conventional chemicals, we screened microorganisms for insecticidal activity against *Spodoptera frugiperda* and found that *Paenibacillus* sp. GA015 produced insecticidal metabolites. The insecticidal activity of the produced metabolites was evaluated against *S. frugiperda* larvae. The mortality of *S. frugiperda* larvae infected with *Paenibacillus* sp. GA015 (OD = 0.0001) was 94.5%. We prepared cellular fractions of *Paenibacillus* sp. GA015 and tested effects on *S. frugiperda* larvae. More than 80% of *S. frugiperda* larvae infected with cell lysates, and extracellular supernatants of *Paenibacillus* sp. GA015 died at the 48 h feeding period. *Paenibacillus* sp. GA015 induced severe damage in intestinal cells of *S. frugiperda* and disrupted intestinal epithelial integrity, and this mortality assays suggested that *Paenibacillus* sp. GA015 may be applicable to control *S. frugiperda*. These results showed that *Paenibacillus* sp. GA015 could be applicable in controlling fall armyworm for developing novel biopesticides in organic agriculture.

#### C054

## The Isolation of Diazinon Degrading Bacteria, and the Use of a Bacterial Consortium to Enhance the Degradation Effect

Dayeon Kim<sup>1</sup>, Jackson Kilonzi<sup>2</sup>, Ye-eun Kim<sup>1</sup>, Jae-Hyung Ahn<sup>1</sup>, Jaekyeong Song<sup>1</sup>, Sihyun An<sup>1</sup>, Jehyeong Yeon<sup>1</sup>, Joon-Hui Chung<sup>1</sup>, Jeong-Jun Kim<sup>1</sup>, and Hang-Yeon Weon<sup>1\*</sup>

<sup>1</sup>Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration, <sup>2</sup>Kenya Agricultural and Livestock Research Organization Tigoni, Limuru, Kenya

Optimization of yields to curb food insecurity concerns globally rely on extensive application of chemicals including organophosphates to manage biotic stresses. Undoubtedly, the inevitable use of pesticides implicates environmental and human health apprehensions. To the contrary, the potential of adapted and resilient microbes in soil pesticides-degrading has attracted partial research interest. In response, bacterial strains were isolated from soil and evaluated as single isolate and as consortium in degrading diazinon. Results revealed that, out of the 46 strains isolated from the soil, three isolates herein referred to as S6, S36 and S37 exhibited an average diazinon degradation rate of 76.4%, 76.7% and 76.8% respectively, of the initial dose (50 ppm) within 11 days of incubation in mineral medium. However, strains S36 and S37 were not significantly different and were found to be more effective in degrading diazinon at average rate of 3.45 mg/L/day and 3.21 mg/kg/day in broth mineral medium and soil aliquot respectively after 11 days. The strains S36 and S37 possessed catalase and lacked oxidase enzyme. Phylogenetically, the closest species for S36 and S37 were *Priestia megaterium* and *P. arybattia*, respectively, based on 16S rRNA gene similarity (>99%). Combination of three bacterial strains increased diazinon degradation ability by 45%.

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**Investigation of Secondary Metabolites Compounds Produced from Halotolerant Bacteria and Their Applications** Yeonjae Yoo, Sunlul Kwon, Chul-Hwan Kim, Sang Hyun Lee, Minseo Cho, and Jae-jin Kim<sup>\*</sup> *Division of Environmental Science & Ecological Engineering, College of Life Science & Biotechnology, Korea University* 

Coastal wetlands are a transitional environment between land and aquatic habitats. In wetlands, salinity is a limiting factor in the growth of many living organisms such as plants, algae, and microorganisms. Hypersaline environments such as coastal sediments, solar salt, and tidal flats have significantly lower prokaryotic biodiversity than ordinary environments due to salinity. Despite their low diversity, bacteria living in harsh environments are known to produce secondary metabolites as a way of adapting to their environment, making them more valuable as resources. The discovery of unknown secondary metabolites from extreme halophiles in harsh environments is expected to enable further industrial development. In this study, we performed the isolation of secondary metabolites and reveal the new compounds, as well as investigated the optimization conditions for producing these metabolites for halophilic bacteria isolated from salterns of Yeongjong-do (37°26'N, 126°23'E), Republic of Korea.

## C056

#### Predatory Bacteria and Violacein as Alternative Antibiotics under Microgravity

Hyochan Jang<sup>1</sup>, Seong Yeol Choi<sup>1</sup>, Wonsik Mun<sup>1</sup>, Seok Hoon Jeong2<sup>2</sup>, and Robert J. Mitchell<sup>1\*</sup> <sup>1</sup>School of Life Sciences, Ulsan National Institute of Science and Technology (UNIST), <sup>2</sup>Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine

As mankind moves toward permanently inhabiting outer space and other planetary bodies, alternatives to antibiotic that can effectively control drug-resistant pathogens are needed. The activity of one such alternative, *Bdellovibrio bacteriovorus* HD100, was explored here, and was found to be as active or better in simulated microgravity (SMG) conditions as in flask and normal gravity (NG) cultures, with the prey viabilities decreasing by 3-to 7-log CFU/ml in 24 h. The activity of *B. bacteriovorus* HD100 under SMG was also appraised with three different antibiotic resistant pathogens. In addition to being more efficient at killing two of these pathogens under SMG conditions (with losses of 5- to 6-log CFU/ml), we also explored the ability of *B. bacteriovorus* HD100 to hydrolyze the antibiotic resistant gene pools, i.e., mcr-1, bla<sub>KPC-2</sub> and bla<sub>OXA-51</sub>, present in pathogens. We found removal efficiencies of 97.4±0.9%, 97.8±0.4% and 99.3±0.1%, respectively, in SMG cultures, while similar reductions were also seen in the flask and NG cultures. However, predatory bacteria cannot attack Gram-positive bacteria. Violacein is a bisindole antibiotic which is effective to several Gram-positive bacteria. Under SMG, antimicrobial activity of violacein increased against *S. aureus* compared to NG. These results illustrate the potential applicability of *B. bacteriovorus* HD100 and violacein as an antibiotic to combat the ever-growing threat of multidrug-resistant (MDR) pathogens during spaceflight.

# An Investigation of the Effects of Antibiotic Administration on Obesity-induced Mice via Metagenomic Analysis of Gut Microbiota and Resistome

Jungman Kim<sup>1,2</sup>, Gwangpyo Ko<sup>2</sup>, Minwoo Kim<sup>2</sup>, and Tatsuya Unno<sup>1,2\*</sup> <sup>1</sup>Subtropical/tropical Organism Gene Bank (SOGB), Jeju National University, <sup>2</sup>Faculty of Biotechnology, College of Applied Life Sciences, SARI, Jeju National University

The unhealthy condition including obesity is closely associated with the disruption of gut microbiota in hosts. Many studies related to the interaction of gut microbiota in obese states have been designed using a mouse model fed with a high-fat diet (HFD). However, few papers have recently reported on the relationship between obese gut microbiota, antibiotic efficacy, and antibiotic resistance genes (ARGs). In this study, we investigated the interaction between gut dysbiosis and resistome using an obese mouse model fed with HFD for 13 weeks. After antibiotics administration (ciprofloxacin, amoxicillin, and erythromycin) for 3 days, we monitored the shift in gut microbiota and resistome based on metagenomic analysis for 2 weeks. All antibiotics treatment showed a lower recovery speed of gut microbiota with more decreases in bacterial richness and evenness in HFD-fed groups compared to ND-fed groups. HFD-fed groups had a significantly higher abundance of *Lactococcus* which was a dominant species that survived against antibiotic administration. In addition, the analysis of ARGs showed that the levels of ARGs were higher in HFD-fed groups than in ND-fed groups, which was increased by obesity-related *Lactococcus* which was the most frequently identified host of ARGs such as ImrD. These findings suggest that gut dysbiosis induced by HFD is more affected by antibiotics treatment than normal health conditions, which may be associated with changes in gut resistome.

#### C058

**Investigation of Bacterial Diversity Isolated from a Tidal Flat in Julpo Bay, Republic of Korea** Sang Hyun Lee, Yeonjae Yoo, Chul-Hwan Kim, Sun Lul Kwon, Minseo Cho, and Jae-Jin Kim<sup>\*</sup> *Division of Environmental Science & Ecological Engineering, College of Life Science & Biotechnology, Korea University* 

Getbol are unique tidal flats found on the western and southern coast of the Republic of Korea. Tidal flats such as Getbol are known to have diverse ecological roles such as habitats of coastal organisms, purification of pollutants, and regulation of nutrients. Microorganisms are known as a key part of the diverse ecological roles of tidal flats, so isolation and study of diverse microorganisms in tidal flats are important to understand the complicated ecological roles of tidal flats. Also, the diversity of microorganisms in tidal flats gives great opportunities for researchers to seek novel species and molecules.

In this study, we isolated 49 bacteria strains from sediments by serial dilution method on starch-casein agar medium. 22 strains of Bacillaceae, 20 strains of Streptomycetaceae, 4 strains of Halomonadaceae, and 1 strain of Planococcaceae and Micrococcaceae each were identified. Among those strains, *Streptomyces* sp. JPGA2-3 showed a unique feature of blue pigment production. Antioxidant activity of bacterial extract from *Streptomyces* sp. JPGA2-3 was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The results showed high radical scavenging activity, with 92% radical scavenging activity compared to ascorbic acid of the same concentration. Since pigments produced by bacteria are known to have high bioactivities, further studies of the strain *Streptomyces* sp. JPGA2-3 and its pigment are required.

#### Screening of Indigenous Bacterial Strains for Alleviating Salinity Stress in Cucumber Plants

Eun Hye Na, Jeong Hyeon Suh, In Ho Cho, Hwa Jin Jang, and Sung-Je Yoo<sup>\*</sup> Sangju Agricultural Technology Extension Center

Plants are constantly exposed to a variety of abiotic stress conditions. To protect themselves from stresses, plants could interact with soil beneficial bacteria, including plant growth-promoting rhizobacteria (PGPR). In the present study, 112 bacterial strains were isolated from plant rhizosphere soil in four cucumber farms located in Sangju province, Korea; seven strains were selected based on four plant growth-promoting (PGP) traits (phosphate solubilization, aminocyclopropane-1-carboxylate (ACC) deaminase activity and productions of indole-3-acetic acid (IAA) and ammonia) and growth ability under salinity stress condition. In plant tests, three among seven strains (SAG04, SAS07 and SAS24) increased total fresh weight under non-stressed condition and decreased stress severity (%) of cucumber plants under salinity stress condition (EC 6.2 ds/m); SAS07 and SAS24 strains enhanced enzyme activities of salinity soil including urease and acid phosphatase, respectively, compared to untreated plants. These findings demonstrated that both strains SAS07 and SAS24 play important roles in alleviating salinity stress by regulating soil activity, and both can be used as potential bio-fertilizers for sustainable agriculture.

## C060

# Alteration of Symbiotic Fungal Community Following the Decline of Korean Fir (*Abies Koreana*) in Regions of Mt. Hallasan, Jeju Island, the Republic of Korea

Minsoo Jeong<sup>1</sup>, Setu Bazie Tagele<sup>1</sup>, Min-Ji Kim<sup>1</sup>, Suk-Hyung Ko<sup>2</sup>, Kwon-Su Kim<sup>2</sup>, Jung-Goon Koh<sup>2</sup>, Da-Ryung Jung<sup>1</sup>, YoungJae Jo<sup>1</sup>, Yeong-Jun Park<sup>1</sup>, Min-Sueng Kim<sup>1</sup>, Kyeongmo Lim<sup>1</sup>, and Jae-Ho Shin<sup>1,3\*</sup> <sup>1</sup>Department of Applied Biosciences, Kyungpook National University, <sup>2</sup>Hallasan Research Department, World Heritage Office, Jeju Special Self-Governing Province, <sup>3</sup>NGS Core Facility, Kyungpook National University

The *Abies koreana* (Korean fir) population is declining at an accelerating rate on Mt. Hallasan in Jeju Island, Republic of Korea. Several prior studies have reported various inconclusive reasons, indicating that additional data, such as data on microbial, are required to further understand the phenomena. To the best of our knowledge, this is the first investigation that documents the changes in the microbial and fungal community as the result of the decline of the Korean fir forest. Interestingly, HKF soil comprised dominant symbiotic fungi, whereas the microbial composition in DKF samples comprised abundant saprotrophs. Judging from the standard total effects, the structural equation model showed that the symbiotic fungal community had greater effects on the Korean fir decline. Our findings suggest that the lager-scale decline of Korean fir might be due to changes in the soil symbiotrophs that were indirectly affected by the surrounding environment. Based on these results, further studies are needed to elucidate the scientific causal relationship between the Korean fir decline and the symbiotrophs. [This research was supported by a project to train professional personnel in biological materials by the Ministry of Environment and the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ015697)" Rural Development Administration, Republic of Korea. Sequencing was performed at the KNU NGS core facility.]

### Can We Predict Preterm Birth by Analyzing the Vaginal Microbiome Using Machine Learning Techniques?

Sunwha Park<sup>1</sup>, Jeongsup Moon<sup>2</sup>, Taesung Park<sup>2</sup>, Young-Ah You<sup>1</sup>, Young Min Hur<sup>1</sup>, Soo Min Kim<sup>1</sup>, Gain Lee<sup>1</sup>, Young-Han Kim<sup>3</sup>, and Young Ju Kim<sup>1\*</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Ewha Womans University Mokdong Hospital, <sup>2</sup>Interdisciplinary Program in Bioinformatics, Seoul National University, <sup>3</sup>Department of Obstetrics and Gynecology, Yonsei University College of Medicine

In this case-control study, the subjects were pregnant women with 54 with preterm birth and 96 with term birth. Cervicovaginal fluid was collected and their demographic profiles and white blood cell count were recorded. The microbiome profiles were analyzed with 16S rRNA gene amplification of V3-4 region. Amplicon Sequence Variants analysis, grouping, and classification were conducted using DADA2. After univariate analysis with Wilcoxon ranksum test, EdgeR, DESeq2, ZIBSeq, metagenomeSeq, ANCOM and CLR Permutation, 17 markers were selected. Samples were randomly divided into a training set and a test set by the ratio of 2 to 1. The machine learning models such as logistic regression, random forest, XGBoost and support vector machine were used. When forward variable selection was performed using the whole markers, the maximum validation AUC was 0.94. When using 10 selected markers, including *Lactobacillus* spp., *Gardnerella vaginalis, Ureaplasma parvum, Atopobium vaginae, Peptoniphilus grossensis, Prevotella timonesis,* the AUC was 0.73. In the model developed using XGboost, test AUC was 0.72 with 60% of sensitivity and 83% of specificity. Our study demonstrates that several candidate bacteria could be used as a potential predictor for preterm birth and we confirmed that predictive rate can be increased as a model through machine learning technique.

[This study was supported by the NRF, the BK21 and a grant from the Korea Health Technology R&D Project through KHIDI]

## C062

**Plant Growth Promoting Rhizobacteria Improve Growth and Biological Control of Pepper (***Capsicum Annum***)** Tino Bashizi<sup>1</sup>, Kyeongmo Lim<sup>1</sup>, Setu Bazie Tagele<sup>1</sup>, Min-Ji Kim<sup>1</sup>, GyuDae Lee<sup>1</sup>, and Jae-Ho Shin<sup>1,2\*</sup> <sup>1</sup>Department of Applied Biosciences, Kyungpook National University, <sup>2</sup>NGS Core Facility, Kyungpook National University

Growing demands for agricultural production have become a big challenge as people also realize the importance of reductions in the use of synthetic chemical fertilizers. Plant Growth Promoting Rhizobacteria (PGPR) are proven biofertilizers to increase crop yields by promoting plant growth through various direct or indirect mechanisms. However, a cumulative or synergistic effect does not always follow when a bacterial consortium is used. This work evaluates the mechanisms of Phytophthora blight disease suppression by PGPRs and evaluates their potential to promote plant growth, in particular on Pepper (Capsicum Annum). We also bring proof of the potentialities of the various stocks at the biochemical level as well as their compatibilities to constitute a consortium. Finally, we tested the strains in pots in consortium to see their effectiveness in comparison with a second treatment containing only the pathogen. From the results, we concluded on the applicability of these strains in the consortium in order to fight against Phytophthora capsici, but also of carrying out a field experiment for more efficient applicability in agriculture.

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#### **Neutralization Suitability Study for High Concentration Surfactants**

Hyun Gee Lee Amorepacific

In the case of high concentrations of surfactants, such as shampoo, it is difficult to evaluate the microbial limit. The reason is that the neutralizers does not completely neutralize the shampoo. The purpose of this study is to find a dilute medium that is completely neutralized even in formulations containing high concentration surfactants. Most of them failed when evaluated by the ISO standard test method. However, when MLA medium was used instead of TSA, neutralization was confirmed except for some bacteria and formulations. It was confirmed that the acceptance rate was increased when tween and lecithin were added to the neutralization medium (MLB). In

particular, it was confirmed that TSA neutralized over a certain level. In conclusion, in the case of a formulation with a high concentration of surfactant, it is suggested to use MLB with Tween80/lecithin = 4%/1% as a neutralization medium and to use MLA as an agar medium.

#### C064

## Soybean Core Bacterial Microbiota Involved in Nodule Formation

Amani Sliti<sup>1</sup>, Minsoo Jeong<sup>1</sup>, and Jae-Ho Shin<sup>1,2,3\*</sup>

<sup>1</sup>Department of Applied Biosciences, Kyungpook National University, <sup>2</sup>Department of Integrative Biology, Kyungpook National University, <sup>3</sup>NGS Core Facility, Kyungpook National University

The soybean is an important source of food hence; its production has been increasing worldwide. Therefore, to boost soybean production, the identification of its microbiome is crucial to investigate functional core microbes that could be applied as biofertilizers. The NGS analysis of Korean soybean hybrids from the rhizosphere, bulk, endosphere, and nodule microbial communities revealed the presence of differently abundant microorganisms such as the higher abundance of *Bradyrhizobium* (94%) in nodules. The 16S rRNA sequencing showed as well that bacterial communities belonging to phylum *Proteobacteria, Bacteroides, Firmicutes,* and *Actinobacteria* exist in different proportions in bulk, rhizosphere, and endosphere. This distribution is affected by several factors including plant genotype, plant growth stage, and plant metabolites. The genera *Bradyrhizobium* is a symbiotic bacteria, involved in the fixation of atmospheric nitrogen to make it assimilable by plants. Based on these findings we propose that these genera might be having the potential to be used as biofertilizers for soybean crops, yet further studies are needed to confirm it.

[This research was supported by a project to train professional personnel in biological materials by the Ministry of Environment and the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ015697)" Rural Development Administration, Republic of Korea. Sequencing was performed at the KNU NGS core facility.]

## Functional Differences of Peach [Prunus persica (L.) Batsch] Tree Phyllospere Associated with Peach Gummosis

Tae-Hyung Park<sup>1</sup>, YoungJae Jo<sup>2</sup>, Da-Ryung Jung<sup>2</sup>, Dokyung Lee<sup>1</sup>, Min-Kyu Park<sup>2</sup>, Kyeongmo Lim<sup>2</sup>, Wan-Ro Kim<sup>1</sup>, Yeon-Kyeong Lee<sup>1</sup>, Amani Sliti<sup>2</sup>, GyuDae Lee<sup>2</sup>, and Jae-Ho Shin<sup>1,2,3\*</sup>

<sup>1</sup>Department of Integrative Biotechnology, Kyungpook National University, <sup>2</sup>Department of Applied Biosciences, Kyungpook National University, <sup>3</sup>NGS Core Facility, Kyungpook National University

Peach gummosis is one of the most important and fatal symptoms that occur in these peaches, and many countries have attempted to control it. In our previous study, we compared the differences of microbiome and mycobiome according to gummosis in peach trees. In summary, by identifying the microbial community of the peach tree, it was possible to confirm the difference. Interestingly, we were able to find that there were a variety of microbes that can help plant growth in unhealthy peach trees. In this study, microbiome and mycobiome from unhealthy groups were analyzed and compared to functional differences with healthy groups. We performed functional profiling of bacteria and found on unhealthy peach tree bark with gummosis, that some types of bacteria can help plant growth. Although the abundance of disease-causing plant pathotropic fungi was high in unhealthy groups, it was confirmed that the functional properties of the microbiome induce nitrogen fixation and polysaccharide production.

[This research was supported by a project to train professional personnel in biological materials by the Ministry of Environment and the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through the Crop Viruses and Pests Response Industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA)(321097-3). Sequencing was performed at the KNU NGS core facility.]

## C066

## Diversity and Bioprospecting of Cold Adapted Antarctic Fungi Isolated from the Antarctica

Ji Hyeon Lee<sup>1</sup>, Rye Gyeong Park<sup>1</sup>, Ji Eun Lee<sup>1</sup>, Ju Hong Kang<sup>1</sup>, Jae Won Lee<sup>1</sup>, Won Ho Jeong<sup>1</sup>, Sung Ju Cho<sup>2</sup>, Hyun Cheol Oh<sup>3</sup>, and Jae Hak Sohn<sup>1,2\*</sup>

<sup>1</sup>Maior in Food Science and Culinary Arts, College of Health and Welfare, Silla University, <sup>2</sup>Seafood Research Center, IACF, Silla University, <sup>3</sup>Institute of Pharmaceutical Research and Development, College of Pharmacy, Wonkwang University

We surveyed the diversity of Antarctic fungi separated from other regions, including King George, Jangbogo Antarctic Research Station, and the Ross Sea, and their capability to produce bioactive compounds. Sample diluted by 10 fold dilution method after homogenization, were inoculated on nutrient agar (NA), 0.1% NA, R2A, PDA, YMA, ISP2, ISP4, GYA and Marine Agar plate and then incubated at 10°C for 20 days. 359 fungal colonies were isolated and stored in 15% (v/v) glycerol solution. Isolates were tested for their ability to grow at temperatures (5, 10 and  $25^{\circ}$ C). As a result, the 92 isolates were psychrophiles, 123 isolates were psychrotolerant and 132 isolates were mesophile. 117 fungal strains were incubated at 15°C for 10 to 15 days and then extracted with ethyl acetate. As a result of the PTP1B inhibition assay using the extract, 117 strains showed strong inhibitory activity. From the results of identification using the internal transcription spacer (ITS) sequence, the fungal strain consisted of 196 taxa in 62 genera. These results suggest that Antarctic fungi might be a valuable resource for the screening of physiological active material.

#### Differences in Bacterial Composition between Public and Private Restrooms

Yeon-Kyeong Lee<sup>1</sup>, Da-Ryung Jung<sup>2</sup>, and Jae-Ho Shin<sup>1,2,3\*</sup>

<sup>1</sup>Department of Integrative Biology, Kyungpook National University, <sup>2</sup>Department of Applied Biosciences, Kyungpook National University, <sup>3</sup>NGS Core Facility, Kyungpook National University

Human in industrialized areas, spend more time in shared spaces than in their residences. Human microbiota is influenced around us, and at the same time affects microbial communities in that space. To investigate microbial communities in shared spaces, our study focuses on the analysis of the differences between bacterial composition in men's public and private restrooms. Furthermore, 54 samples were collected using sterile cotton swabs from three public and private restrooms at Kyungpook National University (KNU) and each studio. Nine sites were sampled in each place. Sequencing of 16S rRNA genes was performed using Illumina Miseq after the amplification of the V4-V5 region aiming to investigate the composition of microbiota in each sampled site.

As a result, samples collected from public restrooms have a higher relative abundance of bacteria which are associated with the fecal and skin microbiota including *Staphylococcus* and *Prevotella* genera. On the contrary, in private restrooms, microbial communities are related to the skin and environmental bacterial flora such as *Methylobacterium* and *Sphingomonas* genera. Our research highlights the existence of various microbial communities that can differ depending on the location, users, and environmental factors.

[This research was supported by a project to train professional personnel in biological materials by the Ministry of Environment. Sequencing was performed at the KNU NGS core facility.]

#### C068

## The Tumor Therapeutic Effects of Enterotoxin-secreting Tumor Target Bacteria in a Claudin-4 Expression Tumor Models

Jam-Eon Park, Seung-Hyeon Choi, Ji Young Choi, and Seung-Hwan Park<sup>\*</sup> Biological Resources Center, Korea Research Institute of Bioscience and Biotechnology

Bacteria-mediated cancer therapy was a way in which tumor target bacteria penetrate the tumor, suppress and degenerate the tumor, and expect anticancer effects. In addition, the development of molecular biology and genetic engineering had increased the efficiency of cancer therapy by allowing anticancer agents to be delivered through tumor-targeting bacteria. In order for bacteria to specifically deliver anticancer agents to tumors, a mechanism to precisely regulating the gene expression of anticancer agents was required. In this study, a pBAD promoter system in which gene expression is controlled with L-arabinose was used for safe delivery of anticancer agents. Attenuated bacteria was transformed with plasmids encoding the Clostridium perfringens (CPE) gene: CPE was already known as cytolysin, which binds to the claudin-4 (CLDN-4). The engineered bacteria successfully expressed and secreted CPE by L-arabinose, and the secreted-CPE specifically bound to CLDN-4, induced cell necrosis. In vivo study, CPE-expressing bacteria successfully targeted tumors and produced CPE in tumors. The CLDN-4 expression tumor was suppressed and degenerated by produced CPE form the attenuated bacteria in tumor-bearing mice, and necrotic cells were observed. Therefore, CPE-expressing attenuated bacteria can be used as a powerful anticancer agent that can be successfully targeted and treated for CLDN-4 expressing tumors. This study supported by grants from Ministry of Trade, Industry & Energy.

## A Self-interacting Fusarium graminearum virus 1 ORF2 Protein Is Involved in Transcriptional Regulation of RNAi-Related Genes in *Fusarium graminearm*

Jisuk Yu<sup>1</sup> and Kook-Hyung Kim<sup>1,2,3\*</sup>

<sup>1</sup>Plant Genomics and Breeding Institute, Seoul National University, <sup>2</sup>Department of Agricultural Biotechnology, Seoul National University, <sup>3</sup>Research Institute of Agriculture and Life Sciences, Seoul National University

Fusarium graminearum virus 1 (FgV1) is a positive-sense single-stranded RNA virus infecting *Fusarium graminearum*, the primary causal agent of Fusarium head blight. FgV1 confers hypovirulence-associated traits in fungal hosts and inhibits RNAi responses mediated *by FgDICER-2* and *FgAGO-1*. This study identified molecular mechanisms of pORF2-mediated antiviral suppression activity and characterized the function of pORF2 (~17 kDa). Comparing hairpin RNA construct-induced responses at transcript levels of *FgDICER-2* and *FgAGO-1* in each ORF expressing mutant, only ORF2 expressing mutant did not show increases in transcript levels of those genes. Electrophoretic mobility shift assays showed that pORF2 binds DNA non-specifically *in vitro* via its C-terminal region. In yeast two-hybrid assay, we confirmed that pORF2 interacts with itself, and the N-terminal region was required for self-interaction. We also found that ORF2-GFP localized in mycelia granules and sometimes colocalized with H1-RFP. This study indicates that pORF2 of FgV1 inhibits RNAi defense response by transcriptional repression of RNAi-related genes. Further investigation will elucidate whether the properties of pORF2 (DNA-binding and self-interaction) play a vital role in suppressing RNAi response.

[This research was supported by grants from the National Research Foundation of Korea funded by the Ministry of Science and ICT (NRF-2020R1C1C1011779) of South Korea.]

#### C070

**Nematicidal Activity of Microbial Control Agents against Clover Cyst Nematode** Eun-Hyung Park, Se-Geun Park, Byeong-Yong Park, and Hyoung-Rai Ko<sup>\*</sup> *National Institute of Agricultural Sciences* 

Clover cyst nematode (CCN) is one of the important plant-parasitic nematodes worldwide, and mainly damage to Kimchi-cabbage production in Korea. However, nematicides to manage the CCN are no available during Kimchi-cabbage cropping season because of the residual toxicity. This study was conducted to develop the bio-control agents (BCAs), which could be used during the cropping season, against the CCN. We assessed the control efficacy of two bacterial candidates (BC1 and BC2) to CCN second-stage juveniles (J2s) using *in vitro* assay. The assay result showed the culture solution of the BC2 was highly toxicity to the J2s with 100% mortality. To verify the *in vivo* efficacy of nematicidal bacterial strain, we performed pot experiment in a temperature-controlled room (25°C). As a result, the total fresh weights of BC2 treatments treated twice at 15-day intervals were 1.4 and 2.2 times higher than those of the TSB (medium alone) and NemaO (nematode alone), respectively. The female proliferation in BC2 treatment in Kimchi-cabbage roots was inhibited by 77% compared with control (NemaO). The BC2 treatments reduced the cyst size compared to NemaO treatment. These results showed that the BC2 has nematicidal activity against CCN and has a potential as BCAs.

#### Library Construction for Compounds Isolated from Actinomycetes with Harmful Nematode Activity

Min-Kyoung Kang<sup>1</sup>, Jong-Hoon Kim<sup>1</sup>, Hyeon Ji Jeong<sup>1,2</sup>, Byeong Min Lee<sup>1,3</sup>, Bong Hyun Sung<sup>4</sup>, Dong-Jin Park<sup>1</sup>, and Kwang-Hee Son<sup>1\*</sup>

<sup>1</sup>Microbiome Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>2</sup>Department of College of Pharmacy, Chungnam National University, <sup>3</sup>Department of Bio Environmental Chemistry, Chungnam National University, College of Agriculture and Life Science, <sup>4</sup>Synthetic Biology and Bioengineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB)

Due to the annual increase in nematode damage, the concerns of farmers are increasing. Nematodes survive regardless of the environment such as soil, irrigation water, and soil, and they parasitize plants and damage crops. Nematodes are the most abundant multicellular species on Earth and play an important role in the maintenance and conservation of ecosystems. However, crop damage from nematodes is estimated at around \$130 billion in global agricultural productivity. Among the methods for controlling nematodes, chemical control is the most used. Chemical control is a method of treating nematodes such as pesticides and organic materials. Chemical control is the most used at replace it. The development of biological pesticides is global, and recently, pesticides and pharmaceutical companies are entering the pesticide market using microorganisms or their metabolites by switching from chemical-based pesticides to biological fields. In our study, nematode activity screening was performed from 8,000 soil actinomycetes that produce useful metabolites. Actinomycetes with activity were selected and identified based on 16S rRNA to construct an nematicidal active strain library. By separating the active compound from the selected strain, it was attempted to secure a data base that could be used in the future nematode control market.

[Supported IPET and NRF & MSIT]

#### C072

## Isolation and Characterization of Melatonin Producing Rhizobacteria that Enhance Growth of Oriental Water Melon (*Cucumis melo* ssp. agrestis var. makuwa)

Ji-In Woo, Jin Ryeol Jeon, Eun-Hae Kwon, Sang-Mo Kang, and In-Jung Lee<sup>\*</sup> Department of Applied Biosciences, Kyungpook National University

The global climate change and application of synthetic fertilizers have been significantly imposing environmental stress, phytotoxicity and low quality yield. Since optimization of nutrients is crucial for sustainable crop production; ; biostimulants such as plant growth promoting bacteria and melatonin are emerging as an efficient tool for nutrients supplement and stress reliever in plants. Based on these fact, we isolated altogether 586 bacterial strain from greenhouses located in Seongju-gun, Gunwi-gun, Uiseong-gun, and Buan-gun, Jeollabuk-do, Gyeongsangbuk-do, Republic of Korea. Moreover, we tested their plant growth promoting traits such as 1) EPS production ability, 2) Phosphate solubilizing activity, 3) IAA production ability, 4) Siderophore production ability, 5) ROS scavenging ability, 6) Catalase activity, and 7) melatonin production. It was observed that 100 strain have considerable plant growth promoting traits and Nine strain showed a melatonin production ability. These nine strains were finally selected and treated to oriental water melons (*Cucumis melo* ssp. agrestis var. makuwa) with pellet and culture medium. Our results showed that inoculation of these strain significantly enhanced the total biomass of the *Cucumis melo* plants especially Oryzae KJW44 and Bacillus safensis KJW143 showed higher beneficial effect. The current findings showed that the bacterial strain used in this experiment could be employed as an environmental friendly biofertilizer tool to improve plant physiology and growth.

[Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2022R1A2C1008993).]

### Inoculation of Melatonin-producing Rhizobacteria Promotes Growth of Soybean (Glycine max L.)

Jin Ryeol Jeon, Ji-In Woo, Eun-Hae Kwon, Sang-Mo Kang, and In-Jung Lee<sup>\*</sup> Department of Applied Biosciences, Kyungpook National University

Melatonin has been reported as an important molecule for regulating the internal physiology of plants. In addition, optimizing the nutrient uptake efficiency in the plant is crucial for quality crop production. Since synthetic fertilizer's natural source and its uptake efficiency in plants is limited, biostimulants such as plant growth promoting microorganisms are considered an important substitute to synthetic fertilizers for organic and sustainable agriculture production. In light of these facts, we screened several bacterial strains from soil and tested their plant growth promoting traits. Among 874 isolates, 100 strains showed considerable traits such as phosphate solubilization, ROS scavenging and catalase activity, and indole-3-acetic acid, EPS, and siderophore production in which nine isolates showed higher melatonin production ability. These nine bacteria were treated to the soybean seedlings with pellet and culture media. Overall, all the strains enhanced the plant biomass in which the treatment with KJW126 pellets and KJW143 and KJW342 culture medium significantly higher increase in biomass of the soybean seedlings. These findings suggest that the melatonin producing bacterial could be a significant biofertilizer tool to enhance the growth and development of commercial agriculture crops.

[Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2022R1A2C1008993).]

#### C074

**Functional and Genomic Study of Violacein Producing Microbe, Janthinobacterium** Chun-Zhi Jin, Min-Ho Jeon, and Hyung-Gwan Lee<sup>\*</sup> *Cell Factory Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB)* 

Natural microbial pigment has been considered to be alternatives for the synthetic pigment owe to high safety for healthy, high productivity and easily being genetic engineered comparing with pigment from plant. While conducting a project for screening of natural pigment produced by indigenous bacteria, 12 *Janthinobacterium* strains were isolated and they were phylogenetically showed 99.5-100% similarity based on 16S rRNA sequence, but their inhabiting environments and physiological properties were distinctly different. Interestingly, some crude extracts of them showing effects on recovery of damaged cell by UVB and reduction of melanin contents, thus they can be used bioactive compounds for anti-photo-aging and whitening. To gain insight how *Janthinobacterium* survive in diverse environmental habitats based on genomic adaptation and production of secondary metabolite, we performed genome compare, phenotypic characterization, and metabolite analysis in 12 isolated strains. Overall, this study may provide insights into the potential mechanisms of violacein biosynthesis and bioactive compound production depend on their environment habitats.

[Korea Environment Industry & Technology Institute (KEITI) through project to develop eco-friendly new materials and processing technology derived from wildlife, funded by Korea Ministry of Environment (MOE) (2021003240004) and the National Research Foundation of Korea (NRF) in grant funded by the Korean government (NRF-2018R1C1B3009513)]

## New Discovery of Skin Whitening Effects from an Actinomycetes, Streptomyces

Chun-Zhi Jin, Dawon Kyung, and Hyung-Gwan Lee<sup>\*</sup> Cell Factory Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB)

Actinomycetes, are widely distributed in natural ecosystem habitats and could produce diverse secondary metabolites which have the primary importance on cosmetic, medicine, agriculture and food production. A brown pigment producing *Streptomyces* sp. AN XXX which was isolated from soil sample, showed whitening effects through decrease melanin content and tyrosinase activity on α-MSH stimulated B16-F10 cells. Ferment extract significantly decreased protein and mRNA expression level of TRP-1, 2 and MITF. Protein expression level of p-ERK/ERK, p-P38/P38, p-CREB/CREB and p-PKA/PKA were decreased while p-JNK/JNK was decreased. Effective compound formula was determined to be compound A through LC/MS and NMR analysis. Other secondary metabolites were also studied from genome sequences on antiSMASH, RAST and KEGG. Functional group study of compound A is the next step to improve or alter effects. This study may provide a new insight into the more stable, healthy natural pigment from actinomycetes as the component of cosmetics.

[Korea Environment Industry & Technology Institute (KEITI) through project to develop eco-friendly new materials and processing technology derived from wildlife, funded by Korea Ministry of Environment (MOE) (2021003240004) and the National Research Foundation of Korea (NRF) in grant funded by the Korean government (NRF-2018R1C1B3009513)]

## **C076**

## A Gut Microbiome-based Classification Model for Obesity Prediction

Hyun Ji Sim, Jae Won Lee, Kwang Moon Cho, Hye Ri Kim, and Sun Jae Kwon<sup>\*</sup> Accugene, Inc.

The incidence of obesity continues to increase worldwide, and obesity has associations with various diseases, including inflammation, hypertension, Type 2 diabetes, and cancers. Conventionally, the correlation between obesity and gut microbiome has been analyzed with the ratio between two major phyla, Bacteroidetes, and Firmicutes. However, the correlation remains obscure due to inconsistent results from different researches. We conducted feature selection and built obesity prediction models using random forest machine learning algorithms and 16S rRNA amplicon sequencing data in efforts to validate the correlation and secure the list of microorganisms that have high correlation with obesity. We trained and tested the random forest classifier using samples with BMI values lower than 22 or higher than 28. The accuracy of the constructed prediction models was assessed using the AUC value of the ROC curve. A literature search was conducted on the microbial characteristics used in the model made using a random forest classifier.

# Comparative Analysis of Physiological and Cosmeceutical Characteristics of Isolated *Chlorella* spp. from Indigenous Freshwater

Min-Ho Jeon<sup>1</sup>, Yun-Yeong Kim<sup>1,2</sup>, Chun-Zhi Jin<sup>1</sup>, and Hyung Gwan Lee<sup>1,3\*</sup>

<sup>1</sup>Cell Factory Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>2</sup>Kyungpook National University, <sup>3</sup>Department of Environmental Biotechnology, University of Science & Technology (UST)

During investigation on a research project for screening of natural pigment produced by indigenous microbes, we have initially isolated hundreds of microalgae from different freshwaters in South Korea. To obtain microbes which could produce diverse secondary metabolites, 108 candidates were selected and be exposed to abiotic stress for second screening. We classified them phylogenetically based on 18S rRNA, ITS, and rbcL genes; 19% of the isolates were *Chlorella* sp. We constructed microalgal ethyl acetate extract library and profiled their secondary metabolites by LC/MS. Biological activity (UV protection, whitening, and antioxidants) as cosmetics and medicine (zebrafish gastrulation genotypic assay) were also checked. Among around 20 strains of *Chlorella* sp., 7 strains had high skin-whitening, UV-protection, and antioxidant effect. After optimizing phototropic cultivation, we analyzed their physiological and cosmeceutical properties. Furtherly, by identification of active compounds and its overproduction, these indigenous *Chlorella* sp. are great bioresource candidates for cosmeceutical industry. [Supported by Korea Environment Industry & Technology Institute (KEITI) through project to develop eco-friendly new materials and processing technology derived from wildlife, funded by Korea Ministry of Environment (MOE) (2021003240004) and the National Research Foundation of Korea (NRF) in grant funded by the Korean government (NRF-2018R1C1B3009513).]

#### C079

## Screening of the Nematicidal Activity of Microbes Isolated from Korean Soil: *Nocardia* sp. HJ31 and *Streptomyces* sp. HJ94

Hyeon Ji Jeong<sup>1,2</sup>, Min-Kyoung Kang<sup>1</sup>, Jong-Hoon Kim<sup>1</sup>, Bong Hyun Sung<sup>3</sup>, Dong-Jin Park<sup>1</sup>, MinKyun Na<sup>2\*</sup>, and Kwang-Hee Son<sup>1\*</sup>

<sup>1</sup>Department of Microbiome Convergence Research Center, Korea Research Institute Bioscience & Biotechnology (KRIBB), <sup>2</sup>Department of College of Pharmacy, Chungnam National University, <sup>3</sup>Department of synthetic Biology and Bioengineering Research Center, Korea Research Institute Bioscience & Biotechnology (KRIBB)

The pine wood nematode (PWN; *Bursaphelenchus xylophilus*) is a plant-parasitic nematode that causes fatal damage to forests to the extent that it is called pine AIDS that has spread all over the world. It invades the host pine tree and blocks the passage of water, and the absorption of nutrients, causing the pine tree to wither and die. Although chemical nematicides such as emamectin benzoate and abamectin are used as control agents for pine wilt nematodes, complete control is still difficult. So, safe and effective biological control agents must be developed. Actinomycetes are very valuable because they are natural products used in the pharmaceutical, agriculture, and biotechnology industries by producing various secondary metabolites. Also, more than 70% of the known antibiotics were isolated from actinomycetes. Therefore, we isolated actinomycetes from soil to find the strain that can be used as a PWN control agent.

The 92 actinomycetes were isolated from Korean soil. In order to search for the strains with better nematicidal activity against PWN, the actinomycetes whole broth was extracted with acetone and the supernatant was concentrated and dissolved in DMSO to prepare the screening samples. After screening, only two strains showed a mortality rate of 90% or more among 92 strains. The two strains were identified as *Nocardia* sp. (HJ31) and *Streptomyces* sp. (HJ94) through 16S rRNA sequencing.

[Supported by grants from IPET, MAFRA, NRF, MSIT]

#### Construction of Nematicidal Library from Soil-borne Actinomycetes in Nematode-damaged Areas

Hyeon Ji Jeong<sup>1,2</sup>, Min-Kyoung Kang<sup>1</sup>, Jong-Hoon Kim<sup>1</sup>, Bong Hyun Sung<sup>3</sup>, Insoo Choi<sup>4</sup>, Dong-Jin Park<sup>1</sup>, MinKyun Na<sup>2\*</sup>, and Kwang-Hee Son<sup>1\*</sup>

<sup>1</sup>Department of Microbiome Convergence Research Center, Korea Research Institute Bioscience & Biotechnology (KRIBB), <sup>2</sup>Department of College of Pharmacy, Chungnam National University, <sup>3</sup>Department of Synthetic Biology and Bioengineering Research Center, Korea Research Institute Bioscience & Biotechnology (KRIBB), <sup>4</sup>Department of Plant Bioscience, College of Natural Resource and Life Sciences, Pusan National University

Plant parasitic nematodes cause approximately \$173 billion in crop losses annually worldwide. However, it is difficult to confirm the infection until the root of the plant is damaged for a while, or until the plant is dead, so the prevention of the infection is known to be difficult. Chemical nematicides have many side effects such as the generation of resistant insects and nematodes, accumulation in the human body, and environmental pollution. Therefore, it is necessary to develop new eco-friendly biocontrol agents based on microorganisms. The secondary metabolites produced by actinomycetes are highly valuable because they are very diverse and abundant in structure and function. So, they have been widely used in medicine, agriculture, and biotechnology industries. In this study, we isolated actinomycetes from the soil of the nematode-damaged areas to construct a new library of Actinomycetes with nematicidal activity. The 92 actinomycetes were selected. In order to search for the elite strains with nematicidal activity, experiments were conducted against *C. elegans*, *M. incognita*, and *B. xylophilus*. Isolated strains were cultivated and extracted in acetone to concentrate the bioactive part. The section guideline was namaticidal activity over 90% or more. We selected four strains from *C. elegans* screen, five from *M. incognita*, and two from *B. xylophilus*.

[Supported by grants from IPET, MAFRA, NRF, MSIT]

#### C081

## Changes in Microbial and Physicochemical Quality During the Storage Period of Fresh-cut Fruits Purchased from Online and Offline Markets

Rye Gyeong Park<sup>1</sup>, Ji Hyeon Lee<sup>1</sup>, Ji Eun Lee<sup>1</sup>, Ju Hong Kang<sup>1</sup>, Jae Won Lee<sup>1</sup>, Won Ho Jeong<sup>1</sup>, Sung Ju Cho<sup>2</sup>, Il Kwon Bae<sup>3</sup>, and Jae Hak Sohn<sup>1,2\*</sup>

<sup>1</sup>Maior in Food Science and Culinary Arts, Silla University, <sup>2</sup>Seafood Research Center, IACF, Silla University, <sup>3</sup>Department of Companion Animals, Silla University

This study evaluated the microbial and physicochemical factors affecting the quality change during storage period for fresh-cut fruits sold in online and offline markets. Samples of melon, pineapple and mixed fruit were purchased and stored at two temperatures (10, 18°C) for 9 days. And samples were taken every 3 days, and physicochemical and microbiological (total aerobic bacteria (TAB), fungi, psychrotrophic bacteria (PB) and lactic acid bacteria (LAB)) analysis were performed. In the case of fresh-cut melon, the number of TAB, PB and LAB increased to 7.4, 7.5, and 7.4 log CFU/g on the third day of culture and the main genera were *Mammaliicoccus, Lactococcus, Serratia*, and *Lactococcus*. The number of TAB, PB, LAB and fungi in fresh-cut pineapple increased to 4.4, 6.2, 4.2, and 4.4 log CFU/g on day 3 of culture, and the main genus was *Lactiplantibacillus*. The numbers of TAB, PB and fungi in the mixed fruit increased to 4.4, 6.7, and 5.5 log CFU/g on day 6 of culture, and the main genus was *Lactiplantibacillus*. From the above results, since the shelf life of fresh-cut fruit is relatively short, it is considered that a careful review of spoilage-inducing microorganisms is necessary to extend the shelf life.

#### Development of Spectinabilin Over-productivity Mutant Strain by a Genetic Mutation

Kyung Ho Moon<sup>1,2</sup>, Min-Kyoung Kang<sup>1</sup>, Jong-Hoon Kim<sup>1</sup>, Hyeon Ji Jeong<sup>1,3</sup>, Byeong Min Lee<sup>1,4</sup>, Bong Hyun Sung<sup>5</sup>, Jeong Sang Yi<sup>6</sup>, Dong-Jin Park<sup>1</sup>, Yeo Joon Yoon<sup>6</sup>, and Kwang-Hee Son<sup>1\*</sup>

<sup>1</sup>Microbiome Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>2</sup>Department of Microbiology Chungbuk National University, <sup>3</sup>Department of College of Pharmacy, Chungnam National University, <sup>4</sup>Department of Bio Environmental Chemistry, Chungnam National University, <sup>5</sup>Synthetic Biology and Bioengineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>6</sup>College of Pharmacy, Natural Products Research Institute, Seoul National University

Soil actinomycetes are bacteria that produce a variety of natural substances, such as antibiotics and anticancer drugs. Spectinabilin, a rare substance produced by Streptomyces spectabilis among these actinomycetes, is known to have antiviral, antimalarial, and nematicidal activity. Spectinabilin has various potentials, but there is a lack of research on its application due to its scarcity. Various studies of spectinabilin require strains that overproduce spectinabilin. Therefore, we tried to improve the spectinabilin-producing strain through gene mutation. There are various methods for generating genetic mutations, but the method using NTG, a chemical that acts on the DNA binding site, was used. Actinomycetes have a high GC ratio in their genome, so the possibility of genetic mutation is low, and it is difficult to secure various mutants because they often recover after mutation. Therefore, a method of increasing the mortality rate was selected to secure genetically diverse strains. Mutants were selected only for experiments with a mortality rate greater than 99.999%. As a result, more than 100 mutants were selected, and among them, the S-N87 strain, which increased spectinabilin production by more than 10-fold compared to the wild-type AN091965 strain, was selected. Selected strains were cultured in 5 ml, 50 ml and 150 L volumes to confirm spectinabilin overproduction.

[Supported by grant from IPET and NRF&MSIT]

## C083

Streptomyces sanglieri Extract Inhibits Melanogenesis via PKA/CREB/MITF Signaling Pathway in α-MSH-Stimulated B16F10 Murine Melanoma Cells Hyunjeong Lee and Seunghee Bae<sup>\*</sup> Cosmetics Engineering, Konkuk University

*Streptomyces sanglieri* (*S. sanglieri*), a bacterium species from the genus of Streptomyces, has been reported to produce metabolites with various biological activities. However, the effect of *S. sanglieri* extract on melanogenesis has not been studied. The aim of this study is to examine the inhibitory effect of *S. sanglieri* extract on melanogenesis and elucidate the underlying mechanism. *S. sanglieri* extract inhibited melanin synthesis and tyrosinase activity in α-melanocyte stimulating hormone-stimulated B16F10 murine melanoma cells. Immunoblotting analysis and qRT-PCR indicated that *S. sanglieri* extract suppressed the expression of tyrosinase, tyrosinase-related protein (TRP)-1, TRP-2, and MITF. And the inhibitory effect of *S. sanglieri* extract is mediated by suppression of PKA and CREB phosphorylation. Taken together, our data suggest that *S. sanglieri* extract has antimelanogenic effect via PKA/CREB/MITF signaling pathway and can be applied as a cosmetic material. [This work was supported by Korea Environment Industry & Technology Institute (KEITI) through project to develop ecofriendly new materials and processing technology derived from wildlife, funded by Korea Ministry of Environment (MOE) (2021003240004), and Commercializations Promotion Agency for R&D Outcomes (COMPA) grant funded by the Korea government (MSIT) (No. 2021B401).]

# Valorization of Leftover Green Tea Residues through Conversion to Bioactive Peptides Using Probiotics-aided Anaerobic Digestion

Yi-Jee Hong<sup>1</sup>, Ji-Young Lee<sup>2</sup>, Jae-Eun Lee<sup>2</sup>, and Dong-Woo Lee<sup>1,2\*</sup> <sup>1</sup>Department of Bioindustrial Engineering, Yonsei University, <sup>2</sup>Department of Biotechnology, Yonsei University

Bioactive peptides (BPs) are protein fragments that benefit human health. To assess whether leftover green tea residues (GTRs) can serve as a resource for new BPs, we performed *in silico* proteolysis of GTRs using the BIOPEP database, revealing a wide range of BPs embedded in GTRs. Comparative genomics and the percentage of conserved protein analyses enabled us to select a few probiotic strains for GTR hydrolysis. The selected probiotics digested GTRs anaerobically to yield GTR-derived peptide fractions. To examine whether green tea (GT) peptide fractions could be potential mediators of host-microbe interactions, we comprehensively screened agonistic and antagonistic activities of 168 human G protein-coupled receptors (GPCRs). NanoLC-MS/MS analysis and thin-layer chromatography allowed the identification of peptide sequences and the composition of glycan moieties in the GTRs. Remarkably, GT peptide fractions produced by *Lactiplantibacillus plantarum* APsulloc 331261, a strain isolated from GT, showed a potent binding activity for P2RY6, a GPCR involved in intestinal homeostasis. Therefore, this study suggests the potential use of probiotics-aided GTR hydrolysates as postbiotic BPs, providing a biological process for recycling GTRs from agro-waste into renewable resources as health-promoting BPs. [Supported by AMOREPACIFIC and grants from KHIDI and NRF]

#### C085

**Anti-melanogenesis Effect of** *E. scabrispora* **Extract via CREB/Mitf Pathway in B16F10 Murine Melanoma** Hee-Jae Shin and Seunghee Bae<sup>\*</sup> *Department of Cosmetics Engineering, Konkuk University* 

This study investigates the anti-melanogenic effects of *Embleya scabrispora* (*E. scabrispora*) extract in B16F10 murine melanoma cells and demonstrates the underlying mechanism. The anti-melanogenic effects of the *E. scabrispora* extracts were analyzed using intracellular melanin contents and tyrosinase activity assays. The effects of the extracts on Mitf and tyrosinase expression were analyzed at the mRNA and protein levels. WST-1-based cytotoxicity assay indicated that the concentration of  $\leq 100$  ng/ml for *E. scabrispora* extract has no cytotoxicity in B16F10 cells. Additionally, intracellular melanin contents assay indicated that *E. scabrispora* extract significantly inhibited  $\alpha$ -MSH-induced melanin synthesis. Furthermore, cellular tyrosinase activity and *in vitro* mushroom tyrosinase activity were significantly inhibited by the *E. scabrispora* extract. Additional investigations revealed that tyrosinase activity reduction was mediated by downregulation of Mitf and inhibition of CREB phosphorylation. Our data suggest that *E. scabrispora* extract showed that the extract inhibits tyrosinase activity and melanogenesis *via* CREB/Mitf signaling pathway.

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# The Protective Effect of *Embleya scabrispora* Extract against UVB-induced Cell Damage in Normal Human Dermal Fibroblasts

Yejin Lim, Jaeho Lee, and Seunghee Bae<sup>\*</sup> Cosmetics Engineering, Konkuk University

Ultraviolet (UV) radiation has been known to have various side-effect on the skin, such as skin wrinkles, ROS production, and skin cancer. *Embleya scabrispora* (*E. scabrispora*) is a bacterium species from the genus of *Embleya* and belongs to the class of *Actinomycetia*. The effect of *E. scabrispora* extract on human dermal fibroblasts has not been studied. The aim of this study is to examine whether the *E. scabrispora* extract has a protective effect on UVB-irradiated normal human dermal fibroblasts (NHDFs). First, our results showed that *E. scabrispora* extract has no cytotoxicity on NHDFs. Then, the cells were irradiated with UVB (15 mJ/cm<sup>2</sup>) and confirmed that the viability was reduced significantly (~30%) compared with the control. Interestingly, *E. scabrispora* extract recovered the impaired viability depending on the concentration of *E. scabrispora* extract. Collectively, our data confirmed that the *E. scabrispora* extract has a photo-protective effect in UVB-irradiated NHDFs and suggests the potential to be used as a material for cosmetics or drugs.

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#### C087

## Target-mismatched Guide RNA Aids CRISPR-mediated Microbial Pinpoint Genome Editing

Ho Joung Lee, Hyun Ju Kim, and Sang Jun Lee<sup>\*</sup> Department of Systems Biotechnology, and Institute of Microbiomics, Chung-Ang University

CRISPR-Cas system has emerged as one of the most used genome manipulating tools from microbes through humans. Especially in microbial genome editing, it has been used to eliminate unedited or unchanged target DNAs during site-specific mutagenesis, and leave the edited target DNAs to make it easy to obtain microbial cells with desired mutations. In this study, we aimed to investigate the genome editing efficiency of Cas9 and Cpf1 systems in bacteria. Cas9 system successfully introduced 2- to 4-base mutations in the *galK* gene in *Escherichia coli*, and Cpf1 can edit the *crtEb* gene in *Corynebacterium glutamicum* with high efficiencies (-95%). However, single-nucleotide edited cells were rarely obtained by either Cas9 or Cpf1, possibly owing to mismatch tolerance of CRISPR-Cas system. To solve this issue, 1- or 2-base mismatches were introduced in the target recognizing sequence in guide RNAs in advance. Consequently, the genome editing efficiencies of Cas9 and Cpf1 were dramatically increased using target-mismatched guide RNAs. These results indicate that the target-mismatched guide RNA method can be extended to a variety of CRISPR-Cas genome editing systems in other bacteria.

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#### Antimicrobial Activity of Prodigiosin from Hahella chejuensis against Cutibacterium acnes

Hyun Ju Kim<sup>1</sup>, Moo-Seung Lee<sup>2,3</sup>, Se Kyoo Jeong<sup>4</sup>, and Sang Jun Lee<sup>1\*</sup>

<sup>1</sup>Department of Systems Biotechnology, Chung-Ang University, <sup>2</sup>Environmental Diseases Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>3</sup>Department of Biomolecular Science, KRIBB School of Bioscience, Korea University of Science and Technology (UST), <sup>4</sup>Research Division, Incospharm Corp.

Prodigiosin, a red pigment produced by *Hahella chejuensis*, a marine-derived microorganism, has several biological functions, including antimicrobial, antiprotozoal, antitumor activities and inflammatory relief. It has high applicability as a pharmaceutical, and further studies on its pharmacodynamics and toxicity are required for its drug application. Because prodigiosin has a red color, we thought that it could be used as a potential cosmetic pigment material. In this study, the antibacterial effect of prodigiosin produced from marine-isolated *H. chejuensis* on skin microorganisms was tested for its applicability as functional cosmetic ingredient. MIC and MBC tests on skin commensal bacterial cells revealed that *Cutibacterium acnes*, an opportunistic pathogen related to acne vulgaris, is highly susceptible to prodigiosin. We analyzed the transcriptome of *C. acnes* whose growth was inhibited by prodigiosin. Our study provided an understanding of the antibacterial mechanism of prodigiosin against *C. acnes* strains.

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## C089

## Effect of Microbial Inocula on Composting Properties and Odor Components Reduction in Livestock Manure Mixture

Yan-Qing Wang, Jae-Hyeong Shin, Min-Gyung Choi, Jeong-Yeon On, and Soo-Ki Kim<sup>\*</sup> Department of Animal Science and Technology, Konkuk University

The compost from livestock manure is returned to the soil and positively affects resource recycling and soil quality improvement. However, odor produced during composting of livestock manure is an acute issue and it still creates various problems. Therefore, this study investigated the effect of microbe strains selected for reducing ammonia gas production on the sizeable compost pile similar to a compost manufacturing site. The introduced strain is a total of 13 strains, including strains with high ammonia utilization such as *Bacillus* sp., and *Rhodococcus* sp. strains, less competitive with each other were used. Two compost piles of 3 tons each were made with the same raw material composition, and the compost pile without 13 strains is the control group (C) and the input 13 strains compost pile is the treatment group (T). As a result, the control group (C) had the highest temperature at 58.9°C on the 9th day of the trial, and the treatment group (T) had the highest temperature at 60.1°C on the 23rd day of the trial. The highest ammonia generation amount is 175.5 ppm/100 ml on the 14th day of the trial in the control group (C), and 91.0 ppm/100 ml in the treatment group (T) on the 11th day of the trial day, respectively. The Mixed ammonia utilization microorganisms suggested that they have an odor control effect in composting.

### D001

## **PE\_PGRS38 Declines Deubiquitination of K48-polyUb in TRAF6 via Interaction with Ubiquitin-specific Protease** Seok-Jun Mun<sup>1,2</sup> and Chul-Su Yang<sup>3,4\*</sup>

<sup>1</sup>Department of Bio-nano Technology, Hanyang University, <sup>2</sup>Center for Bionano Intelligence Education and Research, <sup>3</sup>Center for Bionano Intelligence Education and Research, <sup>4</sup>Department of Molecular and Life Science, Hanyang University

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (Mtb). It is one of the well-known infectious diseases, resulting in a global morbidity and mortality burden. PE\_PGRS, family of unique proteins, possessed by Mtb, related to pathogenesis of Mtb is necessary for understanding of pathogenesis of TB through the findings of functions. Here, we suggested the role of PE\_PGRS38 as a binding partner of herpesvirus-associated ubiquitin-specific protease (HAUSP, USP7) associated to modulating the activities of various proteins with regulating the ubiquitination in substrate proteins. We produced the *Mycobacterium smegmatis* expressing recombinant PE\_PGRS38 (Ms\_PE\_PGRS38) to demonstrate the function of PE\_PGRS38. We demonstrated that the PE domain of PE\_PGRS38 is an essential domain that induces the deubiquitination of TRAF6. Furthermore, Ms\_PE\_PGRS38 regulated the cytokines level in murine bone marrow-derived macrophages (BMDMs) via down-regulation of the deubiquitination of TRAF6 by HAUSP. Ms\_PE\_PGRS38 enhanced the intracellular burden of bacteria through manipulating the level of cytokines *in vitro* and *in vivo*. Altogether, we found that the interaction between PE\_PGRS38 and HAUSP is vital for the intracellular.

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#### D002

## *Bacteroides vulgatus* Attenuates Colitis by Inhibiting CD82 and Increasing Activation of the NLRP3 Inflammasome Hyo Keun Kim and Chul-Su Yang\*

## Department of Molecular and Life Science and Center for Bionano Intelligence Education and Research, Hanyang University

Inflammatory bowel disease (IBD) is a worldwide disease. IBD is known as a chronic immune-mediated intestinal inflammation caused by a variety of causes, such as genetic and microbial factors, but the exact etiology and treatment are unclear and undiscovered. IBD has long been hypothesized that changes in the microbiome are responsible for IBD. Thus, we analyzed each microbiome using pyrosequencing of the colon of normal mice and DSS-induced colitis mice. And we found that the relative abundance of *Bacteroides vulgatus* (*B. vulgatus*) was increased in the colon of DSS-induced colitis mice. Previously, we confirmed that CD82 binds to NLRP3 and BRCC3, with a higher affinity for BRCC3. This binding mediated degradation of NLRP3 by inhibition BRCC3-dependent K63-specific deubiquitination. Therefore, we verified the the effect of *B. vulgatus* on DSS-induced colitis in CD82 KO mice and normal mice. Deficiency of CD82 decreased the severity of colitis in mice with enhancing the level of IL-1 $\beta$  and IL-18. Also, *B. vulgatus* increased the survival through mediating CD82 expression and activating BRCC-dependent deubiquitination of NLRP3 in mice. Taken together, suppression of CD82 reduced the pathogenesis of colitis with elevating the activation of NLRP3 inflammasome by BRCC-dependent K63 deubiquitination. Additionally, *B. vulgatus* may be a novel therapeutic candidate for colitis.

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### Retroductal Salivary Microbiome Reveals Disease-specific Features and Clinical Correlations in Sjogren's Syndrome

Donghyun Kim<sup>1</sup>, Ye Jin Jeong<sup>1</sup>, Jihoon Choi<sup>1,2</sup>, Young Min Park<sup>1,2</sup>, Oh Chan Kwon<sup>2,3</sup>, Yong Woo Ji<sup>2,4</sup>, Sung Jun Ahn<sup>2,5</sup>, Hyung Keun Lee<sup>2,4</sup>, Min-Chan Park<sup>2,3</sup>, and Jae-Yol Lim<sup>1,2\*</sup>

<sup>1</sup>Department of Otorhinolaryngology, Yonsei University College of Medicine, <sup>2</sup>Gangnam Severance Hospital, Yonsei University College of Medicine, <sup>3</sup>Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine, <sup>4</sup>Department of Opthalmology, Yonsei University College of Medicine, <sup>5</sup>Department of Radiology, Yonsei University College of Medicine

Recent studies have demonstrated that the oral microbiome in patients with Sjögren's syndrome (SS) is significantly different from that in healthy individuals. However, the microbial composition is significantly dependent on how the sample is collected. In this study, stimulated intraductal saliva samples were collected from the parotid glands (PGs) of 23 SS and nine non-SS subjects through PG lavage and subjected to 16S ribosomal RNA amplicon sequencing. The correlation between the oral microbiome and clinical features, such as biological markers, clinical manifestations, and functional and radiological characteristics was investigated. Oral microbial composition was significantly different between the anti-SSA-positive and SSA-negative groups. Furthermore, SS subjects with sialectasis exhibited decreased microbial diversity and Firmicutes abundance. The abundance of Bacteroidetes was positively correlated with the salivary flow rate. Bioinformatics analysis revealed several potential microbial biomarkers for SS at the genus level, such as decreased Lactobacillus abundance or increased Streptococcus abundance. These results suggest that microbiota composition is correlated with the clinical features of SS, especially the ductal structures and salivary flow, and that the oral microbiome is a potential diagnostic biomarker for SS.

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#### D004

#### Selection of Resistant Mycobacterium tuberculosis Mutants with Licensed Fluoroquinolones

Bora Shin<sup>1</sup>, Pramila Ghode<sup>2</sup>, Adeline Chua<sup>3</sup>, Suresh B. Lakshminar<sup>4</sup>, Barun Mathema<sup>5</sup>, Karl Drlica<sup>6</sup>, René Wintjens<sup>7</sup>, and Pablo Bifani<sup>1,3,8\*</sup>

<sup>1</sup>Infectious Diseases Translational Research Programme and Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, <sup>2</sup>Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore, <sup>3</sup>A\*STAR Infectious Diseases Labs, 8A Biomedical Grove, Immunos Building, Singapore, <sup>4</sup>Novartis Institute for Tropical Diseases, Singapore, <sup>5</sup>Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA, <sup>6</sup>Public Health Research Institute and Department of Microbiology and Molecular Genetics, New Jersey Medical School, Rutgers Biomedical and Health Sciences, Newark, New Jersey, USA, <sup>7</sup>Unit of Microbiology, Bioorganic and Macromolecular Chemistry, Department of Research in Drug Development (RD3), Faculté de Pharmacie, Université Libre de Bruxelles, Belgium, <sup>8</sup>Department of Infection Biology, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom

Fluoroquinolones (FQs) used to treat tuberculosis (TB) may have intrinsic differences that can lead to faster acquisition of resistance. FQs resistant isolates of Mycobacterium tuberculosis which are known as extensively resistant TB. Here, we report that in the commercially available FQs, ciprofloxacin and ofloxacin exhibited the highest MIC99, the broadest range of mutants selected, and the highest values for mutant prevention concentration (MPC) (>20µM). The calculated values of AUC 24/MPC for these two agents were approximately 1/2-fold and 1/3-fold, 4.65 and 2.45, compared to those of gatifloxacin and moxifloxacin, while the mutation-selection window was 10-fold higher. Ciprofloxacin and ofloxacin administered at the current recommended dose are below the MPC and hence will lead to the acquisition of resistance more readily than gatifloxacin and moxifloxacin. Universal cross-resistance amongst the six antitubercular FQs indicates that the misused of ciprofloxacin and ofloxacin of more potent FQs in the clinic.

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### Genomics and Transcriptomics of Laboratory-evolved Multidrug-resistant *Acinetobacter baumannii* under Nutritional Stresses

Sohyeon Yun, Pureun Kim, and Woojun Park\*

Laboratory of Molecular Environmental Microbiology, Department of Environmental Science and Ecological Engineering, Korea University

Bacterial evolution offers a wide range of genomic mutations and new adaptive pathways, and decreasing fitness costs for new environments is a beneficial product of evolution. We induced the laboratory evolution of multidrugresistant *Acinetobacter baumannii* NCCP 16007 (WT) isolated from patient urine under nutritional stress to obtain two new cell types, Evolved *A. baumannii* 1 (EAB1) and Evolved *A. baumannii* 2 (EAB2). Demonstrated increased fitness through high-dimensional evolution in EAB1 - oligotrophic and EAB2 - eutrophic environments. Genomic analysis shows extensive gene loss, gain, and, rearrangement in evolutionary strains. Gain of genes was caused by insertion sequences, which is a result supported by transcript analysis to interrupt gene coding or increase gene expression level. The *ata* loss of EAB1 and the *bap* overexpression of EAB2 caused differences in bacterial adhesion, demonstrating a decrease in EAB1 and an increased biofilm-forming ability of EAB2. Evolutionary strain maintain all of the antibiotic resistance genes shown in the WT, however, the antibiotic resistance of the polymyxin B and clindamycin has been reduced. We predicted that there is a novel mechanism by which mutations are synergistic to make them sensitive to antibiotics. These data provide the remarkable genetic plasticity of *A. baumannii* and the ability to develop strong adaptive functions to specific environmental niches. [Supported by the National Research Foundation (NRF-2020M3A9H5104237).]

### Differential Diagnostic Method of *Acinetobacter baumannii* and *Acinetobacter* Species Using Cell Wall Binding Domain of Bacteriophage Endolysin

Yoon-Jung Choi<sup>1</sup>, Shukho Kim<sup>1,2</sup>, Seo Yeon Ko<sup>1</sup>, Yu-Jin Lee<sup>1</sup>, Bokyung Kim<sup>1</sup>, Minsang Shin<sup>1,2</sup>, Je Chul Lee<sup>1,2</sup>, and Jungmin Kim<sup>1,2\*</sup>

<sup>1</sup>Department of Biomedical Sciences, The Graduate School, Kyungpook National University, <sup>2</sup>Department of Microbiology, School of Medicine, Kyungpook National University

*A. baumannii* is part of the *A. calcoaceticus-A. baumannii* complex, all of which are highly genetically related and phenotypically difficult to distinguish. In this study, we tried to develop a simple method for differential diagnosis of *A. baumannii* among *Acinetobacter* species using the cell wall-binding domain (CBD) of *A. baumannii*-specific bacteriophage-derived endolysin. The gene encoding the CBD of AbEndolysin of  $\phi$ 1656-2 was cloned into *Escherichia coli* expression vector pET21a (AbPG60\_195-pET21a) and expressed. The purified AbPG was attached to epoxy magnetic beads (MBs), resulting AbPG-MB complex. When *A. baumannii* cells were injected at 3.40×10<sup>5</sup> CFU, 1.6×10<sup>5</sup> CFU (recovery rate of 72%) was bound to the AbPG-MB complex within 1 h. The lowest detectable concentration of *A. baumannii* was 3.4×10<sup>2</sup> CFU. However, other bacterial strains were tested, such as *E. coli, S. aureus*, and *A. calcoaceticus-A. baumannii* complex did not bind to the AbPG-MB complex. SEM microscopy revealed that magnetic bead surfaces were evenly coated with the AbPG protein. The binding of MB-AbPG to *A. baumannii* was observed by confocal fluorescence microscopy using fluorescent red-*A. baumannii*. Here, we developed a simple culture-free method for the specific detection of *A. baumannii*. This simple method allows for differential detection of *A. baumannii* among *A. calcoaceticus-A. baumannii* complex.

#### D008

#### A Study on the Characteristics of Waterborne and Foodborne Viruses Epidemiology in Ulsan Area

Jong Seok Oh, Gwi Ae Lee, Ji Hye Shin, Hye Kyung Moon, Do Hoon Gong, Kyung Sim Park, Sook Nam Hwang, and Soo Mi Choi<sup>\*</sup>

Ulsan Institute of Health & Environment

Norovirus, group A rotavirus, enteric adenovirus, human astrovirus and sapovirus are major cause of vomit, abdominal pain and diarrhea. The weak like children, the elderly and people with a weak immune system particularly vulnurable. These studies have shown that laboratory investigation of patient samples of 8 hospital in Ulasn, Korea. Conventional PCR, real-time PCR and enzyme immuno assay methods used to screen and determine samples. With sequencing and BLAST search, genotype was confirmed.

Testing 1,978 samples in total, 126(6.3%) viral pathogens were detected. The most number of pathogen was Norovirus and 81(4.1%) specimens were isolated. Other isolation volumes of viruses were 22(1.1%) Rotavirus, 22(1.1%) Human Astrovirus and 1(0.1%) Enteric Adenovirus in each. Sapovirus was not detected.

The number of pathogen isolation was more greater during the winter, spring season (from November to April) than summer, autumn season and was highest between February and March. The most dominant genotype in Norovirus was GII.4. GII.4 was the major genotype of norovirus infection in Ulsan until now. However, GII.17 detection increased between 2017 and 2021. G2P[4] was the most prevalent in group A rotavirus, Type 1a was the most in human astrovirus in the same way.

Norovirus has expanded in detected viral pathogens during 5 years. Other viruses decreased gradually. The research should focus on balanced sampling and continuous surveillance to predict viral gastroenteritis pathogens.

### Development of *Mycobacterium tuberculosis* Infection Model Using a Co-culture System with Human Lung Organoids and Macrophages for the Evaluation of Anti-tubercular Drug Efficacy

Seung-Yeon Kim<sup>1,2</sup>, Ji-Ae Choi<sup>3</sup>, Kee Kwang Kim<sup>2</sup>, Chang-Hwa Song<sup>3</sup>, and Eun-Mi Kim<sup>1\*</sup> <sup>1</sup>Department of Predictive Toxicology, Korea Institute of Toxicology, <sup>2</sup>Department of Biochemistry, Chungnam National University, <sup>3</sup>Department of Microbiology, College of Medicine, Chungnam National University

Tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis* (*M. tb*) and is one of the leading causes of death. Although numerous studies on developing TB drugs have been conducted using 2D or animal models, they have limitations on different susceptibility to TB in humans. Recently, 3D human lung organoids (hLOs) that recapitulate cellular composition and functions of lungs have emerged as excellent models for disease modeling and drug screening. Here, we attempt to establish a TB infection model using a co-culture system of hLOs and human macrophages (hM $\phi$ s) for drug efficacy testing. First, we generated hLOs derived from human embryonic stem cells expressing lung-specific markers such as SFTPC and SOX9 using the Matrigel droplet culture method. Next, co-culture condition for hM $\phi$ s-GFP with hLOs was established and H37Rv-RFP, a strain of *M.tb*, was microinjected into hLOs with hM $\phi$ s-GFP. As a result, hM $\phi$ s-GFP surrounded H37Rv-RFP, a granuloma-like form, in the lumen of hLOs expected phagocytosis. In addition, it was observed that the gene expression of pro-inflammatory cytokines such as IL-6 and TNF- $\alpha$  significantly increased in the H37Rv-RFP infected group compared to the non-infected control group. Altogether, these results showed that we successfully established an *in vitro M. tb* infection model using 3D hLOs and hM $\phi$ s, which can be utilized to develop TB drugs.

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#### D010

#### Gender Differences in the Skin Microbiome of Burn Scars

YeonGyun Jung<sup>1</sup>, Eun Kyung Lee<sup>1</sup>, So Young Joo<sup>2</sup>, Cheong Hoon Seo<sup>2</sup>, and Yoon Soo Cho<sup>2\*</sup> <sup>1</sup>Burn Institute, Hangang Sacred Heart Hospital, Hallym University College of Medicine, <sup>2</sup>Department of Rehabilitation Medicine, Hangang Sacred Heart Hospital, Hallym University College of Medicine

Gender differences are observed in various spectrums of skin diseases, and it is known that there are also differences in the rate of wound healing. As the skin microbiome is known to play an important role in skin defense and immune function, there is a growing interest in skin microbiome research. In this study, gender differences were identified for the newly healed skin microbiome of burn patients. Skin bacterial DNA was isolated from normal skin and burn scars of 26 patients (12 men, 14 women) and microbiota analysis was performed. In addition, the correlation between skin microbiota and clinical characteristics was investigated. Trans epidermal water loss (TEWL) showed higher values in burn scars than normal skin in men, but there was no significant difference in women. For Erythema, it was significantly higher in scar in both male and female groups. Alpha diversity did not show any significant difference between men's normal skin and burn scars. However, in the case of women, it was confirmed that the scars were significantly higher than that of normal skin. TEWL was negatively correlated with unclassified Gemellales, and thickness was negatively correlated with *Propionibacterium*. Erythema was positively correlated with *Corynebacterium*. These findings suggest that there are gender differences in the microbiome at burn wound sites, suggesting that different sexes should have different burn treatment strategies.

### The Unique *N*-Glycan-dependent Protein Quality Control System of *Cryptococcus neoformans* is Critical for Cellular Fitness and Pathogenicity

Catia Mota, Kiseung Kim, Eun Jung Thak, Su-Bin Lee, and Hyun Ah Kang<sup>\*</sup> Department of Life Science, Chung-Ang University

Eukaryotes have evolved a highly conserved endoplasmic reticulum glycoprotein quality control system (ERQC) to monitor the folding process of glycoproteins. To investigate the molecular assembly and function of ERQC in the human pathogen *Cryptococcus neoformans*, we constructed and functionally analyzed mutant strains lacking the ERQC genes *UGGT*, *MNS1A* and *MNS1B*, which showed up-regulation upon stress conditions causing misfolded protein accumulation in the ER. The disruption of *UGGT* (*uggt*Δ), encoding the UDP-glucose: glycoprotein glucosyltransferase, generated severe growth retardation with a decrease in the amount of hypermannosylated *N*glycans. In contrast, deletion of *MNS1A* (*mns1A*Δ) and *MNS1B* (*mns1B*Δ) resulted in modest growth defects with distinctively altered *N*-glycosylation profiles, indicating *MNS1A* as the widely known ER- $\alpha$ 1,2-mannosidase I and *MNS1B* as a novel mannosidase involved in mannose trimming in the Golgi, respectively. Notably, the *uggt*Δ and the double *mns1A*Δ1*B*Δ mutants displayed defects in capsule formation, either lacking GXM secretion or increased shedding of the capsule exopolysaccharide, respectively. These defects would contribute not only to a decrease in survival inside phagocytic host cells but also to avirulence in a murine model. Altogether, our data demonstrates that evolutionary unique *C. neoformans N*-glycan-dependent ERQC plays critical roles in cellular fitness and capsule formation.

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#### D012

**Function of Survival Factor A, SvfA, in** *Aspergillus nidulans* **Pathogenesis** Ye-Eun Jung<sup>1</sup>, Cheol-Hee Kim<sup>2</sup>, and Hee-Moon Park<sup>1\*</sup> <sup>1</sup>Department of Microbiology and Molecular Biology, <sup>2</sup>Department of Biology, College of Bioscience and Biotechnology, Chungnam National University

Survival factor A (SvfA) is a protein whose expression is regulated by *veA* gene, a key sexual regulator of *Aspergillus nidulans*. Previous studies have shown that *svfA* gene is involved in the differentiation and growth of *A. nidulans*, especially affecting oxidative-stress response associated with virulence. In this study, the effect of *svfA* gene on the virulence of *A. nidulans* was analyzed using An*svfA*-deficient strain( $\Delta svfA$ ). Cell wall components, PAMP genes expression, immune response with alveolar macrophages and alkaline protease activity, biofilm formation ability were investigated. The amount of  $\beta$ -(1,3)-glucan, the amount of galactomannan and the expression of PAMP genes in  $\Delta svfA$  decreased compared to WT. Phagocytosis assay showed that  $\Delta svfA$  was less eaten than WT, but the killing rate (%) was higher than WT. In both the analysis of alkaline protease activity and biofilm formation ability,  $\Delta svfA$  was lower than WT. When fungal infection assay was analyzed using T-cells deficient zebrafish, the survival rate of zebrafish inoculated with the  $\Delta svfA$  conidia was the highest. Taken together, the *svfA* gene affects cell wall components, interaction with macrophages, protease activity, and biofilm formation. Survival rate analysis using zebrafish suggests that the virulence of *A. nidulans* decreases when *svfA* gene is deficient.

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### Characterization of *Klebsiella pneumoniae*, 48 Strains Registered in National Culture Collection for Pathogens (NCCP)

Joon Ki Kim, Su Yeon Kim, Ji Woong Choi, and Young Sill Choi $^{st}$ 

Korea Disease Control and Prevention Agency (KDCA), National Institute of Health, Division of Pathogen Resource Management

*Klebsiella pneumoniae* is facultative anaerobic and Gram-negative bacteria that causes pneumonia when aspirated. There are 48 strains of *K. pneumoniae* registered by NCCP (Isolated year : 2003 ~ 2016). This study aimed to provide information to researchers on the characterization of *K. pneumoniae*.

The analyses of antibiotic susceptibility, MLST, toxin type, and serotype for 48 *K. pneumoniae* NCCP strains were performed. Antibiotic susceptibility analysis was performed using the MIC method and revealed 34 groups. The result of MLST was classified into 26 types by confirmed using PubMLST (<u>https://PubMLST.org</u>) and ST 11 was the most common (25%). Toxin type and serotype were performed by PCR on the toxin genes *uge, wcaG, rmpA, magA, Aerobactin* and serotype genes K1, K2. Toxin genes were composed of *uge* (25%), *wcaG* (33.3%), *rmpA* (10.4%), *magA* (2.1%), *Aerobactin* (10.4%). Serotype genes were confirmed of K1 (2.1%), K2 (18.8%), non-K1/K2 (79.2%). We expect our molecular and biochemical characterization data and quality assurance resources to be valuable for use as standard materials in the development of diagnostics and therapeutics.

[This study was funded by a grant of the Korea Disease Control and Prevention Agency (No. 2019-NG046-02).]

#### D014

### SARS-CoV-2 Variants Specific Mutations in RBD (Receptor Binding Domain) Impact Infectivity and Susceptibility to Neutralizing Antibodies

Sojung Bae, Taehun Kim, and Jinjong Myoung\* Molecular Virology Laboratory, Korea Zoonosis Research Institute, Jeonbuk National University

The entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the host cells is mediated by the interaction of viral spike protein with Human Angiotensin-Converting Enzyme II (hACE2) and the domain of spike that bind with hACE2 is called the receptor-binding domain (RBD). Currently, various mutations in SARS-CoV-2 result in newly emerging variants. Especially, mutations in spike with RBD of Beta, Kappa, Delta and Omicron (BA.1) variants are prime concern as these variants have high infectivity, transmissibility, and are more resistant to neutralizing antibodies.

In this study, to understand the mechanism of increased infectivity and resistant to neutralizing antibodies of SARS-CoV-2 variants, we developed pseudoviruses bearing spike of wild-type strain or SARS-CoV-2 variants, and evaluated their infectivity and susceptibility to neutralizing antibody. Our results demonstrate that SARS-CoV-2 variants have significantly higher infectivity than wild-type pseudovirus excluding omicron variant, which is mediated by D614G mutation in Spike. And Beta, Kappa and Omicron variants have significantly decrease susceptibility to neutralization compared with wild-type pseudovirus, which is mediated by mutations in RBD.

#### Development of Methods for the Detection of Pathogenic Arcobacter Species

Dong Woo Seo, Yeon Cheol Yu, Seung Hwan Kim, and Soon Han Kim<sup>\*</sup> Food Microbiology Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety

Detection of *Arcobacter* has been continuously reported in food such as pork, beef, poultry, milk, fish and mussels. Among the 22 recognized *Arcobacter* species, *A. butzleri, A. cryaerophilus* and *A. skirrowii* cause infection associated with enteritis and occasionally septicaemia. In this study, culturing methods and PCR methods were developed to detect *Arcobacter* species, and specificity of each method were evaluated. Enrichment broth with supplements comprising cefoperazon, amphotericin B, trimethoprim, novobiocin and 5-fluorouracil and Campylobacter blood free agar with cefoperazon, amphotericin B, Teicoplanin was used to detect *Arcobacter* in culturing methods. Primers the targeting the 16S or 23S rRNA gene were used for detection of *Arcobacter* and identification of *A. butzleri, A. cryaerophilus* and *A. skirrowii* in PCR methods. Total 22 strains (6 target-*Arcobacter* strains and 16 non-target strains) were used for evaluation of each method in this study. It was possible to detect only *A. butzleri, A. cryaerophilus* and *A. skirrowii* through developed culturing methods and PCR methods. Developed methods were applied to food in retail store to monitor pathogenic *Arcobacter*. Through methods for the detection of pathogenic *Arcobacter* species, it will be possible to recognize food poisoning caused by *Arcobacter* species and reduce food poisoning of unknown causes.

[This research was supported by a grant (22191MFDS020) from Ministry of Food and Drug Safety in 2022.]

#### D016

#### pep27 Mutant Immunization Inhibits Caspase-14 Expression to Alleviate Inflammatory Bowel Disease via Treg Upregulation

Hamid Iqbal<sup>1</sup>, Gyu-Lee Kim<sup>1</sup>, Ji-Hoon Kim<sup>1</sup>, Prachetash Ghosh<sup>1</sup>, Masaud Shah<sup>2</sup>, Wonsik Lee<sup>1</sup>, and Dong-Kwon Rhee<sup>1,3\*</sup> <sup>1</sup>School of Pharmacy, Sungkyunkwan University, <sup>2</sup>Department of Physiology, Ajou University, <sup>3</sup>Research Center, DNBIO

Inflammatory bowel disease (IBD) is a highly prevalent gut inflammatory disorder. Complicated clinical outcomes prolong the use of conventional therapy and often lead to compromised immunity followed by adverse events and high relapse rates. Thus, a profound medical intervention is required. Previously, intranasal immunization of pneumococcal *pep27* mutant ( $\Delta pep27$ ) exhibited long-lasting protection against immune-related disorders. System biology analysis has predicted an inverse correlation between  $\Delta pep27$  immunization and gastroenteritis. Recently, we established that  $\Delta pep27$ -elicited Tregs repressed Wnt5a expression and enhanced barrier integrity suggesting restoration of immunological tolerance. Therefore, we evaluated whether  $\Delta pep27$  can alleviate IBD.  $\Delta pep27$  dosedependent response was analyzed in dextran sulfate sodium (DSS)-induced mice using transcriptome analysis.  $\Delta pep27$  significantly attenuated DSS-induced oxidative stress parameters, proinflammatory cytokines, caspase-14 expression level, and upregulated tight junction, anti-inflammatory genes IL-10 and TGF- $\beta$ 1 via upregulation of Tregs to restore healthy gut microbiota. Neutralization studies unveiled that  $\Delta pep27$  had a remedial effect via Treg upregulation. Caspase-14, being important mediator in the pathogenesis of IBD, can be an alternate therapeutic target in IBD.  $\Delta pep27$ -increased Tregs repressed caspase-14 expression and reversed gut microbial dysbiosis aiding to re-establish immunological tolerance.

#### Epoxomicin Regulates MyD88- and TRIF-dependent Signaling Pathways of TLRs

Seokwon Shin<sup>1</sup>, Ye eun Lee<sup>2</sup>, Harbin Ko<sup>2</sup>, and Hyung-Sun Youn<sup>1,2\*</sup>

<sup>1</sup>Department of ICT Environmental Health System, Graduate School, SoonChunHyang University, <sup>2</sup>Department of Biomedical Laboratory Science, College of Medical Sciences, SoonChunHyang University

Toll-like receptors (TLRs) can recognize specific signatures of invading microbial pathogens and activate a cascade of downstream signals to induce the secretion of inflammatory cytokines, chemokines, and type I interferons. The activation of TLRs triggers two downstream signaling pathways: MyD88- and TRIF-dependent pathway. To evaluate the therapeutic potential of epoxomicin, a member of linear peptide a',b'-epoxyketone first isolated from an *Actinomycetes* strain, we examined its effects on signal transduction via TLR signaling pathways. Epoxomicin inhibited the activation of NF-κB and IRF3 induced by TLR agonists, decreased the expression of interferon inducible protein-10, and inhibited the activation of NF-κB and IRF3 induced by overexpression of downstream signaling components of TLRs signaling pathways. These results suggest that epoxomicin can regulate both MyD88- and the TRIF-dependent signaling pathways of TLRs. Thus, it might have potential as a new therapeutic drug for a variety of inflammatory diseases.

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#### iNOS Expression Induced by TLR Agonists is Inhibited by Pristimerin

Seokwon Shin<sup>1</sup>, Su Yeon Kim<sup>2</sup>, Ye Eun Lee<sup>2</sup>, Hanbin Ko<sup>2</sup>, and Hyung-Sun Youn<sup>1,2\*</sup> <sup>1</sup>Department of ICT Environmental Health System, Graduate School, SoonChunHyang University, <sup>2</sup>Department of Biomedical Laboratory Science, College of Medical Sciences, SoonChunHyang University

Toll-like receptors (TLRs) are one of the families of pattern recognition receptors (PRR) operating in the innate immunity. TLRs have the ability to recognize relatively conserved microbial components, which are generally referred to as pathogen associated molecular patterns (PAMPs). The activation of TLRs signaling leads to the activation of NF-kB and the expression of pro-inflammatory gene products such as cytokines and inducible nitric oxide synthase (iNOS). To evaluate the therapeutic potential of pristimerin, which is a naturally occurring triterpenoid compound from *Celastraceae* plants, iNOS expression induced by MALP-2 (TLR2 and TLR6 agonist), Poly[I:C] (TLR3 agonist), or LPS (TLR4 agonist) were examined. Pristimerin suppressed the iNOS expression induced by MALP-2, Poly[I:C], or LPS. These results suggest that pristimerin can modulate TLRs signaling pathways leading to decreased inflammatory gene expression.

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#### D020

### Unraveling the Signaling Networks of a PP2A-like Phosphatase Sit4 Required for Brain Infection of *Cryptococcus neoformans*

Soojin Yu, Hyunjin Cha, and Yong-Sun Bahn<sup>\*</sup> Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

Phosphatases play critical roles in regulating cellular signaling networks involved in the survival and virulence of fungal pathogens. Specifically, protein phosphatase 2A (PP2A) is a highly conserved and abundant serine-threonine phosphatase composed of catalytic, scaffold, and regulatory subunits. In this study, we aim to unravel the signaling networks of a PP2A-like phosphatase *SIT4* in *Cryptococcus neoformans*, an opportunistic fungal pathogen that causes fatal meningoencephalitis. From a previous systematic analysis, we have identified *SIT4* as a virulence-related phosphatase that promotes blood-brain barrier adhesion and crossing. Thus, to elucidate the factors involved in the regulatory subunit, *SAP190 (SIT4*-associating protein 190), was identified. In *Saccharomyces cerevisae*, four copies of the *SAP* genes are present, while in *C. neoformans*, only one copy was identified. As *SIT4* is downstream of the TOR (target of rapamycin) pathway, both *sit4*Δ and *sap190*Δ displayed increased susceptibility against rapamycin. Moreover, under glucose starvation, the expression of both *SIT4* and *SAP190* increased in the wild type, and the expression level of *SIT4* increased in *sap190*Δ while the expression level of *SAP190* increased in the wild type, and the expression level of *SIT4* increased in *sap190*Δ while the expression level of *SAP190* increased in the wild type. The advector of *SIT4* is downstream factors in the signaling networks of *SIT4* to understand its role in brain infection.

### Comparative Studies of Tuberculosis Immunoassay and Protective Ability according to the BCG Immunization Periods in Mouse Models

Kwangwook Kim, Sundong Jang, Soo Ji Kim, Gunho Won, Yookyoung Lee, and In-Ohk Ouh<sup>\*</sup> Division of Vaccine Development Coordination, Center for Vaccine Research, National Institute of Infectious Diseases, National Institute of Health, Korea Disease Control and Prevention Agency

Tuberculosis (TB) is an infectious disease caused by intracellular pathogen *Mycobacterium tuberculosis* (Mtb) that commonly affects the lungs. Presently bacillus Calmette–Guérin (BCG) is the only available vaccine against TB, but its methodologies for preclinical mouse model testing of BCG against Mtb vary from laboratory to laboratory. Previous studies from mouse models show that the duration of BCG immunization varies from 4 to 10 weeks. Accordingly, the immunization period of the BCG vaccine is not accurate, and a standard indicator of BCG efficacy is required because no about a standard indicator of BCG efficacy is required. We designed the duration of mouse BCG immunization into two groups (4-week group and 8-week group) and compared the immunogenicity and defense ability of the two groups after Mtb infection. Mouse Mtb infection models use two mice strains (C57BL/6 and BALB/c). Comparing the ELISpot TB results for IFN-γ between the two groups was not significant. Also, the growth of Mtb was inhibited, but there was no difference between the two groups, and there were no differences in histopathological lesions. Taken together, these data demonstrated that TB protective ability and immune response according to diverse BCG vaccination periods in the mouse models and suggest that specific differences in BCG-induced immunity periods may be uncommon.

#### D022

#### Anti-pseudomonal Effect of a Nephrite-impregnated Contact Lens

Eun Jung Kim<sup>1</sup>, Eun Jung Choi<sup>1</sup>, Tae-Young Jeong<sup>1</sup>, Ji-Eun Lee<sup>2,3</sup>, and Jin-Woo Oh<sup>1,4\*</sup> <sup>1</sup>Bio-IT Fusion Technology Research Institute, Pusan National University, <sup>2</sup>Department of Ophthalmology, Pusan National University Yangsan Hospital, Pusan National University School of Medicine, <sup>3</sup>Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Pusan National University School of Medicine, <sup>4</sup>Department of Nano energy engineering, Pusan National University

This study compared the anti-pseudomonal effects between nephrite-impregnated contact lenses (CLs) and conventional and cosmetic CLs. Conventional, cosmetic, conventional nephrite-impregnated, and cosmetic nephrite-impregnated CLs were prepared. After inoculation with *Pseudomonas aeruginosa* (*P. aeruginosa*), we counted the number of bacteria on the CL surface and observed each surface using atomic force microscopy (AFM) and scanning electron microscopy (SEM). To estimate potential harm of nephrite-impregnated CLs, we conducted a safety test using a rabbit model treated with the four CL types. The attachment of *P. aeruginosa* significantly increased in the cosmetic CLs compared with that in the conventional CLs (p<0.0001). The bacterial adhesion significantly decreased in the nephrite-impregnated CLs compared with that in the conventional and cosmetic CLs (p<0.0001). AFM revealed that the nephrite-impregnated CLs had rougher surface than the conventional CLs and showed nephrite precipitates. In the safety test, there were no significant differences in the findings between four groups, and the clarity and stability of all corneas were preserved. Nephrite may be used as a next-generation substance to reduce infectious keratitis caused by *P. aeruginosa* when added to CLs.

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#### Development of Predictive Models of Pathogenic Escherichia coli in Egg Products

Yu-Na Jo, So Yong Yang, Mi-Gyeong Kim, Yongchjun Park, Sunyoung Hwang, and Soon Han Kim<sup>\*</sup> Food Microbiology Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety

The aim of this study was to develop predictive models of pathogenic *Escherichia coli* (PE) in egg products (Roasted egg, Peeled quail egg, Whole egg liquid, and Steamed egg). Samples were inoculated with five strains of PE and stored at 10, 15, 25 and 37°C to observe the behavior kinetics of PE. To describe the changes in the PE cell counts during storage, the  $\mu_{max}$  [maximum specific growth rate]and *LPD* [lag phase duration] were estimated by fitting with Baranyi model as the primary model. To analyze the effect of the storage temperature on kinetic parameters, square root and polynomial models were used to fitting  $\mu_{max}$  and *LPD* as the secondary model, respectively. As the temperature increased, the  $\mu_{max}$  increased and the *LPD* tended to decrease. In addition, root mean square errors (RMSE) were analyzed to evaluate the suitability of the mathematical models, and the models were considered suitable when the value of RMSE was less than 1. The predictive growth models of PE demonstrated RMSE values of 0.25 in Roasted egg, 0.69 in Peeled quail egg, 0.39 in whole egg liquid and 0.52 in Steamed egg, respectively. The models we developed can provide useful data as an input model for microbial risk assessment in egg products

#### D024

### Characteristic Study of Extended-Spectrum Beta-Lactamase-Producing *Enterobacteriaceae* (ESBL-PE) Isolated from Retail Meat in South Korea, 2021

Jinju Kim, Ji Hye Jeon, Haiseong Kang, Yongchjun Park, and Soon Han Kim<sup>\*</sup> National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety

The widespread of Extended Spectrum Beta-Lactamase-Producing *Enterobacteriaceae* (ESBL-PE) in retail food is a critical worldwide issue. The aim of this study was prevalence and characteristic analysis of ESBL-PE isolated from retail meat in Korea. We collected 1,665 samples (328 beef, 499 pork, 538 chicken meat, 300 duck meat). A total of 843 *E. coli* and 253 *Salmonella* spp. were isolated. We performed minimum inhibitory concentration (MIC) test for antimicrobial susceptibility. We conducted phenotypic ESBL confirmatory test and confirmed genotype of ESBL-producing genes by PCR. Sequence type of each ESBL-PE was confirmed by multilocus sequence typing (MLST). The isolated *E. coli* were relatively high resistant to ampicillin, tetracycline, nalidixic acid and sulfisoxazole. *Salmonella* spp. was showed higher resistance to sulfisoxazole, nalidixic acid, tetracycline, ampicillin and streptomycin. Among the isolated *Enterobacteriaceae*, 29 *E. coli* and 12 *Salmonella* spp. were determined as ESBL-PE. The genotype of 29 *E. coli* included *bla*<sub>TEM</sub> (n=3) and *bla*<sub>CTX M-1</sub> (n=18) and 12 *Salmonella* spp. included *bla*<sub>CTX M-1</sub> (n=6) and *bla*<sub>CTX M-9</sub> (n=6). As results of the MLST, there were 15 and 2 clonal types of *E. coli* and *Salmonella* spp., respectively. These results indicate that ESBL-PE is widespread in retail meat in Korea, suggesting the need for continuous monitoring and management at the national level.

[This study was supported by 20161 위생안 009 from MFDS.]

### Outer Membrane Protein A of *Acinetobacter baumannii* Inhibits Host Xenophagy through Dephosphorylation of the AMPK Pathway a CAMKK2-dependent

Kyungho Woo, Dong-Ho KIm, Ho Sung Park, Mi Kyung Chang, Jaeu Won, and Chul Hee Choi<sup>\*</sup> Department of Microbiology and Medical Science, Chungnam National University College of Medicine

Acinetobacter baumannii has been designated by the World Health Organization as an important pathogen urgently in need of research. Outer membrane protein A (OmpA) plays important roles including bacterial adhesion and evasion of host defenses. Xenophagy is an autophagic phenomenon that specifically involves pathogens. *A. baumannii* triggers xenophagy, yet little is known about the evasion pathway. Here we tried to evaluate the induction of xenophagy in *A. baumannii* infection as well as explore the related evasion mechanisms by OmpA. To determine whether autophagy activity and inhibition were associated with the AMPK pathway, Analysis was performed using both the *A. baumannii* ATCC 17978 Strain, the isogenic OmpA deletion mutant, Exgeonus OmpA. In this study, we demonstrated that the isogenic OmpA deletion mutant has significantly increased AMPK phosphorylation, as well as autophagosome-Lysosome fusion in Raw 264.7 cells, compared to the WT strain. OmpA inhibited phosphorylation by binding to Camkk2, thereby inhibiting the AMPK pathway. Inhibition of the Xenophagy pathway by each inhibitor and exogenous OmpA increased intracellular *A. baumannii*.

Our study has revealed an important role of the evasion mechanism by OmpA in the *A. baumannii*-induced autophagic process. These findings provide basic data for promising therapeutic target against *A. baumannii* infection.

[This work was supported by a research grant from NRF grant (NRF-2019M3E5D1A02068575)]

#### D026

MAB\_0676c Suppress Autophagic Flux through IL-10 Production in *Mycobacterium abscessus* Infection Dong Ho Kim, Kyungho Woo, Ho-Sung Park, Mikyung Chang, Jaeu Won, and Chul Hee Choi<sup>\*</sup> Department of Microbiology and Medical Science, Chungnam National University College of Medicine

*Mycobacterium abscessus* (M. abs) is a rapidly growing non-tuberculous mycobacterial species that infects macrophages of the lung in human. Autophagy is used as a defensive mechanism to combat the infection of host cells by intracellular pathogens. M. abs can inhibits this response to ensure survival within its niche cell. MAB\_0676c was identified as a histidine triad protein, while the function of this protein in infection was not yet defined. In this study, we are investigating the role of MAB\_0676c on autophagy flux. To investigate whether MAB\_0676c played a role in the innate immune response, cytokines were measured by ELISA and qRT-PCR. The autophagic flux in infected BMDM were measured by immunoblotting and confocal microscopy analysis. ELISA analysis showed that MAB\_0676c induced enhanced production of IL-10 in BMDM. The expression of mTOR, Rubicon, ULK and p62 was sustained and LC3-LAMP2 co-localization was decreased in BMDM infected with MAB\_0676c-expressing *M. smegmatis*. Furthermore, the intracellular growth of MAB\_0676c-expressing *M. smegmatis* was significantly higher than *M. smegmatis* mock strain. Blockade of IL-10 signaling allowed the expression of autophagic markers was decreased and the intracellular growth of MAB\_0676c-expressing *M. smegmatis* was decreased. These results suggest that M. abs inhibits autophagy by IL-10 production, and that blocked autophagy flux may enhance intracellular survival of M. abs. [This research was supported by a grant of KHIDI.]

### Propolis Inhibits the Pathogenicity of *Acinetobacter baumannii* and Protects the Structure and Function of Infected Tight Junctions

Ho-sung Park, Dong Ho Kim, Kyungho Woo, Jaeu Won, Mikyung Chang, and Chul hee Choi<sup>\*</sup> Department of Microbiology and Medical Science, Chungnam National University college of Medicine

Treatment of *A. baumannii* infection is difficult because of the high resistance to conventional antibiotics. Although the antimicrobial activity of propolis has been reported, its activity against the virulence of *A. baumannii* is poorly understood. The aim of this study is to investigate antibacterial effect of propolis on *A. baumannii* and protective effect on infected tight junctions. To determine the effect of propolis on *A. baumannii*, we measured bacterial growth curves, surface motility, and biofilm formation. To confirm the effect of propolis on the infection susceptibility of host cells, ROS, ICS, Giemsa analysis and tight junction analysis were performed. Propolis significantly reduced biofilm formation and surface motility of *A. baumannii*, and inhibited bacterial adhesion and invasion into epithelial cells. The ROS production increased after *A. baumannii* infection, and significantly decreased when pretreated with propolis. Infected cells increased cell permeability compared to uninfected cells, and decreased when pretreated with propolis. The permeability changes were similarly observed in the *in-vivo* model. These findings indicate that the use of propolis, which has anti-virulence and anti-oxidative effects, could be promising for the prevention of *A. baumannii*-induced tight junction disruption. [This work was supported by the NRF grant.]

#### D029

### LC-QTOF Dataset on Antibacterial Activity Extract of *Dryopteris lacera* (Thunb.) Kuntze and *Dryopteris bissetiana* (Baker) C. Chr.

Jung-Ae Kim<sup>1,2</sup>, Yangseon Kim<sup>1</sup>, Jeong-Sup Song<sup>1</sup>, Byoung-Hee Lee<sup>3</sup>, and Youn Kyoung Son<sup>3\*</sup> <sup>1</sup>Department of Research and Development, Center for Industrialization of Agricultural and Livestock Microorganisms, <sup>2</sup>Department of Bioactive Material Science, Jeonbuk National University, <sup>3</sup>Biological and Genetic Resources Assessment Division, National Institute of Biological Resources

*Dryopteris* sp. is known for its various pharmacological effects and is used as a traditional medicine in Asia. The present data report investigated the chemical composition and antimicrobial activity of *Dryopteris* sp. distributed in Korea. The chemical compounds in the ethanolic extracts of *Dryopteris lacera* and *Dryopteris bissetiana* were investigated by ultra-high performance liquid chromatography–quadrupole time-of-flight–mass spectrometry analysis and identified by exploring the UNIFI traditional medicine library. Flavonoids such as juglanin, 6-hydroxyluteolin 7-O-laminaribioside, peltatoside, kaempferitrin, hyperoside, and astragalin were identified in both *D. lacera* and *D. bissetiana*. Neochlorogenic acid was identified as a terpenoid in *D. bissetiana*. Both extracts of *D. lacera* and *D. bissetiana* exhibited antibacterial activity against Gram-positive pathogens, *Staphylococcus aureus* and *Streptococcus mutans*. The antibacterial activity was attributed to the identified phenolic compounds, juglanin, 6-hydroxyluteolin 7-O-laminaribioside, kaempferitrin, astragalin, and neochlorogenic acid, which have been reported to have antibacterial effects. Therefore, *D. lacera* and *D. bissetiana* can be used as Gram-positive selective antibiotics for further investigation.

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### Antimicrobial Resistance and Clonal Diversity of Livestock-associated *Staphylococcus epidermidis* Isolated from Pork Production System in Korea

Gi Yong Lee<sup>1</sup>, Ji Heon Park<sup>1</sup>, Jang Won Yoon<sup>2</sup>, Kun Taek Park<sup>3</sup>, and Soo-Jin Yang<sup>1\*</sup> <sup>1</sup>Department of Veterinary Microbiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, <sup>2</sup>College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, <sup>3</sup>Department of Biotechnology, Inje University

The aim of current study was to investigate prevalence and genetic characteristics of antimicrobial-resistant *S. epidermidis*, especially methicillin-resistant *S. epidermidis* (MRSE), in the pork production systems. A total of 89 *S. epidermidis* (22 MRSE and 67 MSSE) strains were isolated from pig farms (n = 75), slaughterhouses (n = 12), and retail pork (n = 2), respectively. Genetic analyses (MLST, *agr*, and SCC*mec* types) of the strains revealed two dominant clonal lineages of ST100 and ST570 (73/89, 82%). The ST100 clonal lineage strains were identified in pigs and workers, indicating transmission between human and animal hosts. All 16 MRSE strains from pig farms were carrying SCC*mec* V, while MRSE isolates out of pig farms were carrying SCC*mec* IVa, II, or V for methicillin resistance. Pig farm-originated *S. epidermidis* strains exhibited higher levels of AMR and multidrug resistance than those of strains from slaughterhouses and pork samples. Higher carriage rates of genetic determinants for resistance to  $\beta$ -lactams, phenicols, and tetracyclines were also identified in pig farm-associated *S. epidermidis* strains exhibited linezolid resistance via harboring the *cfr* gene. These results provide important insight into the genetic diversity of AMR-*S. epidermidis* in the pork meat production chain in Korea. [This work was supported by grants from Research of Korea Centers for Disease Control and Prevention (Project No. 2020ER540500 and 2021ER220100)]

#### D031

### Profiling Oral Microbiome of Patients with Head and Neck Cancer and Constructing Machine Learning Model for Classifying Head and Neck Cancer

Hojun Sung<sup>1</sup>, Dong-Wook Hyun<sup>1</sup>, Jae-Yun Lee<sup>1</sup>, So-Yeon Lee<sup>1</sup>, Jee-Won Choi<sup>1</sup>, Ji-Ho Yoo<sup>2</sup>, Mi-Ja Jung<sup>1</sup>, Hyun Sik Kim<sup>1</sup>, Young Chan Lee<sup>3</sup>, and Jin-Woo Bae<sup>1,2\*</sup>

<sup>1</sup>Department of Life and Nanopharmaceutical Sciences and Department of Biology, Kyung Hee University, <sup>2</sup>Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University, <sup>3</sup>Department of Otolaryngology-Head and Neck Surgery, School of Medicine, Kyung Hee University

Head and neck cancer is the term that includes malignancies developed in several regions of head and neck except for brain and eyes. Recent evidences describe that oral microbiome is highly associated with pathogenesis and progression of head and neck cancer. Oral microbiome harbors high diversity of microorganisms, however, little is known about which taxa mainly drives the pathogenesis of head and neck cancer. In this study, we analyzed oral microbiome profiles of saliva samples from patients with head and neck cancer and those from cancer-free participants using 16S rRNA amplicon sequencing. Overall, the oral microbiome of patients with head and neck cancer were enriched with several genera, including *Capnocytophaga, Peptostreptococcus, Alloprevotella*, and *Abiotrophia*, which is consistent with other studies that linked these genera to a higher risk of tumor. We proposed a classification model using a random forest machine learning algorithm to predict the pathogenesis of head and neck cancer. This model may be used as a non-invasive method to diagnose the malignant status of head and neck cancer.

[This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean government (MSIT) (NRF-2022M3A9F3082331).]

### Identification of a Novel Human Gut Microbiota Inhibiting Growth of *Clostridioides difficile* through Membrane Disruptive Activity and Restoring Gut Microbial Community in *C. difficile* Infection

Byeong Seob Oh<sup>1</sup>, Seoung Woo Ryu<sup>1</sup>, Seung Yeob Yu<sup>1</sup>, Won Jung Choi<sup>1</sup>, Jeong Eun Bak<sup>1</sup>, Ji-Sun Kim<sup>1</sup>, Seung-Hwan Park<sup>1</sup>, Se Won Kang<sup>1</sup>, Mi Kyung Eom<sup>1</sup>, Jam-Eon Park<sup>1</sup>, Seung-Hyeon Choi<sup>1</sup>, Ji Young Choi<sup>1</sup>, Min Kuk Suh<sup>1</sup>, Han Sol Kim<sup>1</sup>, Jung-Sook Lee<sup>1,2</sup>, and Ju Huck Lee<sup>1\*</sup>

<sup>1</sup>Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>University of Science and Technology (UST)

*Clostridioides difficile* is the causal agent of antibiotic-associated diarrhea, called *C. difficile* infection (CDI), in individuals who have dysbiosis of the gut microbiota. As the conventional treatment with antibiotics inducing the gut dysbiosis causes to a high recurrence rate, new approaches that could restore gut microbial diversity are required, such as fecal microbiota transplantation (FMT) and microbiome therapy. To discover a novel gut microbiota (GM) inhibiting the growth of *C. difficile*, we exploited the cell free culture supernatants (CFSs) of 100 GM isolated from healthy human feces for screening antibacterial activity. As a result of the antibacterial assay, CFS of GM86 (GM86CFS), inhibited the growth of *C. difficile* over 80%. Furthermore, GM86CFS was unstable for heat, protease treatments and mechanical stress. Based on these results, we conducted the protein precipitation using GM86CFS to obtain the crude protein, and confirmed that the crude proteins (GM86PPT) showed anti-Cd activity. In addition, we observed that the disruption of *C. difficile* cell wall was induced by the treatment of GM86PPT. Furthermore, live GM86 attenuated the symptoms of CDI by reducing the colonization and toxins of *C. difficile* and inflammatory response (IL-6, TNFa) and restored gut microbial composition in CDI mouse model. Altogether, these findings will provide valuable information for the discovery of potential probiotic candidates that can be applied for prevention of CDI.

#### D033

### Identification of Novel Human Gut Microbiota Preventing Inflammatory Bowel Disease via Anti-inflammatory Activity and Gut Microbiome Modulation

Seung Yeob Yu, Byeong Seob Oh, Seoung Woo Ryu, Won Jung Choi, Jeong Eun Bak, and Ju Huck Lee<sup>\*</sup> Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology

Inflammatory bowel disease (IBD) is a severe chronic, relapsing inflammatory disease of the gastrointestinal tract. An imbalance in the gut microbiota that can disrupt host-microbial homeostasis, called microbial dysbiosis, is implicated in the pathogenesis of IBD. Therefore, modulation of the gut microbiome (GM) may be a strategy for suppressing IBD and inflammatory responses. In this study, we evaluated anti-inflammatory activity using cell-free supernatant (CFS) of isolated GM from healthy human feces. Among 53 GMs that inhibited inflammation in Raw264.7 cells, GM37 had the highest anti-inflammatory activity. We verified the anti-inflammatory activity of GM37 *in vitro* and *in vivo*. As a result, the CFS of GM37 decreased the amount of IL-6, TNF- $\alpha$  and NO via inhibition of NF- $\kappa$ B pathway. Furthermore, the mouse colitis model induced by DSS showed that GM37 prevented body weight loss, colon length shortening, colon permeability, bloody stools, and disease activity index (DAI). In addition, we discovered that GM37 treatment could ameliorate mucosal inflammation and improve the microbial community. All together, these findings suggest the possibility that the GM37 can prevent IBD and be a promising new drug for IBD therapy.

#### Protein Stability of Cytoskeleton Regulator Confers Antibiotic Persistence in Pathogenic Bacteria

Minseong Park<sup>1</sup>, Jia Xin Yee<sup>2</sup>, and Jinki Yeom<sup>1,2,3\*</sup>

<sup>1</sup>Department of Biomedical Science, College of Medicine, Seoul National University, <sup>2</sup>Department of Microbiology and Immunology, College of Medicine, Seoul National University, <sup>3</sup>Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore

Cells have cytoskeleton proteins such as actin and tubulin that controls cell survival by regulating mechanical support and movements. Here, we report that bacteria promote antibiotic persistence by stabilizing cytoskeleton regulator protein and cell morphology. Bacteria actin MreB and tubulin FtsZ control cell elongation and division, respectively. RodZ is required for determination of bacteria cell shape by interacting MreB and FtsZ. Inactivation of *rodZ* compromises bacteria cell morphology. Surprisingly, RodZ produces antibiotic persister bacteria, which is a tolerant population against antibiotics and cause chronic infection in the host. Furthermore, bacteria need RodZ proteins to confer survival under broad stress conditions. This accumulation in bacteria cytoskeleton regulator RodZ proteins resulted from an increase in the RodZ protein stability under antibiotics. Our findings suggest that protein stabilization of cytoskeleton regulator under antibiotic treatment is a bacterial strategy that facilitates chronic infection by promoting formation of antibiotic persistence.

#### D035

#### **Lis-II Shows Antifungal Activity and Reduces Dual-species Biofilms by Fungi and Bacteria** Jonggwan Park and Chang Ho Seo<sup>\*</sup> Department of Bioinformatics, Kongju National University

Antibiotic resistance is one of the biggest issues to human and animals. To resolve this, antimicrobial agents have been developed. Antimicrobial peptides exhibit a wide range of antimicrobial activities against pathogens, including bacteria, fungi, virus and cancer. Lis-II, derived from the venom of the spider *Lycosa singoriensis*, has exhibited antibacterial activity by disrupting membranes. However, the mode of action of Lis-II and its antifungal activity have not been determined. Therefore, we investigated that Lis-II showed antifungal activity against *Candida albicans* (*C. albicans*). To investigate the mode of action, membrane-related assays were performed, including a study of *C. albicans* membrane depolarization and membrane integrity after exposure to Lis-II. These results indicated that Lis-II disrupted the *C. albicans* membrane. Moreover, Lis-II induced oxidative stress through the production of reactive oxygen species in *C. albicans*. Moreover, Lis-II exhibited an inhibitory activity on dual-species biofilm formation by *C. albicans* and *Staphylococcus aureus*, which are the most co-isolated fungi and bacteria. Therefore, Lis-II can be against *C. albicans* and dual-species strain infections.

### **Synergistic Activity of Antimicrobial Peptide SPE-2 with Ciprofloxacin against** *Pseudomonas aeruginosa* Infection Jonggwan Park and Chang Ho Seo<sup>\*</sup>

Department of Bioinformatics, Kongju National University

Antimicrobial resistance is a serious global health issue. To develop novel antibiotics, we designed antimicrobial peptide, SPE analogs with Lys substitutions, resulting in improved amphipathic  $\alpha$ -helical structure and cationicity. Moreover, truncated analogs of SPE and Lys-substituted peptides were designed to produce linear 18-residue amphipathic  $\alpha$ -helices, which were further investigated for their mechanism and functions. These truncated analogs exhibited higher antimicrobial activity and lower cytotoxicity than SPE. In particular, SPE-2 exhibited pore formation, permeabilization of the outer/inner bacterial membranes, and DNA binding. Fluorescence spectroscopy and scanning electron microscopy showed that SPE-2 kills bacterial cells by disrupting membrane integrity. *In vivo*, wounds infected with *Pseudomonas aeruginosa* healed significantly faster when treated with SPE-2 than did untreated wounds or wounds treated with ciprofloxacin. Moreover, SPE-2 facilitated infected-wound closure by reducing inflammation through suppression of interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, and tumor necrosis factor alpha (TNF- $\alpha$ ). These result suggest that the SPE-2 could be useful for future development of therapeutic agents effective against *P. aeruginosa* infection.

#### D037

Investigation of Molecular Characterization and Virulence of Mammalian-adapted Avian H3N2 Virus A-Hyeon Kim<sup>1,2</sup>, Soo-Hyeon Kim<sup>1,2</sup>, Si-Hyun Lee<sup>1,2</sup>, and Su-Jin Park<sup>2\*</sup> <sup>1</sup>Division of Applied Life Science, Gyeongsang National University, <sup>2</sup>Division of Life Science, Research Institute of Life Science, Gyeongsang National University

The H3N2 avian influenza viruses (AIV) continuously circulate in wild migratory birds and are repeatedly reported to spill over to other hosts across the species barrier. Therefore, the H3N2 virus threatens public health and is also considered to be the next potential human pandemic strain due to its rapid evolution and interspecies infection. In this study, to investigate the molecular markers of adaptation from avian to mammalian, we demonstrated the serial lung-to-lung passage of avian H3N2 [A/MDK/South Korea/GNU-1/2021(H3N2)] virus in mice. After the mice adaptation, the virus (P20 MA) demonstrated significantly enhanced virulence with substitutions in PB2, PA, HA, M, and NS segments relative to the wild-type strain. The high virulence associated with the PB2 and PA amino acid mutations corresponded to considerably enhanced polymerase activity. Furthermore, HA mutation also exhibited enhanced human virus-like receptor binding. Thus, these results suggest knowledge of the molecular markers associated with mammal adaptation of avian influenza viruses. [Supported by grants from NRF]

#### Genetic and Pathogenic Diversity of Dabie bandavirus

Si-Hyun Lee<sup>1,2</sup>, A-Hyeon Kim<sup>1,2</sup>, Soo-Hyeon Kim<sup>1,2</sup>, Su-Jin Park<sup>2</sup>, and YoungKi Choi<sup>3\*</sup> <sup>1</sup>Division of Applied Life Science, Gyeongsang National University, <sup>2</sup>Division of Life Science, Research Institute of Life Science, Gyeongsang National University, <sup>3</sup>Center for Study of Emerging and Re-emerging Viruses, Korea Virus Research Institute, Institute for Basic Science

To investigate nationwide Dabie bandavirus infection status, we isolated Dabie bandavirus from patients with suspected severe fever with thrombocytopenia syndrome (SFTS) in 207 hospitals throughout South Korea between 2013 and April 2017. A total of 116 Dabie bandaviruses were isolated from 3137 SFTS-suspected patients, with an overall 21.6% case fatality rate. Genetic characterization revealed that at least 6 genotypes of Dabie bandaviruses were co-circulating in South Korea, with multiple reassortments among them. Of these, the genotype B-2 strains were the most prevalent, followed by the A and F genotypes. Clinical and epidemiologic investigations revealed that genotype B strains were associated with the highest case fatality rate, while genotype A caused only one fatality among 10 patients. Further, ferret infection studies demonstrated varying clinical manifestations and case mortality rates with different strains of Dabie bandavirus, which suggests this virus could exhibit genotype-dependent pathogenicity.

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#### D039

#### Infection Route Impacts the Virulence of Dabie bandavirus in Ferrets

Soo-Hyeon Kim<sup>1,2</sup>, A-Hyeon Kim<sup>1,2</sup>, Si-Hyun KIm<sup>1,2</sup>, and Su-Jin Park<sup>2\*</sup> <sup>1</sup>Division of Applied Life Science, Gyeongsang National University, <sup>2</sup>Division of Life Science, Research Institute of Life Science, Gyeongsang National University

The threat of severe fever with thrombocytopenia syndrome (SFTS) to public health has been increasing due to the rapid spread of the ticks that carry the causative viral agent. The Dabie bandavirus (DBV) was first identified in China and subsequently detected in neighboring countries, including South Korea, Japan, and Vietnam. In addition to the tick-mediated infection, human-to-human transmission has been recently reported with a high mortality rate; however, differential study of the pathogen has been limited by the route of infection. In this study, we investigated the pathogenic potential of DBV based on the infection route in aged ferrets, which show clinical signs similar to that of human infections. Ferrets inoculated with DBV via the intramuscular and subcutaneous routes show clinical signs comparable to those of severe human infections, with a mortality rate of 100%. Contrastingly, intravascularly infected ferrets exhibit a comparatively lower mortality rate of 25%, although their early clinical signs are similar to those observed following infection via the other routes. These results indicate that the infection route could influence the onset of SFTS symptoms and the pathogenicity of DBV. Thus, the infection route should be considered in future studies on the pathogenesis of DBV infection.

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### Bactericidal Activity and Mechanism of Action of Novel Antimicrobial Peptide SHP20 and Its Analog Peptides against *Pseudomonas aeruginosa*

Jonggwan Park and Chang Ho Seo\*

Department of Bioinformatics, Kongju National University

SHP20, derived from the venom of the scorpion *Heterometrus petersii*, exhibits antibacterial activity with cytotoxicity. Several analogue peptides were designed based on the parent peptide SHP20 to reduce cytotoxicity and increase activity (deletion of glycine and phenylalanine, substitution with leucine and lysine). The analogue peptides developed comprised 12 amino acids and showed amphipathic α-helical structures, with higher hydrophobic moments and net positive charge than those of the SHP20. The analogues showed less hemolytic and toxic effects toward mammalian cells than the SHP20, especially SHP20-te, which exhibited particularly potent antibacterial and antibiofilm activities against multidrug-resistant *Pseudomonas aeruginosa* (MRPA) strains. The analogue peptide SHP20-te was more stable against salt and trypsin than the SHP20. SHP20's mechanism of action involves binding to lipopolysaccharide (LPS), thereby killing bacteria through membrane disruption. SHP20-te kills bacteria more rapidly than SHP20 and not only seems to bind more strongly to LPS but may also be able to enter bacterial cells and interact with their DNA. Moreover, SHP20-te can show antimicrobial activity against MRPA *in vivo*. The results of this study indicate that SHP20-te not only displays antimicrobial activity, but is also functional in physiological conditions, confirming its potential use as an effective therapeutic agent against MRPA.

#### D041

### D-SP25, an Antimicrobial Peptide Derived from Venom of Scorpion, Exerts Potent Antimicrobial Activity against Zoonotic Bacteria

#### Da Dam Kang<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

Antibiotic-resistant bacteria has been an increasing public health threat in recent years. Thus, antimicrobial peptides (AMPs) have been considered for substitutes for antibiotics. In this study, the AMP D-SP25, isolated from the venom of *Centruroides suffuses suffusus*, was found to exhibit antimicrobial activity against bacteria such as *Listeria monocytogenes*, *Streptococcus suis*, *Campylobacter jejuni*, and *Salmonella typhimurium* that cause zoonotic diseases. Moreover, the cytotoxicity and hemolytic activity of D-SP25 was lower than that of melittin isolated from bee venom as control peptide. Circular dichroism assays showed that D-SP25 has an  $\alpha$ -helix structure in an environment mimicking that of bacterial cell membranes. We examined the effect of D-SP25 on bacterial membranes using *N*-phenyl-1-naphthylamine, 3,3'-dipropylthiadicarbbocyanine iodides, SYTOX green, and propidium iodide. Our findings suggest that the D-SP25 peptide kills bacteria by disrupting the bacterial membrane. Moreover, D-SP25 exhibited antibiofilm activity against *L. monocytogenes*. Thus, D-SP25 may be useful as an alternative to antibiotics in humans and animal husbandry.

### Antimicrobial Activity and Biofilm Eradication Effect of Antimicrobial Peptide SAP and Therapeutic Potential for Skin Infections and Wounds against Methicillin-resistant *Staphylococcus aureus*

Da Dam Kang<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

The antibiotic resistance crisis has led to a pressing need for alternatives such as antimicrobial peptides (AMPs). AMPs are one of the most promising choices for next-generation antibiotics. In this study, we found SAP peptide derived from the venomous gland of scorpion. The scorpion peptide SAP was investigated to have antimicrobial activity, outer membrane permeability, and membrane polarization. SAP had excellent antimicrobial activity and membrane activity below amount to cause toxicity. Moreover, SAP killed multidrug-resistant (MDR) pathogens, inhibited biofilm formation and eliminated established biofilms. *In vivo* data showed that treatment of SAP reduced skin wound size and significantly reduced the bacteria burden on skin and other tissues. SAP eradicated acute infections with methicillin-resistant *Staphylococcus aureus* (MRSA) from wounded murine skin *in vivo*. Collectively, SAP not only exhibits antibacterial activity, but also has effective activity *in vivo*, confirming its potential as an effective therapeutic agent for MRSA.

#### D043

#### **Antimicrobial Peptide Hag-2 and Hag-3 Regulate STAT3 and MAPKs to Promote Wound Healing** Da Dam Kang<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

Skin wounds are continuously exposed to bacteria and can easily become infected. Infected wounds require antibiotic treatment, and infections caused by drug-resistant bacteria are an important public health problem. Antimicrobial peptides have broad-spectrum antibacterial activity, induce little or no drug resistance and may be suitable for treating skin infections caused by drug-resistant bacteria. We previously reported the design and function of Hag and Hag analogues. Here we showed that Hag-2 and Hag-3 exhibit antimicrobial and anti-biofilm activities against antibiotic-resistant *Staphylococcus aureus, Acinetobacter baumannii*, and *Pseudomonas aeruginosa* in high salt environments and in gelatin. Moreover, these peptides facilitated infected wound healing by decreasing inflammation through suppression of IL-6, IL-8, and TNF- $\alpha$  and regulation of downstream mediators such as STAT3, p38, JNK, and EGFR. In a mouse skin wound model infected with antibiotic-resistant bacteria, Hag-2 and Hag-3 eliminated the infection and enhanced wound healing. We therefore propose the use of these peptides for treating infected wounds and burns.

#### Cholera Toxin Production in Vibrio cholerae O1 El Tor Biotype Strains in Single-phase Culture

Eun Jin Kim<sup>1,2</sup>, Dong Hyun Lee<sup>1,2</sup>, Jonghyun Bae<sup>1,2</sup>, Seonghyeon Son<sup>1,2</sup>, Hunseok Choi<sup>1,2</sup>, and Dong Wook Kim<sup>1,2\*</sup> <sup>1</sup>Department of Pharmacy, College of Pharmacy, Hanyang University, <sup>2</sup>Institute of Pharmacological Research, Hanyang University

*Vibrio cholerae* O1 serogroup strains have been classified into classical and El Tor biotypes. Cholera, a lifethreatening diarrheal disease, can be caused by either biotype through the cholera toxin (CT) that they produce. To increase our knowledge of the pathogenicity of bacteria, we must understand the toxigenicity of bacteria. CT production by classical biotype strains in simple single-phase cell cultures has been established; however, special culture media and growth conditions that are not appropriate for mass production of CT are required to facilitate CT production in El Tor biotype strains. In this report, we produced CT in El Tor biotype strains using simple media and single-phase culture conditions. A single point mutation in ToxT, a transcriptional activator of toxin coregulated pilus (TCP) and CT, enabled the El Tor biotype strains to produce CT in similar quantities as classical biotype strains in single phase laboratory culture conditions. CT production capacity varied between El Tor biotype strains. Wave 2 and 3 atypical El Tor strains tended to produce more CT than prototype Wave 1 strains. The Wave 3 strain that caused the 2010 cholera outbreak in Haiti produced CT only when neutral fermentation was abolished. The disparity in CT production between the seventh cholera pandemic strains highlight the differences in virulence between strains and the cause of population changes in *V. cholerae*.

#### D045

#### The Role of the Extracellular Loops of Outer Membrane Protein A in Acinetobacter baumannii

Kyeongmin Kim, Md. Maidul Islam, Dooyoung Kim, Seunghyeok Bang, Jeongah Kim, Samia Akter, Je Chul Lee, and Minsang Shin<sup>\*</sup>

Department of Microbiology, School of Medicine, Kyungpook National University

Multidrug resistant *Acinetobacter baumannii* is a global problem as opportunistic pathogen causing nosocomial infections. The development of new therapeutic agents for this bacteria is urgently needed. The outer membrane protein A (OmpA) is a versatile virulence factor of *A. baumannii*. The OmpA has four extracellular loops exposed to the outer membrane surface, and it is associated with the pathogenesis of *A. baumannii* through interactions with host molecules. However, role of the individual loops is unclear. To identify various pathogenic roles of individual loops we constructed mutant strains as a deletion of six amino acids at individual loops. We examined biofilm formation, cell adhesion and invasion, surface motility, serum resistance, mouse experiments and minimum inhibitory concentration (MIC) test using each mutant strains. All mutant strains decreased biofilm production. Adherence assay on A549 cell line was decreased in loop2 and loop3 mutant strains. As a result of surface motility, only loop4 mutant was reduced like an OmpA-deficient strain. The loop3 and loop4 mutant strains were susceptible to normal human serum in comparison with the wild-type. Interestingly, only the loop4 mutant strain had fewer bacterial number in the blood than the wild-type in a neutropenic mouse model and showed susceptibility in various antibiotics using MIC test. These results may be the use of an antibody against loop4 of OmpA as a therapeutic target in *A. baumannii*.

#### Cyanidin Chloride Inhibits Expression of Virulence Factors against Acinetobacter baumannii

Jaeu Won, Mikyung Chang, Ho-sung Park, Kyungho Woo, Dong Ho Kim, and Chul Hee Choi<sup>\*</sup> Department of Microbiology and Medical Science, Chungnam National University School of Medicine

*Acinetobacter baumannii* (*A. baumannii*) is a representative opportunistic pathogen among Gram-negative bacteria that can damage patients with compromised immune systems. Among flavonoids, cyanidin chloride, an anthocyanidin family, is reported to have cytoprotective and anticancer effects. However, the antibacterial mechanism of cyanidin chloride against *A. baumannii* has not been reported. To confirm the effect of cyanidin chloride against *A. baumannii* has not been reported. To confirm the effect of cyanidin chloride on A. baumannii, bacterial growth was measured with CFU. Surface motility, biofilm formation and AHL inhibition was measured to confirm pathogenic inhibition. Inhibition at gene level was measured through qRT-PCR. Synergistic effects were measured with conventional antibiotics. Results showed that cyanidin chloride did not affect the growth of ATCC 17978, the standard strain *A. baumannii*, but significantly inhibited the biofilm formation, motility and AHL production. Cyanidin chloride inhibited the expression of biofilm-related and motility-related genes (*bap, bfmR, csuAB*) and QS system-related genes (*AbaR, Abal*) at the genetic level, and also down-regulated *ompA*, a major virulence gene of *A. baumannii*. In the subsequent synergy test, it was confirmed that cyanidin chloride exhibited additive effects with the antibiotics; tetracycline, amikasin and ciprofloxacin. These results indicate the possibility that cyanidin chloride can be used as a pathogenic inhibitor against *A. baumannii*. [This work was supported by the NRF grant.]

#### D047

### Antimicrobial and Anti-inflammatory Activities of Boana and Its Analogs against Acinetobacter baumannii Myeongjin Lee<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

The number of multi-drug resistant bacteria has been increasing rapidly, threatening human health. As the limitations of antibiotics have been widely documented, there is growing interest in developing new therapeutic agents, such as antimicrobial peptides (AMPs). In previous study, A novel 18-amino acid AMP Boana was identified in the South American frog *Hypsiboas albopunctatus*. In a recent study the analog peptides were designed based on the parent peptide Boana to improve antimicrobial efficacy and to decrease toxicity. The analog peptides were replaced with alanine and lysine resulting in the formation of  $\alpha$ -helical structures in bacterial membrane-mimicking environments and in the induction of net charge and hydrophobicity. In addition, the analog peptides exhibited lower toxicity and mammalian cell selectivity than Boana. In particularly Boana-a1 and Boana-a2 exhibited broad-spectrum antimicrobial and anti-biofilm activities against carbapenem-resistant *Acinetobacter baumannii* (CRAB). Permeability assays indicated that analog peptides killed bacteria by binding to lipopolysaccharide and by disrupting the bacterial membrane. Moreover, Boana-a1 and Boana-a2 reduced inflammation by suppressing pro-inflammatory cytokines expression by *Acinetobacter baumannii* (*A. baumannii*). Our results suggest that the analog peptides substituted with several residues based on Boana have antibacterial and anti-inflammatory activity, and can be an alternative treatment for CRAB.

### Enhanced Therapeutic Index of MJ27-2 in Mice by Improving Synergy Effect with Antibiotic and Activity against *Staphylococcus aureus*

Myeongjin Lee<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

Antimicrobial peptides (AMPs) are central components of the innate immune system providing protection against pathogens. MJ27, a 27-amino acid peptide release *Streptococcus pneumoniae*, is major toxic factor. To develop a clinically applicable AMPs, we designed MJ27 analogs with Trp substitutions to enhance its antimicrobial activity compared to that of MJ27. Moreover, MJ27-2 exhibited strong antibacterial activity against multidrug-resistant (MDR) bacteria. We found that MJ27-2 acts as an effective cell-penetrating peptide in bacterial and mammalian cells. Combination treatment with MJ27-2 and Ceftriaxone showed synergistic effect, remarkably reducing abscess size and improving the bacterial antimicrobial rate from the *Staphylococcus aureus* infection site. In addition, MJ27-2-antibiotic combination treatment reduced inflammation, lowering levels of tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, inducible NO synthase (iNOS), and cyclooxygenase (COX-2) in skin abscess tissues. In conclusion, we suggest that MJ27-2 peptide is a potential theraphy agent for combating MDR bacterial improving effect of antibiotic.

#### D049

### Anti-biofilm Activity and Antibacterial Mechanism of Pamp1 and Pamp1 Analogs against Multidrug-resistant *Klebsiella pneumoniae*

#### Myeongjin Lee<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

Antimicrobial peptides (AMPs) have been used as substitute to conventional antibiotics. AMPs are key components of the innate immune system and represent the first line of defence against infectious pathogens. Pamp1 is AMP purified from *Branchiostoma japonicum*, commonly known as the lancelet or amphioxus. We purified protein organized of 97 amino acids, but analyses using peptide cutter enabled found of the mature form, Pamp1. Notable, Pamp1 has antibacterial activity, it is not toxic to mammalian cell. In the recent study, we designed Pamp1 analogues by adding the several amino acid sequence to the peptide's N-terminus. Compare of their antibacterial activity exhibited that Pamp1-A performed better than did Pamp1. We then designed 10 additional analogues based on the Pamp1-A model to further enhance the peptide's antimicrobial activity. Among these analogues, Pamp1-A-a10 showed potent antimicrobial and antibiofilm activities against multidrug-resistant *Klebsiella pneumoniae* (*K. pneumoniae*), as well as its cytotoxicity activities against MRC-5 cells derived from human lung tissue. Also, the mechanism of Pamp1-A and Pamp1-A-a10 against *K. pneumoniae* were showed in membrane-related experiments and DNA binding assay. Finally, we suggest the peptides designed in this study have the potential effective activity against infection by MDR *K. pneumoniae*.

Effect of ppGpp on ROS Stress Defense Mechanism of *Acinetobacter baumannii* in Macrophage

Dooyoung Kim, Kyeongmin Kim, Md. Maidul Islam, Samia Akter, Jeongah Kim, Seunghyeok Bang, Je Chul Lee, and Minsang Shin<sup>\*</sup>

Department of Microbiology, School of Medicine, Kyungpook National University

*Acinetobacter baumannii* is an opportunistic pathogen that causes respiratory infections in immunocompromised patients. ppGpp is a secondary messenger that response to various stress conditions in bacteria and regulates the balance of bacterial growth and maintenance functions. Macrophages generate reactive oxygen species (ROS) to eliminate invading bacterial pathogens under the condition of oxidants such as hydrogen peroxide. We examined the mRNA expression of catalase activity-related genes in H2O2 environment using ppGpp-deficient strain. The growth of the ppGpp-deficient strain in the medium containing H2O2 showed a slower recovery rate compared to wild-type strain. *In vitro*, we observed the difference in phagocytosis of macrophages, paying attention to the fact that the ppGpp-deficient strain lowered the defense against H2O2. We found that the intracellular viability was decreased in ppGpp-deficient strain against mouse macrophages (RAW264.7) and human macrophages (THP-1). To visualize intracellular survival rate we imaged using confocal microscopy. And each strain carrying the KatGp-gfpOVA biosensor was used to detect Catalase-related gene expression in macrophage and imaged by fluorescence microscopy. This study suggest that ppGpp deficiency decreases the viability of *A. baumanii* in the H2O2 environment, which also reduces the viability of ROS in macrophages. Therefore, the ppGpp may be a therapeutic target for novel drug design and can promote phagocytosis of macrophages.

### Complete Genome Sequence of Clinical *Acinetobacter baumannii* KBN10P05679 Isolate: Identification of Antimicrobial Resistance Genotype and Potential Virulence Traits

Jeongah Kim, Md. Maidul Islam, Kyeongmin Kim, Seunghyeok Bang, and Minsang Shin<sup>\*</sup> Department of Microbiology, School of Medicine, Kyungpook National University

*Acinetobacter baumannii*, a causative agent of nosocomial infection and, its increasing multi-drug resistance properties are of major concern worldwide. Owing to its genomic complexity and inadequate knowledge on the antibiotic-resistant mechanisms and virulence factors, development of new therapeutic agents is still challenging. This study investigated the genomic diversity, drug resistance pattern and virulence potential in MDR *A. baumannii* KBN10P05679 strain by whole-genome sequencing (WGS) and *de novo* assembly. The results showed that complete genome of KBN10P05679 consists of a circular chromosome 3990428 bp (39.0% GC content), two plasmids 74294 bp and 8731 bp respectively, and was assigned to ST451. Moreover, Clusters of Orthologous Genes (COG) annotation identified 3,810 genes including amino acid transport and metabolism, transcription, inorganic ion transport, energy production and conversion, replication, recombination and repair, carbohydrate and protein metabolism. The antibiotic resistant genes were investigated by searching in the Comprehensive Antibiotic Resistance Database (CARD) and the genome harbors thirty antibiotic resistant genes. Eighty-six virulence factor genes were identified in the genome by searching for virulence factors in the pathogenic bacteria databases. Overall, the antibiotic-resistant genotype and potential virulence factors obtained in this study will help to direct future studies for developing control measures for this MDR pathogen.

#### D053

#### Antimicrobial Activity and Mechanism of Action of Red Sea Bream-derived Antimicrobial Peptide CAP2 Seyoung Lee<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

*S. aureus*, the most common cause of skin and soft tissue infections, is particularly problematic due to its ability to withstand environments with high salt concentrations, and thus effective novel antibiotics are urgently needed. Antimicrobial peptides (AMPs) are an alternative to conventional antibiotics due to their ability to disrupt bacterial membranes and cause cell death. CAPs are amphipathic alpha-helical antimicrobial peptides derived from gill cells of red sea bream. According to previous studies, among CAP analogs, CAP1 has been reported to have antibacterial and antiendotoxin effects. However. There is no research on the antibacterial effect of CAP2, so we tested the antimicrobial and anti-biofilm activities of CAP2 against *S. aureus* strain. Circular dichroism analysis indicated that the CAP2 appeared as α-helical structures within bacterial membrane-mimetic environments. Moreover, we conducted NPN uptake, DiSC<sub>3</sub>-5, PI uptake, and flow cytometry assays to confirm that the CAP2 killed *S. aureus* cells by permeabilizing the cell membrane and damaging the integrity of the membrane envelope. Importantly, the CAP2 was stable in physiological environments. Finally, we confirmed the *in vivo* effects of the peptide, and as a result, CAP2 reduced wound lesions against *S. aureus* skin infections. Collectively, these results suggest that CAP2 may be an effective antibiotic that can replace conventional antibiotics against *S. aureus*.

### Antimicrobial and Anti-inflammatory Activities of LCP-II Isolated from Spiders against Multidrug-resistant Bacteria

Seyoung Lee<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

Antibiotics have been developed to extend human lifespan and improve quality of life, but due to the misuse or overuse, the number of multidrug-resistant bacteria is rapidly increasing worldwide. Among them, there are few options for treating infections caused by the rapidly spreading oxacillin-resistant strains and carbapenem-resistant strains. Antimicrobial peptides (AMPs) that modulate immune responses and have antimicrobial activity can be used as an alternative to antibiotics. The AMP LCP-II is a 21 amino acid peptide isolated from *Lycosa singoriensis*. LCP-II showed strong antibacterial activity and biofilm inhibition effects against Gram-positive and Gram-negative bacteria including oxacillin-resistant *S. aureus* and meropenem-resistant *P. aeruginosa* isolated from patients. In addition, LCP-II did not show cytotoxicity to mammalian cells and hemolysis to sheep red blood cells at concentrations exhibiting antibacterial activity. The LCP-II has a mechanism of action that destroys the bacterial membrane by binding to lipoteichoic acid and lipopolysaccharide present in the membranes of Gram-positive and Gram-negative bacteria. Moreover, LCP-II showed anti-inflammatory effects by inhibiting the expression of pro-inflammatory cytokines that are increased during bacterial infection in mammalian cells. These results suggest that LCP-II can utilize as an alternative treatment for antibiotics against multidrug-resistant *S. aureus* and *P. aeruginosa* strain infections.

#### D055

### Antibacterial and Anti-biofilm Properties of Antimicrobial Peptide BMP Derived from Bee Venom by Damaging the Plasma Membrane against Drug-resistant Bacteria

Seyoung Lee<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

Antibiotics developed to treat diseases and increase lifespan have been developed, but due to misuse and abuse, multidrug-resistant bacteria have appeared worldwide, and accordingly, the development of novel therapeutic agents is urgent. Antimicrobial peptides (AMPs) found in all organisms can be an alternative to conventional antibiotics due to their broad spectrum of antimicrobial activity and low cytotoxicity. In a previous report, BMP, isolated from bee venom, exhibited antimicrobial activity against both Gram-positive and negative bacteria. In the present study, BMP was synthesized and its antibacterial and anti-biofilm activities were tested against bacterial strains, including Gram-positive and negative bacteria, and drug-resistant bacteria. Moreover, BMP did not exhibit hemolytic activity and cytotoxicity to keratinocytes, whereas Melittin, as a positive control, showed very high toxicity. Circular dichroism assays showed that BMP has an  $\alpha$ -helical structure in membrane mimic environments. BMP binds to peptidoglycan and lipopolysaccharide and kills the bacteria by disrupting their membranes. Moreover, the fractional inhibitory concentration index indicated that BMP has additive and partially synergistic effects with conventional antibiotics against drug resistant bacteria. Therefore, these results suggest that BMP has the potential to be used as an antimicrobial agent against infectious bacteria, including drug-resistant bacteria.

### A Fractional Extract of *Paederia scandens* Regulate Intracellular *Mycobacterium tuberculosis* Growth in Murine Macrophage via Enhanced NF-κB Signal Pathway and Autophagy

Eui-Kwon Jeong<sup>1</sup>, Hyo-Ji Lee<sup>2,3</sup>, Heejung Yang<sup>4</sup>, and Yu-Jin Jung<sup>1,2,3\*</sup>

<sup>1</sup>Department of BIT Medical Convergence Graduate Program, Kangwon National University, <sup>2</sup>Department of Biological Sciences, Kangwon National University, <sup>3</sup>Kangwon Radiation Convergence Research Support Center, Kangwon National University, <sup>4</sup>College of Pharmacy, Kangwon National University

Tuberculosis (TB) is one of the leading causes of death worldwide, threatening public health consistently. Conventional TB treatment requires a long-term regimen and has side effect problems. Moreover, the efficacy of anti-TB drugs has decreased with the emergence of drug-resistant TB, thus the development of new anti-TB drugs is urgently needed. In this study, we first screened plant-derived natural products that can control *Mycobacterium tuberculosis* (Mtb) infection via pilot experiments. Among them, *Paederia scandens* extract significantly reduced intracellular Mtb growth in infected macrophages without cytotoxicity. To identify the active constituents thought to be effective in controlling Mtb infection, *P. scandens* extract was divided into 4 fractions depending on the polarity of the solvent. Of these fractions, the butanol fraction increased secretion of TNF-α and nitric oxide by sustaining NF-κB signaling, and decreased secretion of IL-10. Furthermore, we observed that the butanol fraction inhibits apoptosis of macrophages induced by Mtb and enhances autophagy to contribute to controlling Mtb infection, and this indicates that *P. scandens* extract contains active constituents that can control Mtb infection, and this indicates that *P. scandens* extract contains active constituents that can control Mtb infection, and this indicates that *P. scandens* extract has the potential to be developed as a novel anti-TB drug or a drug for host-directed therapy.

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#### D057

#### Polychlorinated Biphenyl 77, an Endocrine Disruptor, Attenuates Anti-tuberculosis Activity by Elevating Production of the Anti-inflammatory Cytokine IL-10 in Mouse Macrophages during *Mycobacterium tuberculosis* Infection

Han-min Kim<sup>1</sup>, Eui-Kwon Jeong<sup>1</sup>, Hyo-Ji Lee<sup>2,3</sup>, Su-Hyun Cho<sup>1,4</sup>, and Yu-Jin Jung<sup>1,2,3\*</sup>

<sup>1</sup>Department of BIT Medical Convergence Graduate Program, Kangwon National University, <sup>2</sup>Department of Biological Sciences, Kangwon National University, <sup>3</sup>Kangwon Radiation Convergence Research Support Center, Kangwon National University, <sup>4</sup>Department of Physiology, College of Medicine, Kangwon National University

Tuberculosis is a respiratory disease caused by the infection of *Mycobacterium tuberculosis* (Mtb), which is still a leading infectious disease after outbreak of COVID\_19 pandemic in worldwide. Macrophages induce phagocytosis, cell death, and inflammatory cytokines to control invading Mtb. Polychlorinated biphenyls (PCBs) are an industrial material banned worldwide in 1979. PCBs can still affect the dysregulation of endocrine system, because there is a risk of exposure by remaining in environments. In this study, to elucidate the effect of PCB77 in innate immune response, mouse macrophages were treated with PCB77 before and after Mtb infection. In the results, the intracellular bacterial growth increased in PCB77-treated cells compared to untreated cells during Mtb infection. However, there were no significant differences in the secretion of inflammatory mediators. But the production of IL-10 was increased in PCB77-treated cells compared to untreated cells during Mtb infection. In addition, phosphorylation level of STAT3 was increased in PCB77-treated cells compared to untreated cells during Mtb infection. But the treatment of PCB77 did not induce macrophage polarization. These results indicate that treatment of PCB77 suppresses the antibacterial activities by promoting the production of anti-inflammatory cytokines in Mtb-infected macrophages.

[Supported by grant from through the National Research Foundation of Korea (NRF-2020R1I1A1A01066916 and 2021R1A2C100452511)]

### Photosensitizer DH-I-180-3 Enhances the Anti-bactericidal Activity in *Salmonella* Typhimurium-infected Murine Macropahges

Hyo-Jung Kim<sup>1</sup>, Hyo-Ji Lee<sup>2,3</sup>, and Yu-Jin Jung<sup>1,2,3\*</sup>

<sup>1</sup>Department of BIT Medical Convergence Graduate Program, Kangwon National University, <sup>2</sup>Department of Biological Sciences, Kangwon National University, <sup>3</sup>Kangwon Radiation Convergence Research Support Center, Kangwon National University

The intestinal pathogen *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is recognized through patternrecognition receptors (PRRs) of macrophage. Stimulated PRRs enhance the inflammatory response by activating NF- $\kappa$ B and MAPK signaling pathways. Photodynamic therapy (PDT) is known as a cancer treatment method in which a photosensitizer (PS) activated by light damages cancer tissue by generating ROS. In addition, it has been reported that PDT can be used as a new therapeutic strategy to control antibiotic-resistant pathogen. In a previous study, DH-I-180-3, a PS, effectively controlled multidrug-resistant *Mycobacterium tuberculosis*. In this study, to determine whether photoactivated DH-I-180-3 enhances the bactericidal activity of macrophages against *S*. Typhimurium. Photoactivated DH-I-180-3 regulated intracellular bacterial growth in *S*. Typhimurium-infected cells. DH-I-180-3 treatment during *S*. Typhimurium infection not only enhanced the phosphorylation level of ERK1/2 and IkB $\alpha$ , but also increased the transcriptional level and protein secretion of TNF- $\alpha$ . DH-I-180-3 was also observed to increase the production of intracellular ROS. These results suggest that DH-I-180-3 can improve the bactericidal activity against intracellular bacterial infection by increasing the proinflammatory response through activation of MAPK and NF- $\kappa$ B signaling pathways.

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#### D059

### Interleukin-10 Regulates the Bactericidal Activity against *Salmonella* Typhimurium Infection by Attenuating the Process of Phagocytosis

#### Hyo-Jung Kim<sup>1</sup> and Yu-Jin Jung<sup>1,2\*</sup>

<sup>1</sup>Department of BIT Medical Convergence Graduate Program, Kangwon National University, <sup>2</sup>Department of Biological Sciences, Kangwon National University

Macrophages maintain tissue homeostasis through phagocytosis, a defense mechanism that isolates pathogens [eg, *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium)], cellular debris, and small particles into the phagosome. Phagosomes undergo a maturation step and form phagolysosome that eliminates foreign substances through the fusion with lysosomes. Interleukin (IL)-10 is an anti-inflammatory cytokine that regulates the host immune response. Previous studies have reported that the effect of IL-10 on the host varies according to the type of pathogen, infection situation, and the degree of inflammatory response. In this study, we investigated whether IL-10 affects phagocytosis and intracellular bacterial survival in macrophages during *S*. Typhimurium infection. IL-10 treatment downregulated the activation of inflammatory signaling pathways and decreased the transcriptional levels and protein secretion of pro-inflammatory cytokines. IL-10 treatment inhibited the uptake of *S*. Typhimurium and reduced the protein levels of phagosome maturation markers. These results suggest that IL-10 plays a role in inhibiting bactericidal activity against *S*. Typhimurium in macrophages by regulating inflammatory signaling and phagocytosis.

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# CHLO-Cl, Newly Synthesized Photosensitizer, Regulates Intracellular Bacterial Growth by Improving the Production of Pro-inflammatory Cytokine and Reactive Oxygen Species in Murine Macrophages Infected with *Escherichia coli* or *Staphyloccocus aureus*

Min-jeong Kim<sup>1</sup>, Hyo-ji Lee<sup>2,3</sup>, and Yu-jin Jung<sup>1,2,3\*</sup>

<sup>1</sup>Department of BIT Medical Convergence Graduate Program, Kangwon National University, <sup>2</sup>Department of Biological Sciences, Kangwon National University, <sup>3</sup>Kangwon Radiation Convergence Research Support Center, Kangwon National University

The overuse and inappropriate use of antibiotics contribute to the increase in antibiotic resistance, and thus the number of available antibiotics is decreasing. Accordingly, photodynamic therapy has emerged as a new therapeutic strategy for the control of antibiotic-resistant pathogens. In a previous study, the photosensitizer CHLO-Cl enhanced the ability of macrophages to kill intracellular Mtb. In this study, to investigate the bactericidal activity of newly synthesized CHLO-Cl derived from DH-I-180-3, Raw264.7 cells were pre-treated with CHLO-Cl and then irradiated with light. As a results, the treatment of CHLO-Cl with or without light irradiation has little effect on the survival of Raw264.7 cells. Photoactivated CHLO-Cl decreased the growth of *E. coli* or *S. aureus* as well as bacterial survival in *E. coli* or *S. aureus*-infected cells. Furthermore, in the presence of photoactivated CHLO-Cl, secretion of TNF- $\alpha$  and protein levels of p-MEK1/2 and p-ERK1/2 were significantly increased in *E. coli* or *S. aureus*-infected cells. Under light illumination, CHLO-Cl increased the production of intracellular ROS. These results suggest that CHLO-Cl may help regulate intracellular bacterial growth by increasing pro-inflammatory cytokine through activation of the MAPK signaling pathway and producing ROS. [Supported by grant from the National Research Foundation of Korea (2020R1I1A1A01066916 and 2021R1A2C100452511).]

#### D061

### Potent Antibacterial Activity of Hs26 and Its Analog Peptides against Multidrug-resistant Acinetobacter baumannii-associated Murine Sepsis Model

Hyeongsun Kim<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

Acinetobacter baumannii is an opportunistic pathogen, and the increasing prevalence and incidence of multidrugresistant *A. baumannii* (MDRAB) infections is a major global health problem. Antimicrobial peptides (AMPs) are promising alternatives to antibiotics for the treatment of MDR bacterial infections. Hs26 was isolated from the venom of scorpion. Analog peptides were designed based on Hs26, by substituting amino acids to reduce cytotoxicity and truncating to reduce the length. The antibacterial activity of analog peptides against MDRAB was comparable to that of Hs26. The hemolytic activity and cytotoxicity to mammalian cells of analog peptides were lower than that of Hs26. Calcein leakage from the liposomes showed that Hs26-A3 selectively acted on the Gramnegative bacterial membrane. Moreover, all peptides effectively inhibited biofilm formation in MDRAB strains. The secondary structure of peptides exhibited  $\alpha$ -helical structure, making it easier to act on bacterial membranes. Unlike Hs26, Hs26-A3 was found to exhibit antibacterial action by binding to DNA, and showed antibacterial activity by inducing intracellular reactive oxygen species generation. Notably, Hs26 and Hs26-A3 did not induce bacterial resistance. In the MDRAB-infected sepsis mouse model, Hs26-A3 treatment remarkably reduces tissue bacterial burden and suppresses pro-inflammatory cytokines to mitigate tissue damage. These results suggest that Hs26-A3 is a promising therapeutic agent for combating MDRAB.

### Therapeutic Efficacy and Mechanism of Action of Antimicrobial Peptide Ey17 against Carbapenem-resistant *Klebsiella pneumoniae*

Hyeongsun Kim<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

*Klebsiella pneumoniae* is a major nosocomial pathogen and causes a variety of diseases, including bloodstream infections, urinary tract infection, pneumonia, and sepsis. The global emergence and spread of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is emerging as a serious public health problem. Therefore, it is necessary to find new candidates that can replace the antibiotics. Antimicrobial peptides (AMPs) with broad antimicrobial activity, anti-biofilm, and anti-inflammatory effects are attracting attention as promising therapeutic agents. Ey17 was purified from the solitary bee venom. Ey17 has an α-helical structure in a bacterial membrane and exerts antimicrobial activity against *K. pneumoniae* strains. Furthermore, Ey17 maintained antimicrobial activity stable under the physiological conditions that affect the antibacterial activity. Through the bactericidal mechanism tests, Ey17 exhibits antimicrobial activity by rapidly destroying the bacterial membranes. In addition, Ey17 effectively inhibited and eradicated biofilm formations by CRKP strains. Unlike antibiotics, Ey17 did not induce bacterial resistance. Moreover, Ey17 ameliorated multiple-organ damage and attenuated systemic infection-associated inflammation in an CRKP-induced sepsis mouse model. These results provide that Ey17 can be a promising therapeutic option for CRKP infection.

#### D063

### Enhanced Antimicrobial and Anti-biofilm Effect of CM205 Peptide and Its Analogs against *Acinetobacter* baumannii

#### Hyeongsun Kim<sup>1</sup> and Yoonkyung Park<sup>1,2\*</sup>

<sup>1</sup>Department of Integrative Biological Sciences & BK 21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, <sup>2</sup>Department of Biomedical Science, College of Natural Sciences, Chosun University

Acinetobacter baumannii is an opportunistic pathogen that causes biofilm-associated infections, such as ventilatorassociated pneumonia and catheter-associated infections. Formation of biofilms is one of the major drug resistance mechanisms of *A. baumannii*. The drug resistance rate of *A. baumannii* increases year on year, and the available drugs for the treatment of infection are extremely limited. Antimicrobial peptides (AMPs) are candidates for new antimicrobial agents because of their natural antimicrobial properties and a low propensity for development of resistance by microorganisms. An amphipathic  $\alpha$ -helical peptide, CM205, was isolated from the venomous gland of the scorpion. In this study, we designed analog peptides by substituting amino acids at the C-terminal residues of CM205 to reduce toxicity and improve antibacterial activity. The analog peptides, which had an amphipathic  $\alpha$ helical structure, were active against Gram-positive and Gram-negative bacteria, particularly multidrug-resistant *A. baumannii*, and showed lower cytotoxicity than CM205. *N*-phenyl-1-naphthylamine uptake and DisC<sub>3</sub>-5 assays demonstrated that the peptides kill bacteria by effectively permeating the outer and cytoplasmic membranes. Additionally, the analog peptides inhibited biofilm formation largely than CM205 at low concentrations. These results suggest that the analog peptides of CM205 can be used as suitable therapeutic agents against *A. baumannii* infection.

### The ThiL Enzyme is a Valid Antibacterial Target Essential for Both Thiamine Biosynthesis and Salvage Pathways in *Pseudomonas aeruginosa*

Hyungjun Kim, Hyunjung Lee, Yunmi Lee, Inhee Choi, and Soojin Jang<sup>\*</sup> Institut Pasteur Korea

Thiamine pyrophosphate (TPP) is an essential cofactor for various pivotal cellular processes in all living organisms, including bacteria. Thiamine biosynthesis occurs in bacteria but not in humans, so the enzymes in this pathway are attractive targets for antibiotic development. Among these enzymes, thiamine monophosphate kinase (ThiL) catalyzes the final step of this pathway, phosphorylating thiamine monophosphate (TMP) to produce TPP. Here, we extensively investigated ThiL in *Pseudomonas aeruginosa*, a major pathogen responsible for hospital-acquired infections. We demonstrate that thiL deletion abolishes not only thiamine biosynthesis, but also thiamine salvage capability, and results in growth defects of the  $\Delta$ thiL strain even in the presence of thiamine derivatives, except for TPP. Most importantly, the pathogenesis of the  $\Delta$ thiL strain was markedly attenuated compared with wild-type cells, with lower inflammatory cytokine induction and decreased bacterial load in an *in vivo* infection model where the intracellular TPP level was in the submicromolar range. To validate *P. aeruginosa* ThiL (PaThiL) as a drug target, we further characterized its biochemical properties. These comprehensive biological and biochemical results indicate that PaThiL represents a potential drug target for the development of an augmented repertoire of antibiotics against *P. aeruginosa*.

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#### D066

### Antimicrobial Resistance Pattern and Species Diversity of *Enterococcus* sp. Isolated from Chronic Otitis Externa in Dogs

Jun Kwon<sup>1</sup>, Hyung Jun Ko<sup>2</sup>, and Se Chang Park<sup>3\*</sup>

<sup>1</sup>Department of Veterinary Anatomy, College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, <sup>2</sup>Biosafety Research Institute, College of Veterinary Medicine, Jeonbuk National University, <sup>3</sup>Laboratory of Aquatic Biomedicine, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University

Otitis externa is a common disease in dogs and can be induced by a variety of causes. When the primary cause changes the environmental composition of the ear, various secondary infections such as bacteria, fungi, and yeast occur. Among them, *Enterococcus* is a multi-resistance bacterium that frequently causes opportunistic infections in the dog ear.

We used MALDI biotyper and biochemical test for species identification, and evaluated antibiotic resistance patterns. antimicrobial susceptibility test was conducted according to the Clinical and Laboratory Standards Institute Guideline.

Among 89 *Enterococcus, E. faecium* was the most common, followed by *E. faecalis* (29.2%), *E. hirae* (14.6%), *E. casseliflamus* (7.9%), *E. avium* (6.7%), *E. gallinanum* (5.6%) and *E. canintestini* (2.2%). Of these, 26 E. *faecalis* and 23 *E. faecium* were tested for antibiotic resistance patterns. *E. faecalis* isolates were mostly resistant to linezolid (38.46%) followed by doxycycline (26.9%), chloramphenicol (23.1%), erythromycin (19.2%), ciprofloxacin (15.3%), penicillin (11.5%), ampicillin (3.8%). In case of *E. faecium*, isolates were found to be highly resistant to penicillin (91.30%), ampicillin (86.9%) and ciprofloxacin (86.9%), erythromycin (60.8%), doxycycline (52.2%), linezolid (34.7%), vancomycin (13.1%), chloramphenicol (0%). When it was defined as MDR when resistant to three or more antibiotic classes, 19% of *E. faecalis* and 83% of *E. faecium* were MDR strains.

#### Development of Immunological Assay & Human Standard Serum for HFMD Vaccine

Gunho Won, Hyun Ju In, Heeji Lim, Eunbee Choi, Minsu Yim, Yookyoung Lee, and Byung Chul Kim<sup>\*</sup> Division of Vaccine Development Coordination, Center for Vaccine Research, National Institute of Infectious Diseases, National Institute of Health, Korea Disease Control and Prevention Agency

Hand, Foot and Mouth Disease (HFMD) is a syndrome characterized by fever and characteristic skin rashes and blisters on the hands, feet, and mouth under 5 years old. In severe cases, meningitis, encephalitis, and polio paralysis may occur due to infection of the nervous system. HFMD is caused by Enterovirus71 (EV71) and Coxsackievirus A16 (CVA16), and there is a little development of vaccines. For this reason, the National Institutes of Health of Korea Disease Control and Prevention Agency intends to standardize the standard serum production and immunogenicity evaluation test methods required for the development of a vaccine for preventing HFMD. For the production of standard serum, 250 clinical samples were supplied through Seoul National University Hospital, and a neutralizing antibody assay was performed through the Neutralizing test (NT). For EV71, an international standard material (WHO, 14/140) was used, but for CVA16, a mouse positive serum was prepared and used for the experiment. After repeating the experiment three times, the final titer was calculated using the average value (EV71; 1 X 107 and CVA; 4.4 X 104). For additional experiments, total antibody analysis will be performed through the enzyme-linked immunosorbent assay (ELISA) test, and a standard operating procedure (SOP) will be developed and established based on these.

#### D068

Characterization of Carbapenemase-producing Colistin-resistant *Enterobacter cloacae* Co-harboring NDM-1 and *mcr-9* Isolated from Hospital Sewage

Jaeyoung Oh, Jaehong Jeong, and Jong-Chan Chae<sup>\*</sup> Division of Biotechnology, Jeonbuk National University

Carbapenem-resistant *Enterobacter cloacae* have been commonly reported in Korea and many other countries. However, colistin resistance in *E. cloacae* has not been reported widely. We isolated carbapenem-resistant *Enterobacteriaceae* (CRE) from the sewage of a tertiary hospital and carbapenem-resistant *Klebsiella pneumoniae*, *Enterobacter* spp., and *Citrobacter* spp. were mainly detected. They were highly resistant to beta-lactams and cephems. Among the isolates, the carbapenemase gene KPC-2 was the most common, followed by GES-5, NDM-5, NDM-1, and VIM-2. The *mcr-9* belonging to the colistin resistance gene was found only in *E. cloacae* strains (colistin MIC, 128 µg/ml) harboring the NDM-1. As a result of the whole genome sequencing analysis of *E. cloacae*, both NDM-1 and *mcr-9* found in the chromosome, and the *mcr-9.1* gene, incomplete sequence, was arranged in the forward and reverse directions on the large-size plasmid (about 300 kb). The IS1 family transposase IS1R was located upstream and downstream of these genes. Additionally, the *mcr-9* was detected in other *E. cloacae* isolates harboring NDM-1, while no colistin resistance gene was found in other CRE isolates. [Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2022R1A2C1011800)]

#### The Emergence of Carbapenem-resistant Escherichia coli Carrying bla<sub>KPC-2</sub> in Companion Dogs

Jaeyoung Oh<sup>1</sup>, Jaehong Jeong<sup>1</sup>, Kwang Won Seo<sup>2</sup>, and Jong-Chan Chae<sup>1\*</sup> <sup>1</sup>Division of Biotechnology, Jeonbuk National University, <sup>2</sup>College of Veterinary Medicine, Chungbuk National University

Pathogenic *Escherichia coli* is causative of intestinal infectious diseases. In this study, *E. coli* were isolated from companion dogs to investigate the clonal relationship between human and companion animal sources during the period from April to August 2022. Forty-four *E. coli* were isolated from rectal swabs of 58 companion dogs. Under the phenotypic characterization of them, carbapenem-resistant *Escherichia coli* (CREC) was observed. Resistance to ampicillin was the highest at 65.9% (n=29), followed by cefotaxime 25.0% (n=11) and meropenem 6.8% (n=3). Resistant gene patterns for CREC strains were *bla*<sub>TEM-1</sub>/*bla*<sub>CMY-2</sub>/*bla*<sub>NDM-5</sub>, *bla*<sub>TEM-1</sub>/*bla*<sub>CMY-166</sub>/*bla*<sub>NDM-5</sub>, and *bla*<sub>TEM-1</sub>/*bla*<sub>CHY-11</sub>/*bla*<sub>CTX-M-27</sub>/*bla*<sub>KPC-2</sub>, respectively. In the phylogenetic association analysis, only three of them isolated from companion animals had low genetic associations. In recent years, the appearance of carbapenem-resistant *E. coli* carrying *bla*<sub>NDM-5</sub> in companion animals has been reported intermittently through surveillance studies. However, the appearance of resistant bacteria carrying *bla*<sub>KPC-2</sub> was first confirmed in this study to our knowledge. This suggests that the spread of *Enterobacteriaceae* harboring the carbapenemase gene in companion animals can threaten humans' public health since many companion animals share the residential environment with humans. [Supported by grants from KDCA (Project no. 2022-ER2103-00)]

#### D070

### Genome Analysis of *Enterococcus faecalis* Carrying Oxazolidinone/Phenicol Resistance Genes Isolated from Pig Farms

Jaeyoung Oh, Jaehong Jeong, and Jong-Chan Chae<sup>\*</sup> Division of Biotechnology, Jeonbuk National University

The appearance of linezolid/florfenicol-resistant enterococci (LREFS) carrying *optrA*, *poxtA*, *cfrD*, and *fexA* genes from domestic industrial animals has been increasing for the past 10 years. This study is to reveal the genetic structure and characteristics of oxazolidinone/phenicol resistance (OPR) through whole genome sequencing of LREFS isolated from pigs. Through a longitudinal study over the past two years, linezolid-resistant *Enterococcus faecalis* ST100 and ST476 clones were consistently isolated from several domestic pig farms, and whole genome sequencing analysis was performed with each of these linezolid-resistant bacteria. *E. faecalis* ST100 contained 1 chromosome and 6 plasmids and *E. faecalis* ST476 possessed 1 chromosome and 4 plasmids. All of them had *optrA* and *fexA* in their chromosomal DNA similar to the Tn554 gene sequence. In *E. faecalis* ST100 and ST476, OPR genes, *fexA*, *poxtA*, and *cfrD*, were identified in 21.8 kb and 26.2 kb plasmids, respectively. In addition, these two oxazolidinone-resistant plasmids were identified in the transconjugants. In conclusion, transmittable plasmids carrying OPR genes were identified, and their resistance transfer mechanism appears to be related to various IS family DNA transposases such as IS3 or IS6.

[Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2022R1A2C1011800)]

### Pullulan Nanoparticles Inhibit the Pathogenicity of *Candida albicans* by Regulating Hypha-related Gene Expression

Sujin Hong<sup>1</sup>, Seo-Kyung Kim<sup>2</sup>, Chong-Su Cho<sup>3</sup>, and Won-Ki Huh<sup>1,4\*</sup>

<sup>1</sup>School of Biological Sciences, Seoul National University, <sup>2</sup>Department of Agricultural Biotechnology, Seoul National University, <sup>3</sup>Research Institute of Agriculture and Life Sciences, Seoul National University, <sup>4</sup>Institute of Microbiology, Seoul National University

*Candida albicans* is a common opportunistic pathogenic fungus that resides on the skin and gastrointestinal tract of humans. Under certain conditions, *C. albicans* cells undergo a transition from commensal to pathogenic, leading to superficial and invasive infections. Although systemic candidiasis is a life-threatening disease, only a few antifungal drugs are used as treatment for the disease. In addition, due to the occurrence of resistant strains to antifungal agents, the need for a new treatment is emerging. One promising strategy is therapy using nanomaterials. In this study, we synthesized phthalic pullulan nanoparticles (PPNPs) and examined their ability to inhibit the pathogenicity of *C. albicans*. We observed that PPNPs are internalized into *C. albicans* cells through endocytosis. Internalized PPNPs inhibit *C. albicans* adhesion to abiotic surface and biofilm formation in a dose-dependent manner. This inhibitory effect is mediated by transcriptional modulation, particularly down-regulation of hypha-related genes and up-regulation of stress-responsive genes. Furthermore, we observed that PPNPs inhibit the adhesion of *C. albicans* to human epithelial cells without toxicity to human cells. Taken together, our findings suggest that PPNPs have an inhibitory effect on *C. albicans* pathogenicity and thus have potential as a novel therapeutic agent for candidiasis. [Supported by grants from National Research Foundation of Korea and BK 21 Plus Program from South Korea]

#### D072

## Protective Effects of Flavonoids from Intestinal Inflammation and Disruption of Gut Microbiota by Dextran Sodium Sulfate (DSS) Reveals Its Anti-inflammation Activities for Functional Food Applications

Soo Jeong Lee, Ho-Jeong Choi, and Ju-Hoon Lee\*

Department of Agricultural Biotechnology, Department of Food and Animal Biotechnology, Center for Food and Bioconvergence, Research Institute of Agriculture and Life Science, Seoul National University

Flavonoids are known to be beneficial in several inflammation by reducing several inflammatory mediators through key inhibition of signaling pathways. However, it is unclear whether the gut microbiota exerts an affect when flavonoids attenuates inflammatory bowel disease (IBD). In this study, morin and green tea extract (GT) were evaluated in 5-week *in vivo* experiments using C57BL/6N mice (5-week-old, male) DSS-induced IBD mouse model. Flavonoids were treated for three weeks after two weeks of DSS treatment. Compared to DSS group, morin and GT groups showed the recovery of body weight, suggesting an anti-inflammatory effect. Both of flavonoids down-regulated pro-inflammatory cytokines and inflammation-associated enzymes and up-regulated anti-inflammatory cytokines compared to DSS group. In addition, colon barrier experiment revealed that biomarkers of mucus layer and tight junction were up-regulated. Furthermore, comparative metagenome analysis of mouse fecal samples revealed the decrease of *Prevotella* and increase of *Muribaculum* and Bacteroidales, supporting their protective effects. Taken together, the morin and GT administration showed anti-inflammatory effects by reducing the risk of colitis-associated colon disease via regulation of inflammation, gut barrier, and compositional change of gut microbiota. Consequently, flavonoids, especially morin and GT could be a novel potential health-protective natural agent against IBD.

#### Identification of Novel Inhibitors of Papain-like Protease of Coronaviruses

Sunghyun Oh, Subin Hong, YuJin Shin, JuOae Chang, Inseo Kim, Jihyeon Kim, ChaeYoung Lee, and Wonsik Lee<sup>\*</sup> School of Pharmacy, Sungkyunkwan University

COVID-19 is a respiratory infectious disease caused by SARS-CoV-2, and a large number of confirmed cases have been reported since 2019 causing a global pandemic. In SARS-CoV-2, the papain-like protease is essential to cleavage the polyproteins to generate mature and functional viral proteins. The PLpro also is known to inhibit the host innate immunity by modulating cellular ubiquitination. Therefore, the PLpro has been applicated to be an important target for antiviral therapeutics against SARS-CoV-2.

In this study, we established a high-throughput screen using PLpro and a small 5-mer fluorogenic peptide conjugated with -7-amino-4-methylcoumarin (AMC), and we screened over 6900 small molecules. The potency of inhibitors of PLpro was examined by measuring the fluorescence of cleaved AMC, and the IC50 of selected hit compounds was further calculated. Among hit compounds, potent inhibitors were further validated using molecular docking tests using a crystal structure (PDB ID: 6W9C) of PLpro in the Schrodinger platform. Our results show that our screen platform and hit compounds could be used to develop novel therapeutics to treat COVID-19. [Supported by grants from KDCA (2021-ER1602-01)]

#### D074

**Induction of Antibiotic Resistance Mutation Following Treatment of** *Mycobacterium intracellulare* Du-Gyeong Han, Ji-A Jeong, Sung-Kyoung Lee, Seong-Han Kim, and Se-Mi Jeon<sup>\*</sup> Department of Bacterial Disease Research, Korea National Institute of Health

Non-tuberculous mycobacteria (NTM) are one of the causative bacteria that cause pulmonary disease and are mainly distributed in the environment. Recently, it has been reported that when quinolone antibiotics were treated with pathogens such as *Escherichia coli* or *Salmonella* spp., resistance to antibiotics other than quinolone antibiotics occurred under the influence of reactive oxygen species (ROS). The purpose of this study was to investigate the effect of quinolone antibiotic treatment on resistance to other antibiotics in NTM. The MICs of the tested antibiotics against *M. intracellulare* ATCC 13950. Next, we performed the kill-curve assay, and harvested the bacteria at a specific time point and used it for CFU-, ROS-quantitative assay, and WGS. A significant association between the three factors such as results of time-point, CFU-, and ROS-quantitative assay was not confirmed by sequencing analysis. At 15 days after treatment, amino acid mutations in *gyrA* gene at condon D95G and increased ROS expression levels were confirmed in some experimental groups, but this was confirmed to be insignificant compared to the control group. Further studies are needed to confirm the results of using combination regimen drugs for treatment *M. intracellulare* infection.

### Development of SARS-CoV-2 Pseudovirus-based Compound Screening and Identification of Antivirals against Coronavirus

YuJin Shin, JuOae Chang, SungHyun Oh, Subin Hong, Sun Ju Kim, RhoEun Lee, Ji-Hoon Kim, and Wonsik Lee<sup>\*</sup> Sungkyunkwan University School of Pharmacy

An unprecedented global pandemic caused by SARS-CoV-2 has posed a serious threat to public health and world economy. Recently, several drugs have been documented for clinical use to treat COVID-19, however, administration of these drugs has been limited due to the toxicity in patients with other disease. In addition, in light of emergence of various human coronavirus in the past several decades, another novel human coronavirus could be spilled through retransmissions from various zoonotic reservoirs. Therefore, there is an urgent need to develop broad-spectrum antivirals against coronavirus.

In this study, we have developed a screening system based on pseudovirus that has spike-protein of SAR-CoV-2, and we screened on compound libraries including FDA-approved library. Pseudoviruses were generated by transfecting lentiviral plasmids, pLP1-HIV-1 gag/pol and pLP2-HIV-1 rev, and SAR-CoV-2 S-protein plasmid into 293T cells. For the screen, we used ACE2 overexpressed HEK293T cells. The screening system was optimized, and confirmed by remdersivir. We identified potent compounds and validated hit compound by measuring IC<sub>50</sub> and cytotoxicity. Among hit compounds, Clioquinol and berbamine were found to be highly potent antiviral activity while the cytotoxicity was not significant. Our pseudovirus-based screen is safe and suitable to perform in non-BSL-3 lab, therefore, this system can be applicable in identifying antivirals rapidly.

[Supported by grants from KDCA (2021-ER1602-01)]

#### D076

**Utilization of Luminescence Protein Expressing Strains an Assessment of Anti-tuberculosis Therapeutic Efficacy** SooMin Kim, jinseung Yun, Jaeseon Jeon, Sohee Park, Dokeun Kim, Hye-sook Jeong, and Eunkyung Shin<sup>\*</sup> Division of Infectious Disease Vaccine Research, Center for Vaccine Research, Korea National Institute of Health, Korea Disease Control and Prevention Agency

*Mycobacterium tuberculosis* (*M. tb*), the causative organism for tuberculosis, still represents a threat to worldwide public health problems. It is necessary to solve the issues of TB by developing effective drugs and vaccine, but one enormous obstacle is the prolonged growth of TB. Utilization of reporter gene, luminescence was an alternative method to overcome.

In this study, we constructed *M. tb* strains presenting luminescence designed using two types of promoters (Hsp and L5) and then the growth of recombinants compared to wild type (WT) strains to confirm the stability *in vitro*. The infectivity of the strains was analyzed using *In Vivo* Imaging System by detecting luminescence in living mice *in vivo* and lungs *ex vivo*.

We confirmed the stability of the recombinant strains by reporter signal and duration of growth compared to WT. *Ex vivo* luminescence in lungs correlated to numbers of bacteria in tissue, and the effect of treatment of mice with the antibiotic could be visualized through luminescence measurements and was confirmed by CFU assays. In addition, *in vivo* luminescence was measured similarly to the *ex vivo* results.

Furthermore, it is supposed that using this method instead of or in co-occurrence with the standard tuberculosis CFU assay reduces the time and cost of evaluating drug and vaccine development in animal models, which would help in the prevention and treatment of tuberculosis.

[Supported by Korea Disease Control and Prevention Agency (grant: 2021-NI-016-01)]

### Development of Live Attenuated Mumps Vaccine Candidate Using Predominant F Genotype

Seo Yeon Kim, Hyeran Won, You-Jin Kim, Dokeun Kim, and Tae-Young Lee<sup>\*</sup> Division of Infectious Disease Vaccine Research, National Institute of Health

Mumps, caused by mumps virus, is still prevalent to the children and teenagers in many countries despite high vaccination rates. Among the 12 genotypes of mumps virus, F, H and I genotypes are the most detected in East Asia and G genotype in the United States and Europe. However, the conventional mumps vaccine strain, Jeryl Lynn is based on A genotype which is no longer predominant. To overcome the genotypic difference between the vaccine strains and the predominant strains, the F genotype was selected for a new vaccine candidate.

In this study, we developed a new attenuated mumps virus through multiple passages of genotype F in Vero cells and investigated its characteristics and immunogenicity. We performed whole genome sequence analysis of wild type and attenuated F virus(F30). The F30 showed a lower titer in growth pattern and a smaller plaque size than the wild type virus. In the humoral immune response, the F30 induced a significantly higher neutralizing antibody titer than the Jeryl Lynn against F, H, I, G genotypes except for A genotype. Also, F30 showed higher cellular immunity than the Jeryl Lynn.

It was confirmed that the new attenuated vaccine F30 had higher immunogenicity than the conventional vaccine. It is considered to be an alternative to the problems of breakthrough infection after Jeryl Lynn vaccination by enabling cross-protection against various genotypes.

[Supported by Korea Disease Control and Prevention Agency (grant : 2021-NI-004-01)]

### D078

**Characterization of Korean** *Helicobacter pylori* Isolates Carrying EPIYA-C CagA Instead of East Asian EPIYA-D CagA Sacheera Angulmaduwa, Kavinda Tissera, Myeong-A Kim, and Jeong Heon Cha<sup>\*</sup> Department of Oral Biology, Oral Science Research Center, Department of Applied Life Science, the Graduate School, BK21 FOUR Project, Yonsei University College of Dentistry

*Helicobacter pylori* is a Gram-negative microaerophilic bacterium that colonizes the stomach of about 50% of the world's population causing various gastric diseases. It has been reported that the severity of the disease is linked to the presence of the CagA toxin. The CagA shows geography-dependent variation in Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs at the C-terminus; East Asian *H. pylori* isolated in Korea, Japan, and China commonly has an EPIYA-ABD while the rest of the world carries EPIYA-ABC. We reported that about 10% (24/260) of Korean *H. pylori* isolates were found to carry the EPIYA-ABC.

To characterize Korean *Helicobacter pylori* isolates carrying EPIYA-C CagA instead of East Asian EPIYA-D CagA, four of these EPIYA-C CagA isolates were selected to study further by whole genome sequencing and phylogenetic analysis. Interestingly, three belonged to the hspEastAsia while one of these belonged to the hspWAfrica. Out of 3 hspEastAsia isolates, the strain K154 had ABCCCC, multi copies of EPIYA-C, which was studied further to understand the change of the multi-copy EPIYA-C motif genotype. The 287 single colonies from the first-generation subculture were examined and found that 1% of single colonies (3/287) had variations in the EPIYA-C motif: ABCCCCC, ABCCC, and AB. This suggests that K154 changes dynamically the EPIYA-C repeats of CagA generating intra-species diversity in *H. pylori*.

# Immune Responses and Single Cell Transcriptional Analysis of Heterologous COVID-19 Vaccination with mRNA and Adenoviral Vector Vaccines

Jinah Yeo<sup>1</sup>, Ji Hyang Jeon<sup>1</sup>, Seongryong Kim<sup>2</sup>, Yun-Ho Hwang<sup>1</sup>, Si Hyun Kim<sup>1</sup>, Jong-Eun Park<sup>2</sup>, You-Jin Kim<sup>1</sup>, and DoKeun Kim<sup>1\*</sup>

<sup>1</sup>Division of Infectious Disease Vaccine Research, National Institute of Health, <sup>2</sup>Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST)

Heterologous COVID-19 vaccination has been recommended for several potential benefits, including reduced adverse events, enhanced immunogenic effect, and programmatic flexibility. Various clinical trials and observation studies have been showed that heterologous vaccination provided a potent immune response. Here, we investigated the innate and adaptive immune responses to heterologous prime-boost COVID-19 vaccination with mRNA and adenoviral vector vaccine (AdCLD-CoV19-1) in mice. The heterologous vaccination resulted in significantly higher neutralizing antibodies levels and antigen-specific T-cell responses. To further understand the molecular mechanisms involved in the immunogenic effects, we performed single cell RNA sequencing in the mouse draining lymph node and vaccine-injected tissue 1 day after intramuscular injection. Single cell analysis revealed that heterologous vaccine schedule triggered strikingly enhanced innate responses in tissues after secondary immunization compared with the homologous strategy. Notably, we found that spike mRNA expression by multiple cell types was associated significantly with interferon- and innate immune-related gene expression, suggesting a robust activation of innate immune signaling by the heterologous booster vaccination. In summary, heterologous vaccine strategy induces stronger innate and adaptive immune responses than homologous vaccination.

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### D080

# RAF as a Key Mediator of Helicobacter pylori-induced Cell Elongation

Ashansa Ramanayake, Jinmoon Kim, Yunhui Choi, and Jeong-Heon Cha<sup>\*</sup> Department of Oral Biology, Oral Science Research Center, Department of Applied Life Science, the Graduate School, BK21 FOUR Project, Yonsei University College of Dentistry

*Helicobacter pylori* is a bacterium which possesses various virulence factors to induce morphological changes in gastric epithelial cells. These changes are induced by the activation of multiple signaling pathways. The ERK pathway containing RAS, RAF, MEK, and ERK, is widely studied for the *H. pylori* -mediated cell elongation. RAF has three isoforms called A-RAF, B-RAF, and C-RAF which act as homo or heterodimers to activate the substrate, Mek. The purpose of this study is to explore specific roles of each RAF isoform in *H. pylori*-mediated cell elongation. AGS cells were transfected with si-RNA to knock down A-RAF, B-RAF or C-RAF and the cell elongation phenotype was examined upon G27 infection. AGS cells treated with A-RAF or B-RAF si-RNA showed a significant reduction of cell elongation whereas si-C-RAF treated cells showed a wild-type level of cell elongation. To confirm the loss-of-function study, the gain-of-function study was performed. AGS cells treated with A, B, or C-RAF si-RNA were transfected with constructs to rescue expression of A, B, or C-RAF isoform, respectively. The cells rescued A-RAF or B-RAF expression, restored the cell elongation. However, the rescue of C-RAF expression showed a reduction in cell elongation. Also, C-RAF overexpression showed a significant reduction in cell elongation. Also, C-RAF are key mediators in *Helicobacter pylori*-induced cell elongation and suggest a differential role of C-RAF in cell elongation.

# **Properties of** *Burkholderia* **sp. MKH5-5 Having Antibacterial Effect, Isolated from Agricultural Soil** Mingyeong Park and Jaisoo Kim<sup>\*</sup>

Kyonggi University

A yellow colour, non-sporulating, Gram-stain-negative, rod bacterium, designated strain MKH5-5 was obtained from soil sampled at the agricultural field, Korea. Cells were strictly aerobic grew optimally at 20–37°C. Strain MKH5-5 showed antimicrobial activity against Gram-positive pathogen (*Staphylococcus epidermidis*). A phylogenetic analysis based on its 16S rRNA gene sequence revealed that strain MKH5-5 formed a lineage within the family *Burkholderiaceae* and clustered as members of the genus *Burkholderia*. The closest members were *Burkholderia diffusa* R-15930<sup>T</sup> (99.8% sequence similarity), *Burkholderia ambifaria* AMMD<sup>T</sup> (99.7%) and *Burkholderia aenigmatica* LMG 13014<sup>T</sup> (99.5%). The genome was 7,314,820.4 bp long and 6,531 protein-coding genes. The DNA G + C content of the type strain was 67 mol%. This species have resistance to Streptomycin. Based on genomic, chemotaxonomic, phenotypic and phylogenetic analyses, strain MKH5-5 represents antibacterial species in the genus *Burkholderia*.

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### D082

# Recruitment of Cytomegalovirus Factors to Replication Origin through G-Quadruplex

Daegyu Park<sup>1</sup>, Woo-Chang Chung<sup>1</sup>, Shuang Gong<sup>1</sup>, Subramaniyam Ravichandran<sup>2</sup>, Gwang Myeong Lee<sup>1</sup>, Minji Han<sup>1</sup>, Kyeong Kyu Kim<sup>2,3</sup>, and Jin-Hyun Ahn<sup>1,3\*</sup>

<sup>1</sup>Department of Microbiology, Sungkyunkwan University School of Medicine, <sup>2</sup>Department of Precision Medicine, Institute for Antimicrobial Resistance Research and Therapeutics, Sungkyunkwan University School of Medicine, <sup>3</sup>Biomedical Research Institute, Samsung Medical Center

G-quadruplex (G4) structures are often found at replication origins of eukaryotic cells, but how they regulate DNA replication is not fully understood. In this study, we evaluated the role of putative G4-forming sequences (G4 motifs) found in the replication origin (oriLyt) of human cytomegalovirus (HCMV) in DNA replication. G4 formation in the HCMV oriLyt G4 motifs, denoted as oriG4-1 to -7, was observed by circular dichroism analysis and native gel electrophoresis. Analysis of recombinant viruses with mutations in G4 motifs revealed that the G4 formation at oriG4-1 was essential for viral growth. This G4 was also required for replication of oriLyt-containing plasmid in transient-transfection replication assays, indicating its essential role for oriLyt function. G4s were enriched at the viral DNA replication compartments in cell staining with G4-specific antibodies. G4 chromatin immunoprecipitation in virus-infected cells also showed G4 formation in oriLyt including oriG4-1 site. Pull-down assays with G4-structured oligonucleotides revealed that viral DNA replication factors such as IE2, UL84, and UL44 effectively bound to the G4 at oriG4-1 and that the binding by IE2 and UL84 could be interfered with treatment of specific G4-binding ligands. Our results demonstrate that G4 formation in the HCMV oriLyt plays an essential role for initiation of viral DNA replication by promoting recruitment of viral replication factors.

# Drug Repurposing Screening of FDA-approved Drugs as Antimicrobial Potentiators against Carbapenem-resistant Acinetobacter baumannii

Seolhui Kim, Jung Sik Yoo, and Seok Hyeon Na<sup>\*</sup> Division of Antimicrobial Resistance Research, Centers for Infectious Diseases Research, National Institute of Health

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is one of the most urgently needed multidrug-resistant bacteria to develop new therapeutics. Drug repurposing is a process of identifying new uses for approved drugs, and could save the time and cost to find a therapeutic agent. The aim of this study is to discovery the candidates which potentiate cabapenem agents against CRAB from FDA-approved chemical library. A total of 1,424 FDA-approved drugs were screened at 50  $\mu$ M for synergistic effects with 4  $\mu$ g/ml of imipenem to CRAB, followed by MIC test or checkerboard assay for antimicrobial or potentiative chemicals, respectively. Of the 1,424 compounds, six showed the antimicrobial activity against clinical isolate CRAB57 (16  $\mu$ g/ml of MIC) at 50  $\mu$ M (>98% of growth inhibition), and their MICs were in 12.5–50  $\mu$ M (4.0–28.2  $\mu$ g/ml). Another four compounds showed >80% of growth inhibition of CRAB57 in the screening step. Their fractional inhibitory concentration index with imipenem was not determined, because they had no antimicrobial activity up to 50  $\mu$ M solely. Although further studies are needed to identify their mechanism, these four compounds were considered to reduce the carbapenem resistance of CRAB. In conclusion, we demonstrated that 10 FDA-approved drugs exhibited potent antimicrobial activity against CRAB or interfered their carbapenem resistance.

[This study was supported by a grant of the Korea National Institute of Health (2022-NI-016-00).]

### D084

# **Characterization of Lytic Bacteriophages Infecting Multi-drug Resistant** *Escherichia coli* Seolhui Kim<sup>1</sup>, Ryeonhoe Seo<sup>2</sup>, Jung Sik Yoo<sup>1</sup>, and Seok Hyeon Na<sup>1\*</sup> <sup>1</sup>Division of Antimicrobial Resistance Research, Centers for Infectious Diseases Research, National Institute of Health, <sup>2</sup>Optipharm Inc.

Bacteriophage (BP), which infect and kill the bacteria without negative effect on animal cells, have been considered to promising therapeutics to combat multi-drug resistant (MDR) bacterial infections. Sequence type (ST) 131 *Escherichia coli* is a worldwide distributed clone including Korea, and its resistance to  $\beta$ -lactam antibiotics is a great concern in public health. This study aimed to characterize lytic BPs infecting MDR *E. coli* belonging to predominant STs in Korea. A total of 15 lytic *E. coli*-specific BPs were isolated from hospital wastewater in 4 different provinces. Spot test and efficiency of plating analysis were performed to determine the host specificity. Tested 10 MDR *E. coli* produced extended spectrum  $\beta$ -lactamases (ESBLs), and belonged to predominant STs, such as ST131 (n = 4), 69 (n = 2), 38, 95, 1193, and 224. All of 15 BPs were capable of infecting more than one of MDR strain, and 13 BPs infected two ESBL-producing *E. coli* ST131 strains. Notably, BP-P5 infected 7 strains belonging to 4 different STs, including carbapenem-resistant ST131. These BPs are needed to overcome MDR *E. coli* infections, our results demonstrated that lytic BPs from hospital wastewater killed the important MDR *E. coli* strains including carbapenem-resistant ST131.

[This study was supported by a grant of the Korea National Institute of Health (2022-NI-017-00).]

# Primary Transcriptome Analysis on Intra-macrophage *Salmonella* Typhimurium Defined a New Role of LeuO in Virulence Regulation

Eunsuk Kim<sup>1</sup> and Hyunjin Yoon<sup>1,2\*</sup>

<sup>1</sup>Department of Molecular Science Technology, Ajou University, <sup>2</sup>Department of Applied Chemistry and Biological Engineering, Ajou University

Salmonella has become one of the most studied bacterial pathogens due to its exclusive nature of manipulating the host, eventually leading to systematic infections. Detailed strategies of Salmonella to survive inside macrophages have not been fully understood. In order to investigate the comprehensive transcriptional regulatory circuits during intra-macrophage survival, intracellular Salmonella cells were isolated from murine RAW 264.7 macrophage cells and subjected to RNA-Seq. DESeq2-mediated analysis revealed 1,013 differentially expressed genes (DEGs) with a threshold of at least 3-fold change, which consisted of 596 up-regulated and 417 down-regulated genes. Among the DEGs, genes enrolled in transcriptional regulation were further investigated in consideration of their potential to orchestrate the transcription of multiple genes associated with the intracellular survival. Six Salmonella mutant strains lacking (putative) regulatory genes, were attenuated in survival inside macrophages and only  $\Delta leuO$  strain was complemented by the introduction of leuO in trans. Overexpression of LeuO repressed the transcription of Salmonella intracellular survival. ChIP-Seq analysis predicted the presumable LeuO-binding motif in SPI-2 loci as well as in well-known cognate targets. These results give insight into the new role of LeuO in Salmonella virulence regulation inside host cells.

### D086

A CRISPR-Cas12a Based Diagnostic Platform for Carbapenemase-producing Enterobacteriaceae (CPE) Detection Ji Woo Yang, Heesu Kim, Jung Sik Yoo, and Sangrim Kang<sup>\*</sup>

Division of Antimicrobial Resistance Research, Centers for Infectious Diseases Research, National Institute of Health

The emergence and spread of carbapenemase producing *Enterobacteriaceae* (CPE), which are resistant to the carbapenem class of antibiotics, has become a significant global public health problem. For the control and management of CPE spread, development of a simple, rapid and more sensitive diagnostic methods is urgently needed. Recently, the CRISPR (<u>C</u>lustered <u>Regularly Interspaced Short Palindromic Repeats</u>)-Cas system has been reported as the powerful diagnostic tool for molecular detection of infectious diseases due to the high specific, sensitive, simple and rapid characteristics of it. We aimed to develop a useful diagnostic platform based on CRISPR-Cas12a with single-stranded DNA-based fluorophore-quencher(ssDNA-FQ) for the CPE detection. Firstly, the two major genes (*bla*<sub>KPC</sub> and *bla*<sub>NDM</sub>) among the carbapenemase encoding genes were selected and aligned on the homologous sequences of each target gene to design the sgRNA. To test the cleavage activity, *in vitro* cleavage assay was carried out with two different types of the Cas12a family (LbCas12a and AsCas12a) and target sgRNA. We confirmed that the two types of sgRNA targeting KPC and NDM, respectively, exhibited different cleavage efficiencies, and also the Cas12a protein effectively detected the fluorescence signal by ssDNA probe. This result presented that CRISPR-Cas12a-based nucleic acid detection method might be a feasible platform to detect antibiotic-resistant bacteria including CPE.

# Identification of a Small Molecule Inhibitor that Attenuates Curli Fimbriae Expression in Enterohemorrhagic *Escherichia coli*

Ji Hye Park and Byoung Sik Kim<sup>\*</sup>

Department of Food Science and Biotechnology, ELTEC College of Engineering, Ewha Womans University

Adhesive factor responsible for surface attachment is important virulence determinant of diverse pathogens. Curli, the adhesive fimbriae of the foodborne pathogen Enterohemorrhagic *Escherichia coli* (EHEC), is essential for surface attachment and biofilm formation. The expression of biosynthetic genes for curli fimbriae (*csgBAC*) is directly activated by a transcriptional regulator CsgD. In the present study, we confirmed the role of CsgD in curli gene expression in EHEC EDL933 strain and developed a high-throughput screening system to identify small molecule inhibitors of CsgD. The *csgD* mutant showed the attenuated expression of *csg* operon and reduced biofilm formation. Screening of a total 8,201 small molecules identified three hits as putative CsgD inhibitors. Among, the molecule A4 exhibited significantly diminished expression of *csgB* gene and reduced attachment of EDL933 to spinach leaves. These results suggest that CsgD is indeed a crucial transcriptional regulator for curli production, and that the selected molecule A4 could be applied as an anti-adhesive agent for EHEC control.

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### D088

# Quantification of Viable Salmonella enterica by Viability-PCR

Minkyu Park<sup>1,2</sup>, Seil Kim<sup>1,3\*</sup>, and Seung Bum Kim<sup>2\*</sup>

<sup>1</sup>Microbiological Analysis Team, Korea Research Institute of Standards and Science, <sup>2</sup>Department of Microbiology and Molecular Biotechnology, Chungnam National University, <sup>3</sup>Department of Bio-Analysis Science, University of Science & Technology

*Salmonella enterica* is a Gram-negative bacterium with various serotypes. It's the most common pathogen causing foodborne illnesses worldwide. Determining the colony-forming unit (CFU) is the simplest and most widely used method to quantify viable cells. However, the method is time-consuming and labor-intensive. Quantitative polymerase chain reaction(qPCR) and droplet digital PCR (ddPCR) are very accurate and sensitive, but do not distinguish live and dead cells. Therefore, to overcome this limitation, an analytical method termed viabilityPCR (vPCR) was studied. The method uses propidium monoazide (PMA). This photoreactive dye inhibits amplification by covalent binding to the DNA of dead cells. CFU, qPCR and ddPCR were compared. Briefly, the concentration of broth cultured. *S. enterica* suspensions were normalized as an optical density at 600 nm of 1.0. These cultures were treated with PMA and genomic DNA was extracted. The qPCR and ddPCR analyses were performed using the extracted genomic DNA using the InvA primer and probe set, which is the most commonly used primer pairs for the detection of *S. enterica*. Various conditions for the PMA treatments were tested. The activity of PMA was further increased until the concentration of PMA reached 100 μM. Changes in the Ct value of qPCR and copy number of ddPCR were obvious. The addition of detergent enhanced the activity of PMA without effect to viable cell. The findings implicate v-PCR as an excellent method to quantify viable bacteria.

### Prevalence of Severe Fever with Thrombocytopenia Syndrome Virus in Ticks

Jun-Gu Kang<sup>1</sup> and Joon-Seok Chae<sup>2\*</sup>

<sup>1</sup>Korea Zoonosis Research Institute, Jeonbuk National University, <sup>2</sup>Laboratory of Veterinary Internal Medicine, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University

Severe fever with thrombocytopenia syndrome virus (SFTSV) is a zoonotic, tick-borne RNA virus of the genus *Bandavirus* (Family *Phenuiviridae*), mainly reported in China, Japan, and Korea. For the goal of this study, a total of 3,898 adult and nymphal ticks of species *Haemaphysalis longicornis* (94.2%), *Haemaphysalis flava* (5.0%), *Ixodes nipponensis* (0.8%), and one sample of *Ixodes ovatus*, were collected from the Deogyusan National Park, Republic of Korea between April 2016 and June 2018. A One-step reverse transcriptase–nested PCR was performed, targeting the S segment of the SFTSV RNA. Total infection rate (IR) of SFTSV in individual ticks was found to be 6.0%. Based on developmental stages, IR was 5.3% in adults and 6.0% in nymphs. The S segment sequences obtained from PCR were divided into 17 haplotypes. All haplotypes were phylogenetically clustered into genotypes B-2 and B-3, with 92.7% sequences in B-2 and 7.3% in B-3. These results indicate that the Korean SFTSV strains were closer to the Japanese than the Chinese strains. Further epidemiological studies are necessary to better understand the characteristics of the Korean SFTSV and its transmission cycle in the ecosystem.

### D090

# Development of DNA Vaccine Candidates for SARS-CoV-2 Based on the Spike Protein

Seeun Kim, Heeji Lim, Yun Ha Lee, So Hee Park, Gayeoung Kim, Woo Jung Park, Hyeon Guk Kim, You Jin Kim, Do Keun Kim, and Jung Ah Lee<sup>\*</sup> National Institute of Health

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified and isolated in 2019, severely has threatened human life by causing record-breaking numbers of COVID19 infections and deaths in the world. The development of an effective vaccine is the most important need to prevent SARS-CoV-2 pandemic. We developed and evaluated diverse DNA vaccine candidates that were composed of the full-length or truncated S protein. For immunogenicity test, mice were vaccinated three times 3 weeks interval and then we evaluated humoral and cell immune responses including antigen-specific antibodies and T-cell responses. All candidates induced not only elevated levels of total IgG and neutralizing antibodies (NAb), but also multiple cytokine expressions. Especially, the DNA vaccine containing full-length S protein elicited significantly stronger immune responses than other groups. To evaluation of the appropriate antigen dose, mice were vaccinated with 50, 20, and 5 µg of vaccine candidates. As the result, we confirmed that the 50ug of antigen vaccinated group induced highest NAb titer significantly compared to other groups. Also we established the challenge model using transgenic mice and then evaluated protectivity of DNA vaccine. The vaccine candidate including full-length S protein has protectivity against S clade virus. Therefore, data presented in this study demonstrated that the full-length S DNA vaccine can be potent vaccine candidate against SARS-CoV-2 infection.

### Changes in the Incidence of Food Poisoning after COVID-19 in Korea

So-Jung Park, Eun-Seon Hur, Min-Seong Kang, Il-Hyung Jeong, Beom-Ho Kim, Yong-Bae Park, and Sun-Mok Kwon\* *Gyeonggi Province Institute of Health and Environment* 

After the global epidemic of coronavirus disease 2019, public health measures to curb the spread of COVID-19 have also affected the outbreak of other infectious diseases. We aimed to investigate whether there was a change in the incidence of food poisoning in Korea after the spread of the COVID-19 pandemic. We collected the open data supported by the Ministry of Food and Drug Safety for 7 years from 2015 to 2021 to investigate changes in food poisoning between the pre- and post-COVID-19 era cases. When the incidences of food poisoning was compared between the pre-COVID-19 and 2020, 2021 in the COVID-19 periods, they were reduced 52.2% (p<0.001) in 2020 and 24.2% (p=0.007) in 2021. Restaurants where food poisoning usually occurs decreased dramatically by 48.5% (p<0.001) in 2020 and 45.2% (p<0.001) in 2021. In the cases of schools, the incidences of food poisoning in 2020 was decreased to 61.5% (p=0.035) and 14.2% in 2021, but its monthly occurrence rates in 2021 was not significantly decreased. In the cases of food poisoning in 2021 significantly increased to 129.7% (p=0.002) due to an increase in food poisoning caused by norovirus during this period. To curb the spread of COVID-19, the change of dining culture and social distancing also helped to reduce the incidence of food poisoning in Korea. This decrease was dramatically clearer in 2020 than in 2021.

# D092

# G-Quadruplexes Formed by Varicella-Zoster Virus Reiteration Sequences Suppress Expression of Glycoprotein C and Regulate Viral Cell-to-cell Spread

Woo-Chang Chung<sup>1</sup>, Subramaniyam Ravichandran<sup>2</sup>, Daegyu Park<sup>1</sup>, Gwang Myeong Lee<sup>1</sup>, Young-Eui Kim<sup>1</sup>, Youngju Choi<sup>1</sup>, Moon Jung Song<sup>3</sup>, Kyeong Kyu Kim<sup>2,4</sup>, and Jin-Hyun Ahn<sup>1,4\*</sup>

<sup>1</sup>Department of Microbiology, Sungkyunkwan University School of Medicine, <sup>2</sup>Department of Precision Medicine, Sungkyunkwan University School of Medicine, <sup>3</sup>Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, <sup>4</sup>Biomedical Research Institute, Samsung Medical Center

G-quadruplex (G4) formed by repetitive guanosine-rich sequences plays important roles in diverse cellular processes; however, its roles in viral infection are not fully understood. Here, we identified ~150 putative G4-forming sequences (G4 motifs) in the Varicella-Zoster virus (VZV) genome. These sequences were enriched in the internal repeat short and the terminal repeat short regions flanking the unique short region and also in some reiteration (R) sequence regions. A high density of G4 motifs in the R2 reiteration region was found on the template strand of ORF14, which encodes glycoprotein C (gC), a virulent factor for viral growth in skin. Biophysical analyses with oligonucleotides revealed that G4 motifs in ORF14 form stable G4s. In transfection assays, gC expression from the G4-disrupted ORF14 gene was increased at the transcriptional level and became more resistant to suppression by G4-ligand treatment. The recombinant virus containing the G4-disrupted ORF14 gene expressed a higher level of gC mRNA with an earlier expression pattern than the parental virus, while it showed a slightly reduced growth. This G4-disrupted ORF14 virus produced smaller plaques than the wild-type virus. Our results demonstrate that G4 formation via reiteration sequences suppresses gC expression during VZV infection and regulates viral cell-to-cell spread.

# **Ubiquilin 1 Targets Invading** *Salmonella typhimurium* **to the Autophagy Pathway** In-Sook Jeon, Seung-Ryul Kim, and Joong-Kook Choi<sup>\*</sup> *Division of Biochemistry, College of Medicine, Chungbuk National University*

Salmonella enterica serovar Typhimurium is a Gram-negative bacterium and is a major enteric pathogen that infects humans and animals causing typhoid fever. These bacteria form membrane—bound Salmonella-containing vacuoles (SCV) to survive and proliferate in the cytoplasm of host epithelial cells. The host cell uses the autophagy receptor protein to transport SCV to the autophagosome and fuse with the lysosome, where the lysosomal hydrolase degrades and kills the bacteria. Ubiquilin 1 reportedly binds to ubiquitin, targets it for proteasome activity, and contributes to autophagy. This study investigated whether Ubqln1 degrades Salmonella through autophagy process. During Salmonella infection, we found that the level of Ubiquilin 1 were elevated, and moreover, was found to be localized near the Salmonella bacteria under a confocal microscopy analysis. Furthermore, during starvation autophagy, Ubiquilin 1 co-localized with the autophagy marker LC3. Our results suggest that Ubiquilin1 may promote the autophagic degradation of Salmonella typhimurium infected cells. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R11A1A01046728 & 2021R11A3058091)]

### D094

Impact of Diesel Exhaust Particles on Toll-like Receptor Signaling and Host Defense against Bacterial Infection Madeeha Arooj, Abdur Rehman, and Young-Sang Koh<sup>\*</sup>

College of Medicine, and Jeju Research Center for Natural Medicine, Jeju National University

Background: Air pollution is an emerging cause of mortality, affecting nearly 5 million people each year. Exposure to diesel exhaust fine particulate matter (PM<sub>2.5</sub>) aggravates respiratory and skin conditions. However, its impact on the protective immunity of the skin remains poorly understood. This study aimed to investigate whether PM<sub>2.5</sub> impairs toll-like receptor (TLR) signaling and host defense against *Staphylococcus aureus* (*S. aureus*). Methods: Intracellular translocation of TLR9 and CpG-DNA internalization were assessed. Cytokine and nitric oxide production were measured. Skin disease severity and bacterial loads were assessed in cutaneous *S. aureus* infection mouse model.

Results: PM<sub>2.5</sub> interfered with TLR9 activation by inhibiting both TLR9 trafficking to early endosomes and CpG-DNA internalization via clathrin-mediated endocytosis. In addition, exposure to PM<sub>2.5</sub> inhibited various TLR-mediated nitric oxide and cytokine production. PM<sub>2.5</sub> rendered mice more susceptible to staphylococcal skin infections. Conclusion: Our results suggest that exposure to PM impairs TLR signaling and dampens the host defense against staphylococcal skin infections. Our data provide a novel perspective into the impact of PM on protective immunity which is paramount to revealing air pollutant-mediated toxicity on the host immunity.

[Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2022R1F1A1063400)]

# **Deciphering the Antimicrobial Activity of α-Mangostin against** *Staphylococcus pseudintermedius* Seong-Yong Park, Seo Yeon Ko, Nayeong Kim, and Je Chul Lee<sup>\*</sup> *Department of Microbiology, School of Medicine, Kyungpook National University*

Staphylococcus pseudintermedius is an opportunistic pathogen that are leading cause of skin, ear, and postoperative infections in dog and cats.  $\alpha$ -mangostin (AMG) shows an active antimicrobial activity in G (+) bacteria. The aim of the present study was to assess antibacterial effect of AMG against clinical isolates from canine or felis, and to investigate its antibacterial mechanism against *S. pseudintermedius*. AMG showed strong antibacterial activity against both ATCC strains and clinical isolates of four staphylococcal species *in vitro*. No cytotoxicity effect was not observed in CPEK cell line at 1-10 µg/ml of AMG. No resistance was induced when bacteria were subcultured in AMG for 10 passages. AMG induced morphological changes such as shrinkage, surface craters, and disruption of bacteria, suggesting the bactericidal activity. Transcriptome analysis demonstrated that AMG up- or down-regulated many genes associated with metabolic pathways and cell wall biosynthesis. Structure-activity relationship revealed that the 3, 6 hydroxyl group of AMG was essential for antibacterial activity. MAP domain containing protein was the binding partner of AMG in the bacterial surface. Our findings demonstrate that AMG binds to MAP domain containing protein and regulate peptidoglycan biosynthesis and metabolic pathways, which leads to the cell wall disruption and bacterial death. This study provides the comprehensive information of antibacterial effect of AMG on *S. pseudintermedius*.

### D096

# Two-component System PmrAB Mediates Colistin Resistance and Virulence of *Acinetobacter baumannii* under Acidic Conditions

Seo Yeon Ko, Hyo Jeong Kim, Seong-Yong Park, Nayeong Kim, and Je Chul Lee<sup>\*</sup> Department of Microbiology, School of Medicine, Kyungpook National University

Acinetobacter baumannii is an opportunistic nosocomial pathogen and a member of ESKAPE pathogens that are important multi-drug resistant microorganisms. The PmrAB two-component system (TCS) is a major regulator of colistin resistance. PmrAB is induced by environmental signals such as acidic pH, high Fe<sup>3+</sup>, and low Mg<sup>2+</sup> levels, leading to achieve the resistance of colistin through lipid A modification of LPS by the expression of PmrC, a pEtN transferase. This study investigated the role of PmrAB in antimicrobial resistance and virulence of *A. baumannii* under acidic conditions. The wild-type *A. baumannii* strains cultured in the media with a pH 5.5 showed a significant increase of colistin MIC compared with that cultured with a pH 7.0. However, the colistin MICs were not significantly changed in both  $\Delta pmrA$  and  $\Delta pmrB$  mutants grown in the media with a pH 5.5. The mass spectrum exhibited peaks at m/z 2033 only in the WT strain cultured under pH 5.5, corresponding to the m/z +123 shifts of mass unit caused by the addition of pEtN to the hepta-acylated lipid A structures at m/z 1910. *pmrC* gene was upregulated in the WT cultured under pH 5.5. The WT strains cultured in the media with a pH 5.5 showed low biofilm mass compared with the WT strains cultured with a pH 7.0, but biofilm mass was restored in the  $\Delta pmrA$  and  $\Delta pmrB$  mutants. Our results demonstrated that PmrAB contributes to colistin resistance and pathogenesis of *A. baumannii* under low pH.

Suppression of SARS-CoV-2 Nucleocapsid Protein Dimerization by ISGylation and Its Counteraction by Viral PLpro Wonjin Bang<sup>1</sup>, Daegyu Park<sup>1</sup>, Jihyun Lee<sup>1</sup>, Donghyuk Shin<sup>2</sup>, and Jin-Hyun Ahn<sup>1,3\*</sup> <sup>1</sup>Department of Microbiology, Sungkyunkwan University School of Medicine, <sup>2</sup>Department of Systems Biology, Yonsei University, <sup>3</sup>Biomedical Research Institute, Samsung Medical Center

Interferon-stimulated gene 15 (ISG15) protein is conjugated to a lysine residue of proteins, regulating protein functions. Protein ISGylation is thought to play a key role in protecting hosts against viral infection. In this study, we investigated whether severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins are modified by ISG15 and whether ISGylation effects functions of viral proteins. ISGylation assays performed in 293T cells overexpressing an active form of ISG15, and ISGylation enzymes such as UBE1L (E1), UbcH8 (E2), and Herc5 (E3) revealed that nucleocapsid (N) protein is effectively ISGylated at multiple sites. Mutational analysis identified K261 within the oligomerization domain as a major ISGylation site. The structure of N predicted using Alphafold2 suggested that ISGylation at K261 may affect N protein dimerization, which is critical for RNA binding. In pull-down assays using the purified N protein, ISGylation of N indeed inhibited its dimerization. It was also found that the viral papain-like protease (PLpro), which is encoded by NSP3, could delSGylate ISG15 from N and effectively cleaved UbcH8. Collectively, out results demonstrate that the SARS-CoV-2 N is effectively ISGylated, inhibiting nucleocapsid formation, and that the viral PLpro counteracts this ISG15-mediated antiviral activity by delSGylating N and by reducing the cellular ISGylation capability via UbcH8 cleavage.

# D098

Antifungal Activity of Aminopyrrolnitrin (APRN) against *Trichophyton verrucosum* Infection in Guinea Pig Model Han Gyu Lee<sup>1</sup>, Madusha Pramud Sudu Hakuruge<sup>1</sup>, Young-Hun Jung<sup>1</sup>, Tai-Young Hur<sup>1</sup>, Dong-Gyun Kim<sup>2</sup>, Hee Jeong Kong<sup>2</sup>, Young-Ok Kim<sup>2</sup>, and Sang-Ik Oh<sup>1\*</sup>

<sup>1</sup>Division of Animal Disease & Health, National Institute of Animal Science, Rural Development Administration, <sup>2</sup>Biotechnology Research Division, National Institute of Fisheries Science

Several antifungal agents have been used in numerous cases of dermatophytosis throughout the past few decades. However, these antifungals are often associated with adverse effects, including treatment failure and relapses of infection. Therefore, novel antifungal agents are needed for better treatment of dermatophytes. This study aimed to analyze the antifungal effect of a novel substance, aminopyrrolnitirn (APRN), against *Trichophyton verucosum* (TV) in guinea pigs. Sixteen guinea pigs were randomly divided into four groups. A suspension (1 ml, 1.0 × 10<sup>8</sup> cells/ml) of TV conidia was applied to the shaved skin on the mid-back of the guinea pigs (2.5 × 2.5 cm). After 10 days of post-infection (dpi), the infected skins were treated by 1 ml of APRN with different formulations (2, 4, and 8 ppm for group A, B, and C, respectively) and 1 ml of enliconazole (2 ppm, group D) daily during 10 days. Clinical lesions and fungi staining by PAS were investigated at 21 dpi. The clinical scores was 5.25 (A), 3.5 (B), 3.5 (C), and 9.0 (group D). Redness and ulcer lesions in APRN-treated groups (group A, B, and C) were significantly less observed than those in group D. In PAS stain, fungi were less detected in group B and C than in group A and D. The overall results suggested that APRN could be selected as antifungal agents against TV infection. Further long-term *in vivo* studies are needed to clarify the safety of using APRN in various animal models. [Supported by grants from NIAS]

### Identifying Tumor Inhibitory Staphylococcus epidermidis Strain with Immune Boosting Features

Jee Won Hwang<sup>1,2</sup>, Gwang Hee Kim<sup>1,2</sup>, Yoo Jin Lee<sup>1</sup>, and Sang Sun Yoon<sup>1,2,3\*</sup>

<sup>1</sup>Department of Microbiology and Immunology, Yonsei University College of Medicine, <sup>2</sup>Brain Korea 21 Project for Medical Sciences, Yonsei University, <sup>3</sup>Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine

Immunotherapy is a breakthrough cancer therapy utilizing patients' immune system. But due to its low response rates, <40%, adjuvants are highly required for the stronger immune responses. *Staphylococcus epidermidis* is bacteria predominantly dwelling on human skin and reported to protect the host against melanoma or virus infection. In this study, we found out that the one strain of *S. epidermidis*, named SE5, from our previous study of anti-bacterial infection also have anti-tumor effect for both immunogenic and non-immunogenic tumors. In the experiments, the SE5 lysate and supernatants treatments on splenocyte sustained the viability of immune cells. From the FACS analysis *ex-vivo*, we found out that anti-tumor associated immune cells and cytokines were elevated especially in the lysate treatment. SE5 lysate injection *in-vivo* also demonstrated increased populations of anti-tumor related immune cells, including CD8+ T cells. Therefore, we tested anti-tumor effect of the SE5 lysate treated groups. Also, the lysate inhibited non-immunogenic melanoma cells, B16F10, when treated before the tumor cells implantation. Furthermore, we confirmed the increase in CD8+ T cells from the B16F10 tissues. In addition, we will conduct Flow cytometry and metabolomics of the CT26 and assess SE5 strain as an adjuvant through a co-treatment experiment with an immune-checkpoint-inhibitor.

# D100

**Anti-cariogenic and Anti-inflammatory Activities of Hwangbaek (***Phellodendron amurense* **bark) Extract** Jumi Lee, Jiyoung Choi, Jayoung Kim, Gi Yeon Han, and Joonseok Cha<sup>\*</sup> *Research Center, The Garden of Naturalsolution Co., Ltd.* 

Dental and periodontal caries have become most common oral diseases affecting children and adults. *Streptococcus mutans, Streptococcus sobrinus, Streptococcus sanguinis,* and *Porphyromonas gingivalis* are the main cariogenic microorganisms involved in dental caries progression. Biofilm formation, glucosyltransferase (GTase) activity, and antimicrobial tests of hwangbaek extract (HE) were performed using the above pathogens. HE showed higher efficacy than the positive control at the biofilm formation inhibition assay, GTase activity test also showed similar efficacy to the positive control. In addition, HE showed excellent antimicrobial efficacy at concentrations below 100 µg/ml. We analyzed the potential role of HE in the inflammatory condition. Human gingival fibroblasts were stimulated with LPS, a cell wall component of *P. gingivalis*, to induce inflammation and then treated with HE. We could confirm to reduction TLR-4 and COX-2, at both mRNA and protein levels. With this result, we could conclude that HE inhibited biofilm, GTase, and bacterial growth. And suggest its use in the oral care product treating dental caries.

### The Immunogenicity of an Inactivated SFTSV in Mice

Da-Eun Jeong<sup>1,2</sup> and Jun-Gu Kang<sup>1\*</sup>

<sup>1</sup>Korea Zoonosis Research Institute, Jeonbuk National University, <sup>2</sup>Department of Veterinary Medicine, Jeonbuk National University

Severe fever with thrombocytopenia syndrome virus (SFTSV) belongs to the Phenuiviridae family and causes SFTS, a tick-borne infectious disease. Despite the high fatality rate of SFTS, there is no approved therapy or vaccine. For development of SFTS vaccine candidate, inactivated SFTSV mixed with two types of adjuvants (aluminum sulfate and squalene-based oil-in-water nano-emulsion) was used. Total three times immunization has been conducted with 2 weeks interval for 3 months and blood collection was carried out four times in total for one week, one month, two months, and three months after the last immunization in C57BL mice. An enzyme-linked immunosorbent assay (ELISA) was performed to evaluate SFTSV nucleocapsid protein (NP) specific IgG. Total IgG titer was higher in high dose (2 × 10<sup>7</sup>) than medium dose (2 × 10<sup>6</sup>) injection group, and in aluminum sulfate mixed group than squalene-based oil-in-water nano-emulsion mixed group. To analyze humoral immune response and cell-mediated immune response, IgG subtypes, IgG1 and IgG2c were identified by enzyme-linked immunosorbent assay (ELISA). The level of IgG1 and IgG2c antibody against SFTSV NP were higher in aluminum sulfate than in other adjuvant groups. Moreover, aluminum sulfate group exhibited a high neutralizing antibody activity. These data show that the inactivated SFTSV mixed with aluminum sulfate could use as SFTS vaccine candidate.

# D102

**Dysbiosis of Oral Microbiota in a Mouse Model of Head and Neck Cancer Induced by 4-Nitroquinoline 1-Oxide** Euon Jung Tak and Jin-Woo Bae<sup>\*</sup> *Kyung Hee University* 

Head and neck cancers (HNSCC) are mostly caused by tobacco and alcohol use. 4-Nitroquinolon oxide (4NQO) is used for establishment of a chemically induced HNSCC animal model for immunocompetent mouse. The 4NQO is a water-soluble carcinogen, resulting in tumors predominantly in the oral cavity. In our study, the mice were administered 4NQO in the drinking water for 16 weeks, and thereafter they were untreated up to week 22. At week 16, 4NQO-treated mice showed higher relative abundance of the family Streptococcaceae in the oral microbiota than 4NQO-untreated control mice. However, gut microbiome was affected by dietary type rather than 4NQO treatment. As expected, mice exposed to 4NQO for 16 weeks developed oral tumors at 22 weeks. Our results suggest that the carcinogen or carcinogen-induced tumor microenvironment leads to oral dysbiosis, which may be involved in tumor development.

# Seeking for 'The' Staphylococcus epidermidis Strain that Alleviates Atopic Dermatitis

YunJee Park<sup>1,2,3</sup> and Sang Sun Yoon<sup>1,2,3\*</sup>

<sup>1</sup>Department of Microbiology and Immunology, <sup>2</sup>Graduate School of Medical Science, Yonsei University College of Medicine, <sup>3</sup>Brain Korea 21 project for Medical Science, Yonsei University

Atopic dermatitis (AD), also called eczema, is a chronic skin inflammation and its pathophysiology can be explained through epidermal dysfunction, skin dysbiosis, and imbalance of skin immunity such as overreaction of type 2 immunity. *Staphylococcus epidermidis* (SE) is one of the most abundant commensals residing in the skin of healthy people. It is known to promote homeostasis and also innate and adaptive immune system to fight against pathogens. This research proposal was conducted to select a specific SE strain that has the best potential as a treatment for AD among the 49 strains. The 49 strains were collected from the nasal of healthy people or patients who were undergoing chronic allergy disease. They display a distinct growth time and patterns, supporting the 'strain-level diversity' feature of SE. Depending on which environment SE resides in, it exhibits an ambivalent effect on the host's skin health. Therefore, to avoid environmental variables, supernatant or lysate of SE will be used throughout the whole experiments. The total protein density of each sample used in screening was standardized by Bradford assay. For screening methods, *in-vitro* (IL-4 promoter reporter vector transfected in HEK293T for Dual luciferase assay), *ex-vivo* (flow cytometry analysis on wild mouse splenocytes), and *in-vivo* (AD mouse model induced by MC903 (calcipotriol), a low-calcemic vitamin D3 analog) perspectives were adopted to narrow down the strain of interest.

# D104

# Clonal Distribution and Antimicrobial Resistance of *Pseudomonas aeruginosa* Isolates from Three Korean Hospitals from 2011 to 2019

Nayeong Kim, Seo Yeon Ko, Seong-Yong Park, Seong-Yeob Kim, and Je Chul Lee<sup>\*</sup> Department of Microbiology, School of Medicine, Kyungpook National University

*Pseudomonas aeruginosa* causes nosocomial infection in immunocompromised patients. The World Health Organization (WHO) has listed the carbapenem-resistant *P. aeruginosa* as a priority pathogen that needs for new antibiotics development in 2017. This study was aimed to study the epidemiological characterization of *P. aeruginosa* isolates from Korean hospitals from 2011 to 2019. A total of 201 *P. aeruginosa* isolates obtained from three Korean hospitals from 2011 to 2019 were analyzed. The MICs of antimicrobial agents were determined using the VITEK II and broth microdilution method. Carbapenemase and aminoglycosides-resistance genes were amplified by PCR. The *oprD* gene was amplified by PCR and sequenced to verify the mutation. The *ampC* gene expression was analyzed by realtime-PCR. The sequence type (ST) 235, 111, and 245 were predominant. Over 80% of isolates in each of the three predominant STs showed imipenem- or meropenem-resistance. More 80% of *P. aeruginosa* isolates belonging to three predominant STs were resistant to imipenem- or meropenem and showed an MDR phenotype. The *blaIMP* (89%), *blaVIM* (7%), and *blaNDM* (4%) genes were detected in 44 carbapenemaseproducing *P. aeruginosa* isolates. The carbapenemase-producing *P. aeruginosa* isolates have higher MICs of carbapenem than isolates carrying *oprD* mutation or showing overexpression of *ampC*. More attention is required to curb the spread of new clones of carbapenem-resistant *P. aeruginosa*.

# Evaluation of the Anti-inflammatory Effect of Red Clover (*Trifolium pratense*) on PGN-induced Damage in Human Keratinocytes

Jiyoung Choi, Minji Kim, Jumi Lee, Jayoung Kim, Gi Yeon Han, and Joonseok Cha<sup>\*</sup> Research Center, The Garden of Naturalsolution Co., Ltd.

Toll-like receptors (TLRs) are key receptors that recognize bacterial pathogens in the human body. Toll-like receptor-2 (TLR-2) triggers an immune response when a microorganism invades and is significantly activated by peptidoglycan (PGN), a cell wall component of Gram-positive bacteria. *Cutibacterium acnes (C. acnes)* is a Gram-positive bacteria known to cause the inflammatory response of acne vulgaris. The skin inflammatory response induced by acne vulgaris is initiated when TLR-2 recognizes *C. acnes*. In this study, we confirmed the inhibition of TLR-2 expression caused by PGN-induced in human keratinocytes and the inhibitory effect on the skin inflammatory response. Red clover extract (RCE) regulated inflammatory factors such as caspase-1 and interleukin-1 $\beta$  (IL-1 $\beta$ ). RCE inhibited PGN-induced inflammatory mediators, nuclear factor-kappa B (NF- $\kappa$ B) and cyclooxygenase-2 (COX-2). In addition, it was confirmed that phospho-NF- $\kappa$ B (p-NF- $\kappa$ B) and I $\kappa$ B $\alpha$  were upregulated when RCE was treated. With these results, we could demonstrate that RCE exerts anti-inflammatory effects through the regulation of TLR-2 and inflammatory cytokines. RCE is a potential ingredient for preventing and treating skin inflammatory symptoms caused by acne vulgaris.

# D106

**Critical Role of Neutralizing Antibody for SARS-CoV-2 Reinfection and Transmission in Ferret Model** Dongbin Park<sup>1</sup>, Young Ran Kim<sup>1</sup>, Min-Ah Yu<sup>1</sup>, Yeong-Lim Kang<sup>1</sup>, and Young Ki Choi<sup>1,2\*</sup> <sup>1</sup>Center for Study of Emerging and Re-emerging Viruses, Korea Virus Research Institute, Institute for Basic Science (IBS), <sup>2</sup>College of Medicine and Medical Research Institute, Chungbuk National University

Recently, there have increased risk of SARS-CoV-2 reinfection by the Omicron variant. Further, the level of natural immunity induced by SARS-CoV-2 infection is not fully clear, nor is it clear if a primary infection is protective against reinfection. To investigate the potential association between serum antibody titres and reinfection of SARS-CoV-2, ferrets with different levels of NAb titres after primary SARS-CoV-2 infection were subjected to reinfection with a heterologous SARS-CoV-2 strain. All heterologous SARS-CoV-2 reinfected ferrets showed active virus replication in the upper respiratory and gastro-intestinal tracts. However, the high NAb titre group showed attenuated viral replication and rapid viral clearance. In addition, direct-contact transmission was observed only from reinfected ferrets with low NAb titres (<20), and not from other groups. Further, lung histopathology demonstrated the presence of limited inflammatory regions in the high NAb titre groups compared with control and low NAb groups. This study demonstrates a close correlation between a low NAb titre and SARS-CoV-2 reinfection in a recovered ferret reinfection model.

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# Epidemiological Characterization of *Acinetobacter baumannii* Isolates from Korean Hospitals over the Last Decade

Seong Yeob Kim<sup>1</sup>, So Hyun Jun<sup>1</sup>, Da Eun Lee<sup>2</sup>, Hye Ryeong Hwang<sup>2</sup>, Nayeong Kim<sup>1</sup>, Ki Tae Kwon<sup>1,3</sup>, Yu Kyung Kim<sup>1,4</sup>, and Je Chul Lee<sup>1,2\*</sup>

<sup>1</sup>Department of Microbiology, School of Medicine, Kyungpook National University, <sup>2</sup>Kyungpook National University Hospital National Culture Collection for Pathogens (KNUH-NCCP), Kyungpook National University Hospital, <sup>3</sup>Department of Internal Medicine, School of Medicine, Kyungpook National University, <sup>4</sup>Department of Clinical Pathology, School of Medicine, Kyungpook National University

The wide-spread of drug-resistant *Acinetobacter baumannii* is a global health problem. This study investigated the clonal distribution and antimicrobial resistance of 167 *A. baumannii* isolates from two Korean university hospitals from 2009 to 2019 by analyzing the sequence types (STs), antimicrobial resistance, and resistance determinants of carbapenems and aminoglycosides. Two STs, ST191 (n = 77) and ST451 (n = 40), were prevalent, and majority (n = 153) of the isolates belonged to clonal complex 208. The ST191 isolates were detected during the study period, whereas ST451 isolates were detected after 2016. The ST191 and ST451 isolates exhibited higher resistance to antimicrobial agents than that of the sporadic ST isolates. Interestingly, ST451 isolates exhibited lower susceptibility to minocycline and tigecycline than the other ST isolates. All 147 carbapenem-resistant *A. baumannii* (CRAB) isolates, except four, carried the ISAbal-bla<sub>OXA-23</sub> structure. *armA* was detected in all amikacin-non-susceptible isolates (n = 128) except for one isolate. Five aminoglycoside-modifying enzyme (AME) genes were detected, but their carriage varied between STs; *ant*(*3''*)-*Ia* and *aac*(*6'*)-*I* were more common in ST191 than in ST451, while *aph*(*3'*)-*I* was more common in ST451 than in ST191. This study demonstrated the clonal evolution related to antimicrobial resistance in *A. baumannii*.

# Detection of Rift Valley Fever Virus Using RT-qPCR and RT-ddPCR

Changwoo Park<sup>1,2</sup>, Minkyu Park<sup>1,3</sup>, Zohaib Ul Hassan<sup>1,4</sup>, Jinyoung Park<sup>1,4</sup>, and Seil Kim<sup>1,4\*</sup> <sup>1</sup>Microobiological Analysis Team, Korea Research Institute of Standards and Science, <sup>2</sup>Department of Agricultural Biotechnology, Seoul National University, <sup>3</sup>Department of Biological Science, Chungnam National University College of Bioscience and Biotechnology, <sup>4</sup>Department of Precision Measurement, University of Science and Technology (UST)

Rift valley fever virus (RVFV) is a single-stranded RNA with three genomic segments and causes Rift valley fever disease. The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) has been a gold standard method of diagnosis. However, the next-generation quantification using reverse transcription-droplet digital PCR (RT-ddPCR) is a powerful method for absolute quantification compared to the relative quantification by RT-qPCR. In this study, RT-qPCR, and RT-ddPCR were compared for the detection of RVFV. The synthetic RVFV viral RNA and mock clinical RNA were used as the templates to determine the LoQ (Limit of Quantification) and LoD (Limit of Detection) of both RT-qPCR and RT-ddPCR. Three synthetic RVFV strains (ZH548, Kenya56, and BIME01) belonged to lineage A, D, and K, respectively. For mock clinical samples, cultured viral RNA belonging to lineage E was used. For screening and validation of the RVFV primer-probe sets, the negative reference viruses for Zika, Dengue, Japanese encephalitis, Chikungunya, and severe fever with thrombocytopenia syndrome were used. The negative reference viral genomes confirmed that both assays are RVFV-specific. Additionally, we demonstrated that these primer-probe sets can be used for RVFV A, D, E, and K lineages. The usage of serially diluted templates showed that the LoQ and LoD of both assays are equivalent.

# D110

**Comparison of Growth Kinetics of Equine Herpesvirus 1 in NBL-6, RK-13, MDBK, Vero, HeLa Cell Lines** Hye Won Lee<sup>†</sup>, Soo Hyun Kim<sup>†</sup>, Young Hoon Cho, and Jung-Yong Yeh<sup>\*</sup> Department of Life Sciences, Incheon National University

Introduction: Equine herpesvirus-1 (EHV-1) is considered as a major pathogen of Equidae. Use of various cell lines for the growth of these causative agents has been reported, but there is little comparative information among these cell lines and optimum growth conditions.

Materials and Methods: Growth kinetics of EHV-1 (Kentucky D strain) were determined in different cell types commonly used by equine viral diagnostic laboratories. Virus growth was monitored by the development of cytopathic effects (CPE) and virus yield in different cell cultures by time-course titration.

Results: The highest titers of EHV-1 in RK-13, NBL-6, MDBK, Hela and Vero cells at 36 h post-infection (hpi) were found to be 8.2, 8.3, 6.8, 7.2, 5.9 log TCID<sub>50</sub>/ml, respectively. Additionally, compared to the first passage of EHV-1, the viral titer in MDBK cells at the 3<sup>rd</sup> passage was increased 6.2 to 6.8 log TCID<sub>50</sub>/ml and the titer in Hela cells at the 4<sup>th</sup> passage was increased 6.7 to 7.2 log TCID<sub>50</sub>/ml. Compared to other cells, the maximum viral titer in Vero cells was decreased 5.9 to 4.6 log TCID<sub>50</sub>/ml after 1<sup>st</sup> passage. In Hela cells, the time to reach the highest titer was decreased 8.35 to 7.46 and in MDBK cells significantly reduced 18.2 to 10.21 compared with the 1<sup>st</sup> and 5<sup>th</sup> passage. Conclusions: These results suggest that RK13 and NBL-6 cells could be practical application for mass propagation of EHV-1. In addition, and Hela cells were also appropriate for rapid cultivation of EHV-1 *in vitro*.

### Characterization of Fusarium Strains Associated with Fusarium Head Blight in Korea

Eunji Jeong, Jae Yun Lim, Minjoo Kim, and Jeong-Ah Seo<sup>\*</sup> School of Systems Biomedical Science, Soongsil University

Fusarium head blight (FHB) is a fungal disease that leads yield loss of cereal crops caused by *Fusarium graminearum* species complexes (FGSCs). Among FGSC, *Fusarium vorosii* is a rarely isolated species with only four reported cases worldwide. In current study, we aimed to determine the characteristics of major pathogens of FHB in Korea by comparison of *F. asiaticum* and *F. vorosii*. From FHB diseased rice, barley, and corn collected in 2020, *F. asiaticum* with nivalenol genotype (n=337, 62.5%) was the most frequently isolated and *F. vorosii* with nivalenol genotype (n=10) was isolated with 1.9% frequency of entire *Fusarium* isolates. As a result of the aggressiveness assay, *F. vorosii* was less aggressive than *F. asiaticum*. But potential of *F. vorosii* as the causative agent of FHB was confirmed to be more aggressive in rice than wheat. Through sequencing of one *F. asiaticum* and *F. vorosii* strains, we completely assembled each of the two genome sequences. Through comparative analyses, 43 of the 45 secondary metabolite gene clusters identified in two strains were conserved. Apicidin and  $\alpha$ -acorenol biosynthetic clusters showed sequence differences between species. This study is the first report comparing the mycological and genomic properties *F. vorosii* and *F. asiaticum* strains that cause FHB in Korea.

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# D113

**Genetic Variants Associated with Resistance to Bedaquiline and Delamanid in Rifampin-resistant Tuberculosis** Gisu Kang<sup>1</sup>, Seuung-Heon Lee<sup>1</sup>, Seungmo Kim<sup>1</sup>, Hee Joo Lee<sup>2</sup>, and Hyejin Kim<sup>1\*</sup> <sup>1</sup>Korean Institute of Tuberculosis, <sup>2</sup>Seoul Laboratory Medicin Center, Korean National Tuberculosis Association

The role of mutations in genes associated with phenotypic resistance to bedaquiline, delamanid, linezolid in *Mycobacterium tuberculosis* is poorly characterized. Here we investigated the phenotypic and genotypic characteristics of for 1,531 rifampicin resistant *M. tuberculosis* isolates. The MICs for two drugs were determined for drug resistant isolates by 7H9 broth microdilution method. Genes (*mmpR, atpE* and *pepQ* for bedaquiline; *ddn, fgd1, Rv1979c, fbiA, fbiB* and *fbiC* for delamanid) strongly linked to resistance to them were analyzed by target sequencing. The resistant rate for bedaquiline of 1,531 rifampicin resistant isolates was 3.97%. Most mutations associated with a high MIC of bedaquiline were found in the *mmpR* (35/39, 89.7%) gene. We identified 15 unique variants in *mmpR* gene, 2 in *pepQ* gene in clinical isolates. No mutations within *aptE* gene were observed. The resistant rate for delamanid of 1,531 rifampicin resistant isolates was 7.7%. Most mutations associated with a high MIC of bedaquiline within solates was 7.7%. Most mutations associated with a high MIC of bedaquiline were found in the *mmpR* (35/39, gene. We identified 4 variants in *ddn* and 2 *fbiA* in in clinical isolates. No mutations within *fgd1, fbiB, fbiC* and, *Rv1979c* genes were observed. The *in vitro* high MIC results of bedaquiline and delamaind were correlated with resistance to two drugs, and the mutation types of the *mmpR* and *ddn* genes were associated with resistance to bedaquiline and delamanid, respectively.

# Analysis of Changes in the Intestinal Microbiome of Newborn Calves following Administration of Beneficial Lactic Acid Bacteria

Seul Ki Lim<sup>1</sup>, Ji-Yeong Ku<sup>2</sup>, Kwang-Man Park<sup>2</sup>, Min-Sung Kwon<sup>1</sup>, Young Joon Oh<sup>1</sup>, Do-Hyeon Yu<sup>3</sup>, Yeonsu Oh<sup>4</sup>, Hak-Jong Choi<sup>1\*</sup>, and Jinho Park<sup>2\*</sup>

<sup>1</sup>Technology Innovation Research Division, World Institute of Kimchi, <sup>2</sup>College of Veterinary Medicine, Jeonbuk National University, <sup>3</sup>College of Veterinary Medicine, Gyeongsang National University, <sup>4</sup>College of Veterinary Medicine, Kangwon National University

To evaluate the efficacy of administering probiotics to healthy newborn calves, changes in the intestinal microbiome after administration of probiotics were comparatively analyzed. First, *Lactobacillus amylovorus* isolated from the feces of newborn healthy Korean native calves was cultured and 10<sup>9</sup> cfu/g/head was administered 5 times at 2-day intervals to newborn calves (n=8) as the experimental group. And feces were collected 5 and 10 days after the first administration, and at 1 month of age. Feces of control group (n=6) were collected at the same time as the experimental group. Sequence reads were obtained from the collected feces and analyzed for alpha-diversity, beta-diversity, taxonomic composition and linear discriminant analysis effect size (LEfSe) analysis. As a result of detecting for major gastrointestinal pathogens, it was confirmed that the pathogen detection rate was significantly lower in the experimental group compared to the control group. The experimental group on the 10 days of administration showed increase the diversity of intestinal microflora compared to before administration of probiotics and the control group of the same age. In addition, while the ratio of beneficial bacteria such as *Lactobacillus* and *Faecalibacterium* increased, the ratio of harmful bacteria such as *Escherichia* and *Bacteroides* decreased. These results suggest that the administration of probiotics to newborn calves can improve the gut microbiota structure and reduce disease outbreak.

# D115

# Effect of Administration of Useful Lactic Acid Bacteria to Newborn Calves

Ji-Yeong Ku<sup>1</sup>, Seul Ki Lim<sup>2</sup>, Kwang-Man Park<sup>1</sup>, Young Jun Kim<sup>1</sup>, Min-Sung Kwon<sup>2</sup>, Young Joon Oh<sup>2</sup>, Hak-Jong Choi<sup>2\*</sup>, and Jinho Park<sup>1\*</sup>

# <sup>1</sup>College of Veterinary Medicine, Jeonbuk National University, <sup>2</sup>Technology Innovation Research Division, World Institute of Kimchi

After administration of probiotics to newborn calves, pathogen detection rates, blood gas, acute phase protein and serum protein levels were compared. First, *Lactobacillus amylovorus* isolated from the feces of newborn healthy Korean native calves was cultured and 10<sup>9</sup> cfu/g/head was administered 5 times at 2-day intervals to newborn calves (n=13) as the experimental group. And feces were collected 5 and 10 days after the first administration, and at 1 month of age. No probiotics were administered to the control group of newborn calves (n=11), and feces were collected at the same time as the experimental group. As a result, in contrast to the experimental group, severe diarrhea was observed in calves (n=2) of the control group. In addition, it was confirmed that the detection rate of major gastrointestinal pathogens was significantly lower in the experimental group compared to the control group. Blood gas and haptoglobin levels were a significant different between the experimental and the control group on the 10th day of administration. And Serum protein electrophoresis results, total protein and globulin levels were observed to be significantly higher on the 5th and 10th days of probiotics administration. These results suggest the administration of probiotics is beneficial for the immune capacity of newborn calves and can be used for disease management.

# Shiga Toxins from Enterohaemorrhagic *Escherichia coli* Promote Inflammation in Toxin-sensitive Cells via p38MAPK/MK2/TTP Pathway

Seo Young Park<sup>1,2</sup>, Yu-Jin Jeong<sup>1</sup>, Kyung-Soo Lee<sup>1,3</sup>, Jongsun Park<sup>2</sup>, and Moo-Seung Lee<sup>1,3\*</sup> <sup>1</sup>Environmental Diseases Research Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB), <sup>2</sup>College of Medicine, Chungnam National University, <sup>3</sup>Biomolecular Science, Korea University of Science and Technology (UST)

The multifunctional Shiga toxin (Stxs) produced by *Shigella dysenteriae* serotype 1 and certain *Escherichia coli* pathotypes causes hemorrhagic colitis, which may progress to hemolytic uremic syndrome (HUS) and central nervous system (CNS) pathology. We identified a previously unknown role of Tristetraprolin (TTP) as an MK2 substrate in Stxs-intoxicated cells. We demonstrated that Stxs induce phosphorylation of MK2-Thr334 and TTP in toxin-specific receptor globotriaosylceramide (Gb<sub>3</sub>)-positive cells, including macrophage-like differentiated THP-1 (D-THP-1) and the human renal proximal tubule epithelial cell line HK-2, but not in Gb<sub>3</sub>-negative human T84 colon carcinoma cells. After treatment with wild-type (WT) Stx, the activity of p-MK2 Thr334 and TTP persists for up to 8 h, whereas after treatment with Stx2a<sup>mut</sup>, which is deficient in *N*-glycosidase activity, the activity of p-MK2 Thr334 and TTP activation in a Gb<sub>3</sub>-dependent manner. TTP-knockdown using siRNA in D-THP-1 cells treated with Stx2a upregulates expression of tumor necrosis factor (TNF)- $\alpha$ , interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin (IL)-6, interleukin (IL)-8, interleukin (IL)-10, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein (MIP)-1 $\alpha$  at transcriptional or translational levels. In conclusion, the MK2-TTP signaling pathway regulates the Stx-induced inflammatory response in toxin-sensitive cells.

### D119

# An Adhesin Protein Confers Bacterial Survival inside Host Cells by Regulating Immune Response and Lysosomal Degradation

# Juyoung Kim<sup>1</sup> and Jinki Yeom<sup>1,2\*</sup>

<sup>1</sup>Department of Biomedical Sciences, College of Medicine, Seoul National University, <sup>2</sup>Department of Microbiology and Immunology, College of Medicine, Seoul National University

Pathogenic bacteria produce a variety of adhesin proteins, known as a potent virulence factor, which promotes bacteria invasion by connecting the bacteria and host cells. Uropathogenic *Escherichia coli* (UPEC) causes persisting urinary tract infection (UTI), which is the most common infectious disease. Here, we report that, unexpectedly, a prototypical adhesin FimH is required for intracellular long-term survival in bacteria. UPEC CI-5 strain has two *fimH* genes, named *fimH1* and *fimH2*; both induced the mRNA expression of proinflammatory cytokine genes such as interleukin 6 (*il-6*) and interleukin 1 beta (*il-16*) in the human bladder epithelial cells. Also, bacterial survival significantly decreased in  $\Delta fimH2$  inside human bladder epithelial cells, but not  $\Delta fimH1$ . Moreover, the inactivation of *fimH2* gene promotes colocalization of bacteria and the lysosomal compartment, which possibly limits the intracellular pathogen growth inside host cells. Taken together, our findings reveal that adhesin protein enables pathogenic bacteria to cause persisting infection by regulating inflammation and lysosomal degradation of host cells.

Effect of Feeding Raw Potato Starch on the Reduction of *Salmonella* Typhimurium Shedding in Weaned Pigs Seung-Won Yi, Han Gyu Lee, Young-Hun Jung, Eunju Kim, Eun-Young Bok, Yoon Jung Do, Tai-Young Hur, and Sang-Ik Oh<sup>\*</sup>

Division of Animal Diseases & Health, National Institute of Animal Science, Rural Development Administration

Raw potato starch (RPS) is metabolized by intestinal microflora in the large intestine. Our previous study suggested that feeding 5% RPS could alter bacterial community composition and promoted gut health in pigs, which may prevent diarrhea. In this study, RPS feed additive was tested for their ability to reduce *Salmonella* Typhimurium (ST) infection in swine. A total 12 piglets were randomly divided into two groups: CON (negative control group) and TRT (5% RPS feeding group). All pigs were orally inoculated with  $1 \times 10^7$  CFU of *Salmonella* Typhimurium LT2 strain (ATCC 19585). The ST-infected pigs were euthanized after 14 days from bacteria inoculation, and immediately necropsied. Proximal, mid, distal jejunum, ileum, and cecum were collected for bacterial isolation. The intestinal swab samples were inoculated in 9 ml of RV broth and incubated at 42°C for 24 h, and one loop of RV culture was streaked onto CHROMagar plates. The mauve colored isolates were considered as the intestinal tissue containing ST. In TRT, ST isolates were not observed in jejunum (proximal, mid, and distal) and ileum, while those were detected in cecum (16.7%). In CON, ST isolates were detected in proximal jejunum (33.3%), mid jejunum (50.0%), distal jejunum (33.3%, n = 2), and ileum (16.7%, n = 1), and cecum (83.3%). The overall results suggested RPS supplementation could be an effective means to reduce *Salmonella* Typhimurium in pigs. [Supported by grants from NIAS]

### D121

# 23-Valent Polysaccharide Vaccine (PPSV23)-Targeted Serotype-specific Identification of *Streptococcus pneumoniae* Using the Loopmediated Isothermal Amplification (LAMP) Method

Jonghyun Bae<sup>1,2</sup>, Eun Jin Kim<sup>1,2</sup>, Donghyun Lee<sup>1,2</sup>, Seonghyeon Son<sup>1,2</sup>, Hunseok Choi<sup>1,2</sup>, and Dong Wook Kim<sup>1,2\*</sup> <sup>1</sup>Department of Pharmacy, College of Pharmacy, Hanyang University, <sup>2</sup>Institute of Pharmacological Research, Hanyang University

Reports of invasive disease due to *Streptococcus pneumoniae* have declined since the introduction of pneumococcal conjugate vaccines (PCV7 and PCV13). The incidence of invasive diseases due to *S. pneumonia* that are not addressed by the vaccines, however, has increased in children and adults, creating a global public health problem. In the current study, we developed a rapid, simple, and cost-effective assay to detect serotypes in the 23-valent pneumococcal polysaccharide vaccine (PPSV23) using the LAMP method. The reactivity, specificity, and sensitivity of LAMP assays were determined and compared to those of conventional PCR. The feasibility of LAMP assays in clinical application in patients with invasive pneumococcal diseases was validated by defining the detection limit of the LAMP assay with bacterial genomic DNA-spiked blood specimens. Their sensitivity was 100 copies per reaction versus 10<sup>3</sup> to 10<sup>6</sup> copies per reaction for PCR assays. Using DNA-spiked blood specimens, excluding the LAMP assay that targeted serotype 22F (10<sup>3</sup> copies per reaction), the limit of detection of the LAMP assay was similar to that with purified DNA as the template (10<sup>2</sup> copies per reaction), compared with 10<sup>3</sup> to >10<sup>6</sup> copies per reaction for PCR assays. A rapid and simple LAMP-based PPSV23-targeted serotype detection assay was developed for use in many countries. This study is the first report of a LAMP-based assay for identification of PPSV23 serotypes.

# Development of a Multiplex PCR for Rapid and Simultaneous Detection of Staphylococcus Species

Hye Jin Kim<sup>1</sup>, Min Ji Seong<sup>1,2</sup>, Kil-Soo Kim<sup>1</sup>, and Eui-Suk Jeong<sup>1\*</sup>

<sup>1</sup>Preclinical Research Center, Daegu-Gyeongbuk Medical Innovation Foundation, <sup>2</sup>Department of Biological Sciences, Pusan National University

Recent reports suggest that *Staphylococcus* species can cause disease in humans and laboratory animals and are considered important opportunistic pathogens associated with various infections. The growing interest in these opportunistic bacteria is due to the growing number of clinical reports, which is also important to the more accurate identification of these species. In this study, *Staphylococcus aureus* and *Staphylococcus intermedius* from coagulase-positive staphylococci (CoPS) and *Staphylococcus xylosus* and *Staphylococcus epidermidis* from coagulase-negative staphylococci (CNS) were selected. In general, it is difficult to distinguish the four selected bacteria by culture, so we performed multiplex PCR (mPCR) under carefully optimized conditions to measure the expression simultaneously in one reaction. We developed an accurate and discriminable mPCR method targeting species-specific sequences from *S. aureus*, *S. xylosus*, *S. intermedius*, and *S. epidermidis*. By using mPCR, sensitivity and specificity can be increased, and multiple genes can be rapidly detected at the same time, saving time and consumables. This technique could be a useful tool for the monitoring of staphylococcal infections in laboratory animals.

[Supported by Preclinical Research Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Republic of Korea]

# D123

# Next-generation Diagnostic Platform of K-MEDI Hub Preclinical Research Center

Minji Seong<sup>1,2</sup>, Se-Kyung Oh<sup>1</sup>, Hyejin Kim<sup>1</sup>, Kil-Soo Kim<sup>1</sup>, and Eui-Suk Jeong<sup>1\*</sup> <sup>1</sup>Preclinical Research Center, Daegu-Gyeongbuk Medical Innovation Foundation, <sup>2</sup>Department of Biological Sciences, Pusan National University

Identification of pathogen is the most important step in the accurate treatment of various diseases. The development of diagnostic technology is essential to effectively respond to infectious diseases that have rapidly increased due to the recent emergence of new variant of microorganisms such as COVID-19, MERS, and Zika virus. The K-MEDI hub preclinical research center (PRC), which is a specialized preclinical research support facility based on excellent infrastructure, is performing research on the development of biomarkers for pathogen identification, next-generation diagnostic platforms and kits. Pathogen diagnosis is divided into molecular diagnosis by directly detecting pathogen gene (DNA, RNA) and immunodiagnosis by detecting antigen or antibody produced by the pathogens, and appropriate diagnostic techniques can be selected and used according to the time of onset and condition of the outbreak of infectious diseases. We have established various pathogen detection platforms as well as antigen and antibody based multiplex detection systems that increase sensitivity, specificity and efficiency. Furthermore, we are making efforts to improve our diagnostic technology to take a preemptive response to infectious diseases through continuous R&D.

[Supported by the Daegu-Gyeongbuk Medical Innovation Foundation]

# A Study on the Evaluation of Test Vaccine against Commercial Vaccine Using Hepatitis A Vaccine Efficacy Evaluation Test

Ji Eun Lee<sup>1</sup>, Dan Bee Han<sup>1</sup>, Hyun Mi Lee<sup>1</sup>, Yong Ju Chung<sup>2</sup>, Han Sle Kim<sup>3</sup>, and Hyun Joo Lee<sup>3\*</sup> <sup>1</sup>JBRC (Jeonnam Biophamaceutical Research Center), <sup>2</sup>Genematrix, <sup>3</sup>K-VCAST (vaccine center for assisting Safety & Technology)

Hepatitis A is a disease with many socioeconomic losses as well as human life as it can cause various complications even if it does not progress to chronic liver disease.

Although the demand for national vaccinations and personal demand for hepatitis A vaccines have soared, hepatitis A vaccines are not localized, so they are all dependent on imports. There were also situations in which supply was unstable, such as a continuous shortage of vaccines, threatening public health. Just as hepatitis B vaccine was developed independently in Korea in the past to lower costs, national research and efforts are needed to reduce vaccine costs.

It is necessary to properly know the standardized method for evaluating the efficacy of the hepatitis A vaccine and to establish an appropriate method in Korea. It should also be able to understand and apply the new methods that are continuously being developed and presented at home. This study evaluated the equivalence/superiority of existing commercialized products and test vaccines through efficacy evaluation tests. The efficacy evaluation test was conducted with humoral immunity evaluation, cellular immunity evaluation, and virus defense efficacy evaluation. Through this study, it is possible to localize hepatitis A vaccine, and through this, economic and health contributions can be made by lowering vaccine prices and stabilizing supply and demand. [Supported by "KHIDI" & "Vaccine Innovation Technology Aliance Korea"]

### D125

# Interplay between Tagatose 1,6-Bisphosphate Aldolase Activity and Pneumococcal Pathogenesis in *Streptococcus pneumoniae* D39

Tae-Seon Song and Min-Kyu Kwak<sup>\*</sup>

Department of Food and Nutrition, College of Bio-Convergence, Institute of Food and Nutrition Science, Eulji University

Despite substantial research in *Streptococcus pneumoniae*, evidence on the tagatose-1,6-bisphosphate aldolase (LacD) activity-dependent pathophysiological characteristics and mechanisms governing this species' virulence remains elusive. We experimentally observed that  $\Delta lacD$  pathogenesis was coupled with the modification of necessary pathogenic determinants, including changes in colony morphology, shortened-chain length for imperfect colonization, and capsule production with uncertain adherence. Data from  $\Delta lacD$  using a glucose-based sample preparation protocol prominently revealed metabolic shifts in regulatory determinants required for pneumococcal infection; growth defective  $\Delta lacD$  displayed short-chain forms and decreased capsule production. The invasion of  $\Delta lacD$  in the blood was also drastically decreased. Importantly, gene transcripts downregulated in  $\Delta lacD$  encoded many previously defined virulence factors, including exoglycosidase, transcriptional regulators, and the two component system. These findings indicate that *lacD* deficiency causes virulence loss in a murine model of *S. pneumoniae* infection; *lacD* deficiency also resulted in *in vivo* defects in principal pneumococcal events, including growth, adherence, colonization, and transmission to the blood. To our knowledge, this is the first study to report that the reciprocal relationship between *lacD* gene expression and pneumococcal pathogenesis.

# Analysis of the Functions of Severe Acute Respiratory Syndrome Coronavirus 2 Non-structural Protein 1 on the Inhibition of Type I Interferon

Taehun Kim, Sojung Bae and Jinjong Myoung\* Molecular Virology Laboratory, Korea Zoonosis Research Institute, Jeonbuk National University

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is quickly spreading over the world, utilizing a variety of virulence factors to achieve specific objectives. An element designed to evade or inhibit innate immune responses is an example of virulence factors. If SARS-CoV-2 avoids or inhibits the innate immune response, which is the initial line of defense against invading viruses, the circumstances are very unfavorable for the host. Therefore, we emphasize the need of SARS-CoV-2 viral factor research for effective SARS-CoV-2 and COVID-19 control. Among the various SARS-CoV-2 virulence factors that inhibit the innate immune system, non-structural protein 1 (nsp1) is known to inhibit type I interference responses, we focused on the function of SARS-CoV-2 nsp1 for inhibition of type I Interferon response and conduct studies to identify the suppression mechanism and inhibitory residues of nsp1. Our findings show that host shutoff is the fundamental mechanism of nsp1 that reduces Interferon- $\beta$  production and that the major residues for host shutoff are K164/H165 amino acids. Furthermore, we propose through the experiments, that the inhibitory mechanism of nsp1 includes inhibition of IRF3 phosphorylation as well as host shutoff.

### Microbial Profile Comparisons of Saliva and Tonsil Samples in Tonsillectomy Young Patients

Jiwon Park<sup>1</sup>, Da Hyeon Choi<sup>1</sup>, Ju Kwang Choi<sup>1</sup>, Kyeong Eun Lee<sup>1</sup>, Won Hee Lee<sup>2</sup>, Jinho Yang<sup>2</sup>, Ju Yeon Lee<sup>3</sup>, Yoon Jeong Park<sup>3</sup>, Chan Oh<sup>4</sup>, Ho-Ryun Won<sup>4</sup>, Bon Seok Koo<sup>4</sup>, Jae Won Chang<sup>4</sup>, and Yoon Shin Park<sup>1\*</sup> <sup>1</sup>Department of Biological Sciences and Biotechnology, School of Biological Sciences, College of Natural Sciences, Chungbuk National University, <sup>2</sup>Institute of MD Healthcare Inc, <sup>3</sup>Central Research Institute, Nano Intelligent Biomedical Engineering Corporation (NIBEC), School of Dentistry, Seoul National University, <sup>4</sup>Department of Otolaryngology-Head and Neck Surgery, Chungnam National University College of Medicine

Oral microbes can spread throughout the gastrointestinal system and are closely associated with multiple diseases. Because the tonsils are located between the oral cavity and laryngoesophagus at the tonsillar tissue, the tonsils may also be affected by both oral and gastrointestinal tract microbiota. Here, we analyzed and compared the distribution profiles of the microbial community in saliva and tonsil from 29 children who underwent tonsillectomy by hyperplasia using the 16S rRNA gene sequencing. The tonsil group showed higher richness, both observed OTUs and Chao1 diversity indices, but the difference showed low significance. The most enriched genera in saliva group were *Streptococcus, Veillonella*, and *Neisseria*. The most enriched genera in tonsil group were *Fusobacterium*, *Haemophilus*, and *Prevotella 7*. Overall, strong similarities between the tonsil and saliva microbial communities are evident in terms of diversity and composition. Furthermore, the salivary microbiome is expected to significantly affect the tonsillar microbiome. We propose that the identified microbes act as both intracellular and external environmental factors with a major impact on the functioning of tonsils.

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# E002

# Effect of Peptidoglycan Carboxypeptidases on Antibiotic Resistance

Si Hyoung Park<sup>1</sup>, Umji Choi<sup>1</sup>, Su-Hyun Ryu<sup>2</sup>, Han Byeol Lee<sup>1</sup>, Jin-Won Lee<sup>2</sup>, and Chang-Ro Lee<sup>1\*</sup> <sup>1</sup>Department of Biological Sciences, Myongji University, <sup>2</sup>Department of Life Science and Research Institute for Natural Sciences, Hanyang University

Peptidoglycan (PG) is a mesh-like rigid architecture which is necessary for shape maintenance and protection from turgor pressure-mediated cell lysis, but it is continuously exposed to modification and degradation by PG hydrolases. PG carboxypeptidases (CPase) catalyzes the removal of the fifth D-alanine (D-Ala) from the stem peptides of PG and subsequently, the remaining stem peptide is covalently linked with the Lpp lipoprotein or the adjacent stem peptide by LD-transpeptidases. In this study, we analyzed the effect of PG CPase on antibiotic resistance. The deletion of PG CPases induced the inverse effects on β-lactam and vancomycin, β-lactam sensitivity and vancomycin resistance. Through performing diverse experiments to reveal the underlying molecular mechanisms of these phenotypes, we elucidated that vancomycin resistance is caused by increased levels of decoy D-Ala-D-Ala residues, while β-lactam sensitivity is associated with physical interactions between PG CPase and penicillin-binding proteins (PBPs). Notably, both of two mechanisms are independent of LD-transpeptidases. Therefore, we revealed two novel LD-transpeptidase-independent mechanisms of PG CPase-mediated antibiotic resistance and the functional relationship between PG CPase and PBPs.

[This research was funded by a research grant from Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF-2021R1A6A3A01086677)]

# The Ubiquitin-proteasome System Provides a Compensatory Mechanism for Degrading Fatty Acid Synthase under Nitrogen Starvation in the Absence of Autophagic Activity in *Saccharomyces cerevisiae*

Hae-Soo Jang<sup>1</sup>, Yongook Lee<sup>1</sup>, and Won-Ki Huh<sup>1,2\*</sup> <sup>1</sup>School of Biological Sciences, Seoul National University, <sup>2</sup>Institute of Microbiology, Seoul National University

Autophagy and the ubiquitin-proteasome system (UPS) are two major protein degradation pathways, and their complementary relationship is essential for maintaining homeostasis. In general, large proteins are degraded by autophagy, whereas small proteins are degraded by the proteasome. However, recent studies suggest that various substrates can be eliminated by both systems. The yeast fatty acid synthase (FAS) is a macromolecular complex of 2.6 MDa responsible for the synthesis of saturated fatty acids. It has previously been reported that the FAS is selectively degraded by autophagy under nitrogen starvation for cell survival. Here, we show that, even when autophagy is dysfunctional, the FAS can be eliminated under nitrogen starvation by the UPS in *Saccharomyces cerevisiae*. Ubiquitination of Fas1/2, the FAS subunits, is increased in response to nitrogen starvation and MG-132 treatment effectively blocks the degradation of Fas1/2 in autophagy-deficient *atg8Δ* cells. We also find that an E3 ubiquitin ligase Ubr1 is required for the UPS-dependent degradation of Fas2. Other selective autophagy substrates, Rpn2 and Ssn2, are not degraded under nitrogen starvation in *atg8Δ* cells, suggesting that the FAS complex is selectively degraded in this condition. Together, our results reveal an alternative degradation mechanism for the FAS complex and suggest a see-saw relationship of the two major protein degradation systems. [Supported by NRF of Korea and BK 21 Plus Program from South Korea.]

### E004

### Chemotactic Response to Animal Bloods by a Pathogenic Bacterium, Vibrio vulnificus

Yunha Suh, You-Chul Jung, Ji-Hong Yu, Hui-Wan Yang, Yu-Ra Kim, and Kyu-Ho Lee<sup>\*</sup> Molecular Microbiology and Biofilm Lab., Department of Life Science, Sogang University

*Vibrio vulnificus*, a septicemia-causing pathogen, exhibits the apparent tendency migrating towards the animal bloods via polar flagellum-mediated swimming motility. To examine the hemophilic characteristics of *V. vulnificus*, its chemotactic responses to blood were investigated. *V. vulnificus* was attracted by the sera derived from the model animals in a concentration-dependent manner. On a soft-agar plate supplemented with the diluted serum, *V. vulnificus* produced multiple distinct migrating rings. It suggested that some specific components in the serum are sensed as the chemotactic attractants. Exposure of each migrating ring to various serum components revealed that an iron-storage protein, ferritin, was responsible for perturbing the migration of one of the rings. Interestingly, the degree of this perturbation was reduced when the iron chelator-treated ferritins were applied. Furthermore, the migration pattern of this ring was sensitive to the ferrous ions. Therefore, it is proposed that the iron ion itself and/or the iron ions complexed with some compounds play a role in attracting *V. vulnificus* cells to the blood.

# Differentiation and Virulence of Aspergillus fumigatus Velvet A (VeA) Mutant

Subin Kim<sup>1</sup>, Cheol-Hee Kim<sup>2</sup>, and Hee-Moon Park<sup>1\*</sup> <sup>1</sup>Department of Microbiology and Molecular Biology, <sup>2</sup>Department of Biology, College of Bioscience and Biotechnology, Chungnam National University

Aspergillus fumigatus is the most prevalent airborne fungal pathogen because of its ability to cause infections called aspergillosis in immunocompromised hosts. Especially sexual development of fungi allows the organism to develop new features. Therefore, study of sexual development in *A. fumigatus* is very important. VeA has been demonstrated to positively regulate sexual development in ascomycetes fungi. thus, the *veA* deletion mutant strain fails to produce sexual fruiting bodies. The result of sexual development experiment, the number of cleistothecia, an organ produced by sexual development, in WT(*mat1-1*) ×  $\Delta$ AfveA(*mat1-2*) cross were significantly higher than that in the WT(*mat1-1*) × WT(*mat1-2*) cross. So, mating between wild type(*mat1-1*) and  $\Delta$ AfveA(*mat1-2*) is more effective in *A. fumigatus* and it is in contrast to *A. nidulans*. Interestingly, one of the  $\Delta$ AfveA(*mat1-1*) progenies which obtain from WT(*mat1-1*) ×  $\Delta$ AfveA(*mat1-2*) cross can't induce sexual development and it suggests that mating type dependency may have to exist. The result of invasive fungal infection assay using T cell deficient *foxn1* zebrafish, there is no significant difference between *veA* deletion strain and wild type, and it indicates that VeA was not involved in virulence.

[This research was funded by the NRF Korea, grant number 2020R1F1A1073075 to H-MP and zebrafish strain was supported by Zebrafish Center for Disease Modeling]

# E006

**Outer Membrane Porin F (OmpF) in** *E. coli* is Critical for Effective Predation by *Bdellovibrio* Wonsik Mun<sup>1</sup>, Sumudu Upatissa<sup>1</sup>, Sungbin Lim<sup>1</sup>, Mohammed Dwidar<sup>2,3</sup>, and Robert J. Mitchell<sup>1\*</sup> <sup>1</sup>Ulsan National Institute of Science and Technology, <sup>2</sup>Case Western Reserve University, <sup>3</sup>Cleveland Clinic

*Bdellovibrio*-and-like organisms (BALOs) are a unique bacterial group which live by predating on other bacteria, consuming them from within to grow and replicate before the progeny come out to complete the life cycle. The mechanisms by which these predators recognize their prey, and differentiate them from non-prey bacteria, however, are still not clear. Through genetic knockout and complementation studies in different *E. coli* strains, we found *B. bacteriovorus* 109J recognizes outer membrane porin F (OmpF) on the surface of this microbe and that the activity of the *E. coli* EnvZ-OmpR regulatory system significantly impacts predation kinetics. OmpF is not the only signal by which BALOs recognize their prey, however, as *B. bacteriovorus* could eventually predate on the *E. coli* Δ*ompF* mutant after prolonged incubation. Furthermore, recognizing OmpF as a prey surface structure was dependent on the prey strain as knocking out OmpF protein homologues in other prey species including *Escherichia fergusonii, Klebsiella pneumoniae* and *Salmonella enterica* did not always reduce the predation rate. Consequently, although OmpF was found to be an important surface component used by *Bdellovibrio* to efficiently recognize and attack *E. coli,* future work is needed to determine what other prey surface structures are recognized by these predators.

[Supported by grants from National Research Foundation of Korea]

# Elucidation of Coordinated and Distinct Roles of Peptidoglycan Carboxypeptidases in Cell Morphology and Stress Adaptation

Umji Choi, Si Hyoung Park, Han Byeol Lee, and Chang-Ro Lee<sup>\*</sup> Department of Biological Sciences, Myongji University

Peptidoglycan (PG) is an essential bacterial mesh-like structure that is pivotal for maintaining shape and overcoming osmotic stress. Although PG synthesis and modification should be tightly maintained, few related mechanisms have been studied. Here, we revealed the coordinated and distinct roles of PG carboxypeptidases (CPase) DacC and DacA, in shape maintenance and adaptation to alkaline and salt stresses. DacC was required for bacterial growth under alkaline stress, and its protein stability and enzymatic activity were significantly increased under alkaline stress, suggesting that DacC is a PG CPase that is specialized for growth at alkaline pH. Under alkaline stress conditions, both DacA and DacC were necessary for cell shape maintenance, but their roles were distinct. Under normal growth conditions, only DacA was required for cell shape maintenance. Unexpectedly, all these roles of DacC and DacA were independent of Id-transpeptidases, and instead were associated with penicillin-binding proteins (PBPs), dd-transpeptidases. Both DacC and DacA physically interacted with PBPs in a C-terminal domain-dependent manner, and these interactions were necessary for most of their roles. Taken together, we reveal novel PBP-related roles of PG CPases DacC and DacA in stress adaptation and shape maintenance.

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# E008

A DeoR Family Transcriptional Regulator of the *fru* Operon in *Faecalibacterium prausnitzii* A2-165 HyeLim Choi and Yeong-Jae Seok<sup>\*</sup>

School of Biological Sciences and Institute of Microbiology, Seoul National University

*Faecalibacterium prausnitzii*, a dominant member of healthy human gut microbiota, relies on the phosphoenolpyuruvate (PEP):carbohydrate phosphotransferase system (PTS) to import fructose, simultaneouly phosphorylating it to fructose 1-phosphate (F1P). In a recent study, we identified that *F. prausnitzii* has two ditinct HPrs (HPr1 and HPr2) as a general PTS component. While HPr1 has one phosphorylation site (Histidine15) and is majorly involved in the PTS sugar transport, HPr2 has two phosphorylation sites (Histidine15 and Serine46) and may participate in numerous physiological regulations. Here, we show that a DeoR family transcriptional regulator (DeoR) not only interacts with HPr2, but also with F1P. DeoR regulates the fructose (*fru*) operon, recognizing repetitive sequences containing palindromes in the *fru* promoter. One of the phosphorylation site of HPr2, Histidine15, is located close to the binding interface of DeoR and HPr2, causing dissociation of their interaction upon its phosphorylation. F1P interacts with the basic residues located in the C-terminal domain of DeoR and Lysine73 residue is critical for its binding. We discuss the structural modifications of DeoR which are induced by the presence of each interaction partner, respectively. Based on the structural analysis, we suggest that the regulation of the *fru* promoter.

# Functional Analysis of *vosA*, a Velvet Protein Gene, on Sexual Development and Virulence in *Aspergillus fumigatus*

Eun-Hee Park and Hee-Moon Park\*

Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University

AfuVosA is a transcription factor with a specific velvet domain in a human pathogenic fungus, *Aspergillus fumigatus*. It affects asexual development, but It's functions in sexual development and virulence are unknown in *A. fumigatus*. Therefore, we investigated how *Aspergillus fumigatus vosA*(*AfuvosA*) affects sexual development and studied virulence through immune response with murine alveolar macrophage(AM), and alkaline protease activity. In sexual development of wild type(*MAT1-1*) and  $\triangle AfuvosA$ (*MAT1-2*), the size and number of cleistothecium increased compared to wild type. In addition, the maturation of fruiting body progressed faster, and the number of ascospores also increased. The trehalose content of the  $\triangle AfuvosA$  ascospores was reduced, and heat resistance was decreased. The mRNA level of genes (*preB, ppgA, veA, esdC, etc.*), which are known to be essential for sexual development in *Aspergillus spp.*, changed at the specific time. *In vitro* experiments revealed that endocytosis of conidia by AM was increased in the  $\triangle AfuvosA$  strain. It is presumed that this is caused by the increase of the pathogen-associated molecular patterns, such as  $\beta$ -1,3-glucan in  $\triangle AfuvosA$  strain. And  $\triangle AfuvosA$  is a factor influencing sexual development and virulence.

[This research was funded by the NRF Korea, grant number 2020R1F1A1073075]

# The Translational GTPase BipA is Pivotal in Growth of *Salmonella enterica* serovar Typhimurium at Low Temperature

Eunwoo Ryu<sup>1</sup>, Eunsil Choi<sup>2</sup>, Ahhyun Huh<sup>1</sup>, and Jihwan Hwang<sup>1,2\*</sup> <sup>1</sup>Department of Integrated Biological Science, Pusan National University, <sup>2</sup>Microbiological Resource Research Institute, Pusan National University

BPI-inducible protein A (BipA) is highly conserved in bacteria and structurally similar with translational GTPases including IF2, EF-Tu, and EF-G. Expression of *bipA* in *Escherichia coli*, is induced by cold shock and its deletion causes a variety of phenotypical changes such as ribosomal abnormality, capsule production, motility, and biofilm formation at low temperature. In *Salmonella enterica* serovar Typhimurium, the expression of *bipA* is induced by an antimicrobial peptide, BPI. Previously its ribosome binding mode was affected by ppGpp, suggesting that BipA may be involved in stress adaption or regulation of virulence factors. In this study, to elucidate the function of BipA in *S*. Typhimurium at low temperature, we deleted the *bipA* gene in three *S*. Typhimurium strains (SL1344, 14028, and LT2) and investigated the several phenotypic changes. Deletion of *bipA* led to the defects in growth of *S*. Typhimurium strains at low temperature. Furthermore, we found out the ribosome assembly defect at low temperature by polysome profiling. Unlike in *E. coli*, deletion of *bipA* had no effects on the capsule production and biofilm formation at 20°C. Nevertheless, *bipA*-deleted *S*. Typhimurium strains exhibited reduced motility at both 37°C and 20°C. Our results imply that *E. coli* and *S*. Typhimurium BipA proteins may have a common role in ribosome assembly and a differential role in the regulation of outer surface structures at low-temperature. [This work was supported by the NRF grant.]

### E012

# *In Vivo* Characterization and Evaluation of Probiotic Strains Isolated from Korean Traditional Fermented Foods for Industrial Applications

Dong-Geun Park, Sun-Min Lee, Seong-Woo Choi, Eun-Ji Shin, and Ju-Hoon Lee<sup>\*</sup> Department of Agricultural Biotechnology, Department of Food and Animal Biotechnology, Center for Food and Bioconvergence, Research Institute of Agriculture and Life Science, Seoul National University

To develop a new probiotic strain, two *Lactococcus lactis* subsp. *lactis* (JJLC-140, JJLC-167) were initially isolated in fermented clam and evaluated *in vitro*, comparing food processing, and probiotic properties; adhesion, gastric/bile acid tolerance, cholesterol reduction, and immune regulation via *Lb. rhamnosus* GG (LGG) as control. To further understand selected probiotic strains characteristics *in vivo*, selected probiotic strains cholesterol and triglyceride reduction, immune regulation were evaluated by oral administration of selected probiotic strains to high-fat diet (HFD) induced mouse model.

In mouse serum cholesterol and triglyceride reduction via HFD only group, LGG reduced serum triglyceride and total cholesterol. However, JJLC-140 and JJLC-167 reduced LDL cholesterol, and triglyceride. Especially, JJLC-140 reduced serum total cholesterol also. In immune regulation, all probiotic strains and LGG down-regulate proinflammatory cytokines (TNF- $\alpha$ , IL-6) and up-regulate anti-inflammatory cytokines (IL-4, IL-10).

In this study, selected probiotic strains showed significant cholesterol reduction and immune regulation in HFD induced mouse model, which showed consistency with *in vitro* data. Therefore, this strain could be a good candidate as a new functional food additive for serum cholesterol and triglyceride reduction and anti-inflammation.

# Investigation of Antibacterial Activity of *Juniperus chinensis* against Pathogenic Bacterial and Identification of the Major Bioactive Components Using LC-QTOF-MS

Da Jung Lim<sup>1</sup>, Jeong-Sup Song<sup>1</sup>, Yangseon Kim<sup>1</sup>, Byoung-Hee Lee<sup>2</sup>, and Young Kyoung Son<sup>2\*</sup> <sup>1</sup>Department of Research and Development, Center for Industrialization of Agricultural and Livestock Microorganisms, <sup>2</sup>Biological and Genetic Resources Assessment Division, National Institute of Biological Resources

Juniperus chinensis is native to countries in the East Asian region and widely used as an ornamental plant. The chemical compounds of *J. chinensis* are known as the three major groups i.e. flavonoids, lignans, and terpenes. In this study, we showed antibacterial activity through the disk-diffusion fusion method using ethanol extracts to 200,000 mg/kg levels from *J. chinensis* against pathogenic microorganisms such as *Escherichia coli* KCTC 2617, *Salmonella* Enteritidis NCCP 14546, *Staphylococcus aureus* NCCP 14560, and *Streptococcus mutans* KCTC 3065 and investigated their bioactive components. The major bioactive components of the extracts were identified by ultrahigh performance liquid chromatography coupled with quadrupole time-of-flight–mass spectrometry (UPLC/Q-TOF-MS) in combination with UNIFI software data. As a result, the bioactive components in the extract were identified as amentoflavone, apocynoside I, citrusin A, cnidimol F, 3-O-trans-coumaroylquinic acid, curculigoside B, 6-hydroxykaempferol-3-O-glucoside, kaempferol-3-O- $\beta$ -D-glucopyranoside, quercetin 3-(2''-acetylgalactoside), and tinoside. Among them, the antibacterial, antioxidant, and anti-inflammatory activities of amentoflavone have been reported. Therefore the antibacterial activity in this study was attributed to amentoflavone.

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### E014

A Novel Flux Regulation of Lysine Acetyltransferase (PatZ) in *Escherichia coli* Gwanwoo Lee and Yeong-Jae Seok<sup>\*</sup> School of Biological Sciences and Institute of Microbiology, Seoul National University

*Nɛ*-Lysine acetylation is a posttranslational modification, that occurs in diverse bacteria species. Lysine acetylation modulates several biological processes, such as RNA metabolism, enzymatic activity, motility, cell shape and bacterial pathogenesis. However, most of the Lysine acetylation is a nonenzymatic acetylation by Acetyl phosphate (AcP) and enzymatic acetylation of Lysine acetylation is not much known. In nonenzymatic reaction, AcP directly donates its acetyl group to the deprotonated lysine-amino group. In enzymatic reaction, Lysine acetyltransferase (KAT) catalyze the transfer of acetyl group from Acetyl-Coenzyme A. Such enzymatic acetylation has high acetylation site specificity and acts as a key regulation point specific to metabolism. In Escherichia coli, only well identified regulatory role of lysine acetyltransferase (PatZ synonyms YfiQ, Pka) is the regulation of acetate metabolism. Here, we compared the growth of K-12 MG1655 Wild type and *patZ* deletion mutant in various carbon sources and growth rate was enhanced by overexpression of GalP (galactose: H+ Symporter) in the *patZ* deletion mutants.

[Supported by Ministry of Science, South Korea (NRF-2019R1A2C2004143)]

### Investigation of Fur Family Proteins in *Clostridium pasteurianum*

Hyun Ji Noh, Jang Wan Son, Eun Soo Han, Ian Jeong, Hyeon Ji Lyu, and Jin Won Lee<sup>\*</sup> Department of Life Science and Research Center for Natural Sciences, Hanyang University

*Clostridium pasteurianum*, a Gram-positive bacterium producing carboxylic acids, has four fur family proteins. Fur family protein is a group of proteins that exist in bacteria and regulate metal homeostasis, oxidative stress, etc., and mainly acts as a repressor. Gram-positive bacteria are commonly known to have one Fur, Zur, and PerR protein each, but there are exceptional cases of having multiple PerR proteins. Therefore, I wanted to make sure that *C. pasteurianum*, which has four Fur family proteins, also has two or more PerR proteins. First, I performed sequence alignment to identify four proteins, CLPA\_c26700, CLPA\_c21450, CLPA\_c32220, and CLPA\_c05360. PerR protein is a metal-dependent hydrogen peroxide sensor, and it can be confirmed by oxidation of histidine residues. Through this, PerR-like protein was selected by MALDI-TOF MS. As a result, CLPA\_c26700 is estimated to be a PerR-like protein, and CLPA\_c05360 was difficult to distinguish. After purifying each protein through FPLC, the number of structural zinc ion per protein monomer was calculated through PAR assay. Additionally, FA assay and  $\beta$ -galactosidase assay will be performed to determine the interaction between the proteins and their operator DNA. [This work was supported by Science Research Center grant (NRF-2018R1A5A1025077) and 2019R1F1A1061890.]

# Comparative Secretome Analysis of Brown-rot Fungus Fomitopsis palustris Grown on Different Lignocellulosic **Substrates**

Ju-Hee Cha and Chang-Jun Cha\* Department of Systems Biotechnology, Chung-Ang University

Fomitopsis palustris is one of the most prominent wood-decaying brown-rot fungi. To investigate the temporal dynamics and diversity of carbohydrate-active enzymes (CAZys) influenced by various lignocellulosic substrates, the secretomes on woody (aspen and pine) and non-woody (rice and barley straws) substrates were analyzed. Lignocellulolytic enzymes were significantly more abundant in the secretomes with woody and non-woody substrates than glucose. Although a majority of lignocellulolytic enzymes were shared during woody and nonwoody decay, time-dependent differences were observed in the profiles of the secreted proteins from various lignocellulosic substrates. The number of identified proteins peaked at 14-day and 21-day on woody and nonwoody secretomes, respectively. We identified several unique components during the decay of substrates, including expansin-like proteins which mediate plant cell wall loosening. These comparative secretome analyses identify the temporal changes and differences in the secretome on various lignocellulosic substrates and support an understanding of the brown-rot decay mechanism.

### E018

Interacting Proteins in Salmonella enterica Can be Purified with Sequential Peptide Affinity Purification System Hye won Jeong and Iel Soo Bang<sup>\*</sup>

Department of Microbiology and Immunology, Chosun University School of Dentistry

Identification of interacting proteins in the cell mostly depends on *in vitro* immunoprecipitation (IP) methods using purified recombinant proteins. However, this method frequently produces arbitrary results that varies depending on the number of cells or other external factors. In this study aimed to find substrate proteins of a periplasmic chaperone protein Spy in Salmonella enterica, we applied the Sequential Peptide Affinity (SPA) purification system developed previously for analyzing E. coli interactome under physiological conditions without overexpressing genes or purifying target proteins. The spy-SPA gene fusion was constructed in Salmonella chromosome to express fusion proteins in the natural regulation of gene expression, in which the C-terminal end of Spy was sequentially fused with Calmodulin Binding Peptide (CBP) and 3X FLAG tags that are separated by a protease cleavage site. LC-MS/MS analysis on proteins isolated through SPA purification system presented potential substrate proteins that Spy may interact in Salmonella cultured in complex media or nutrient-limited media. Although further study is required to refine purification methods, this study suggests that the SPA purification system may help to study protein interactions related to Salmonella pathogenesis in physiological conditions.

[Supported by grants from KEITI/ MOE and NRF of Korea]

# Combination of *In Silico* Simulation Technology with *In Vitro* Cellular Activity Assay to Predict the Toxicity of Indoor Air Bacteria

Mira Kim<sup>1</sup>, Sung Yoon Ahn<sup>2</sup>, Sang Woong Lee<sup>2</sup>, and Iel Soo Bang<sup>1\*</sup>

<sup>1</sup>Department of Microbiology and Immunology, Chosun University School of Dentistry, <sup>2</sup>Pattern Recognition and Machine Learning Lab, Department of AI Software, Gachon University

As observed in the COVID-19 pandemic, infectious diseases mediated by some pathogens can be more easily contracted by airborne transmission under indoor conditions. Indoor air contaminated by microorganisms including virus, bacteria, and fungi, or by pathogenic substances derived from them can endanger human health in daily life. Thus, identification of potential pathogens residing in the air and analysis on their toxicity are crucial in order to prevent disease dissemination and secure indoor air quality. In this study, we evaluated toxicities of bacteria that are frequently found in indoor air on human cell lines, and applied deep learning technology to analyze and predict potential toxicity of bacteria using their amino acids sequences by training the ProtBert model on toxic bacterial proteins and virulence factor proteins. This combinational analysis of *in vitro* evaluation of bacteria in indoor air by identifying the bacterial population composition.

[Supported by grants from KEITI/ MOE of Korea]

### E020

# **RpoS Activates the Transcription of RpoS-dependent Genes under Nitrosative Stress Conditions** Ji Eun Bae and Iel Soo Bang<sup>\*</sup>

Department of Microbiology and Immunology, Chosun University School of Dentistry

Bacterial should promote their fitness in order to survive against a variety of antimicrobial stresses encountered during infection in hosts. For systemic infection in animals, pathogenic bacteria have to resist to nitric oxide (NO) produced by the inducible nitric oxide synthase (iNOS) of phagocytes that can inhibit bacterial persistence. Reactive nitrogen species (RNS) mediated by NO, such as peroxynitrite and nitrite, inhibit bacterial growth by damaging bacterial macromolecules including enzyme complexes, nucleic acids, and membranes. In response to hostile changes in environment, bacteria utilize alternative sigma factors to rapidly initiate transcription of specific sets of stress-responsive genes to cope with stress. However, little is known about corresponding sigma factor for governing bacterial transcription responding to RNS stress. Through a transcriptome analysis, we found that the transcription of considerable numbers of RNS-inducible genes is regulated by the stationary sigma factor RpoS in *Salmonella enterica*. In this study, we will discuss the role of RpoS in *Salmonella* fitness under nitrosative stress conditions.

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# Regulatory Mechanism of Disaggregating Chaperone ClpG in Pseudomonas aeruginosa

Seong joon Moon, Hyunhee Kim, and Changhan Lee\* Department of Biological Sciences, Ajou University

Molecular chaperones are involved in the cellular protein homeostasis. Among various types of chaperones, disaggregating chaperones can unfold misfolded or aggregated proteins to recycle the proteins. ClpB is the most well-characterized disaggregating chaperone. Since this unfolding activity consumes huge ATP energy and it may unfold the native proteins, the activity of disaggregating activity requires finely tuned regulatory mechanism, for example the interaction between M-domain of ClpB and its cochaperone DnaK-DnaJ is required to activate chaperone activity of ClpB. Recently, ClpG disaggregating chaperone has discovered with novel functional mechanism in Pseudomonas aeruginosa. However, the regulation of its activity is largely unknown. Notably, M-domain of ClpG is shorten than M-domain of ClpB, and ClpG has unique C terminal extension domain (CTE), which is not found in ClpB. In this study, we constructed variants of ClpG containing mutation in M-domain and CTE. We observed the cellular distribution of ClpG by using a fluorescence microscopy. In addition, heat shock assay, and growth curve assay was performed with expression of ClpG variants to examine the regulatory role of M-domain and CTE in ClpG.

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# E022

**Investigation of Organic Hydroperoxide Sensing Mechanism in Xanthomonas campestris pv. campestris OhrR** Ian Jeong, Jang-Wan Son, Eun-Soo Han, Hyun-Ji Noh, Hyeon-Ji Lyu, and Jin-Won Lee<sup>\*</sup> Department of Life Science and Research Institute for Natural Sciences, Hanyang University

OhrR is a transcription factor which senses organic peroxide preferentially using cysteine residue(s). OhrR proteins are divided into two groups, 1-Cys and 2-Cys OhrR based on the peroxide-sensing mechanism. *Xanthomonas campestris* OhrR belonging to 2-Cys OhrR has three cysteines in monomer. C22 acts as a peroxidatic cysteine present in both 1-Cys and 2-Cys OhrR. Between additional cysteine residues (C127 and C131) near the C-terminus, it is known that only C127 can form intermolecular disulfide bond with peroxidatic cysteine and this intermolecular disulfide bond is essential for OhrR inactivation. In order to reveal more evidence, OhrR variants were cloned and purified. Finally, we observed dimerization by intermolecular disulfide bond in mutant OhrR(C127S) by Mass spectrometry. Also, we found the dissociation of OhrR from DNA through fluorescence anisotropy (FA) experiments. As a result, both C-terminal cysteine residues could form intermolecular disulfide bond with peroxidatic cysteine. Furthermore, without any cysteines in C-terminus or free cysteine, mutant OhrR(C127SC131S) could be dissociated from DNA right after CHP treatment. This is a comparable result to 1-Cys OhrR which requires low molecular weight thiols for rapid inactivation. In further study, additional *in vivo* experiments will reveal the exact sensing mechanisms.

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# The Anti-aggregation Activity of OPE1 in Humanized Yeast Model to Study Neurodegenerative Diseases

Juwon Jeong, Hyunhee Kim, and Changhan Lee<sup>\*</sup> Department of Biological Sciences, Ajou University

Neurodegenerative diseases are the facing problem due to the increase of aging population. Parkinson's disease is one of the most common neurodegenerative diseases and is characterized by the progressive cell death by formation of Lewy bodies containing misfolded and aggregated a-synuclein. In previous studies, we have shown surprising effects of bacterial protein OPE1 which can resolve a-synuclein aggregates *in vitro*. Here, we used humanized yeast which expresses human a-synuclein to examine the effect of OPE1 *in vivo*. Since yeast has high similarities to human cells, such as 60% of sequence homology to human orthologues, and also they share fundamental eukaryotic cell biology, it has been widely used to study human diseases. We co-expressed a-synuclein with OPE1, and observed the toxicity of a-synuclein as well as formation of cellular a-synuclein aggregates. Indeed, the expression of OPE1 exhibits protective effect against a-synuclein toxicity and also significantly reduces cellular aggregates. Furthermore, we demonstrate that OPE1 could show protective role in Huntington's disease protein which is associated with protein aggregation.

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# E024

**N-Terminal Truncated-elastase for Inhibition of Elastase Activity of** *Pseudomonas aeruginosa* Fatma Ali Allam and Joon-Hee Lee<sup>\*</sup>

Department of Pharmacy, College of Pharmacy, Pusan National University

*Pseudomonas aeruginosa* is associated with a wide range of infections and it is notorious for its multidrug resistance. *P. aeruginosa* produces several proteases as virulence factors. The major of them are elastase (LasB), elastase A (LasA), protease IV (PIV), and alkaline protease. LasB, a major elastase of *P. aeruginosa* is a metalloprotease that has been considered the key target for anti-Pseudomonas drug development. Here, different N-terminal truncations of LasB propeptide were obtained by deleting N-terminal region of propeptide from the junction site between signal peptide and propeptide. 12 amino acids were serially deleted for each truncation. While truncation N1 had little inhibition effect on the LasB activity, all other truncations (N2-N7) greatly reduced the LasB activity. we also discovered that these N-terminal truncation of LasB propeptide inhibited the production of pyocyanin and the bacterial growth. We think that these modified propeptides can affect the pathogenicity of *P. aeruginosa*.

[This work was supported by a grant from the National Research Foundation of Korea funded by the Korean government (NRF-2019R1A2C1010087)+BK.]

## Functional Study of the Fermented Dahlia Extract Using Specific Environment-derived Bacteria as Active Ingredient in Cosmeceuticals

Joo Young Yang<sup>1</sup>, Min-Kyeong Kim<sup>1</sup>, Ji Yeong Park<sup>1</sup>, Sondor Ganbat<sup>1</sup>, Bo Hyeon Park<sup>1</sup>, Dariimaa Ganbat<sup>1</sup>, Mi Hwa Park<sup>2</sup>, Sung Tae Kim<sup>3</sup>, and Sang-Jae Lee<sup>1\*</sup>

<sup>1</sup>Department of Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University, <sup>2</sup>Department of Food and Nutrition, Silla University, <sup>3</sup>Department of Pharmaceutical Engineering, Inje University

Fermentation using microorganisms has been considered a useful biotransformation method to improve health value. The goal of this study is to develop cosmetics with a fermented extract from *Dahlia pinnata*. The composition of the dahlia extract was found to be high with a protein concentration of 19-20% and a sugar content of 60 Brix. Among 1500 isolates that have been isolated from various specific environments, extracellular protease and amylase-producing strains with qualified presumption safety status belonging to the family *Bacillaceae* were screened for dahlia extract fermentation. Consequently, suitable strains for fermentation of dahlia extract were *Bacillus licheniformis* R450403 derived from salt and *Bacillus amyloliquefaciens* CTC 2-5-1 derived from fermented seafood. Compared with unfermented dahlia extract, the fermented dahlia extract increased water-soluble protein content (BCA), antioxidants (DPPH, ABTS), and total polyphenol content (TPC), indicating that the antioxidant efficacy was increased through fermentation. Dahlia fermented extract can be added to various cosmetics to provide antioxidant activity. In addition, the dahlia petals can be used as a natural colorant for cosmetics. [This work was supported by National Research Foundation of Korea (NRF) grant (2020R1F1A1076624), the Technology Innovation Program (20015807) funded by the Ministry of Trade, Industry & Energy, and by grants from the Ministry of Ocean and Fisheries (PM62830)]

#### E026

## Enhancement of Anti-inflammatory and Anti-osteoporosis Effects of Fermented *Abelmoschus Manihot* L. by *Bacillus licheniformis* CP6

Min Kyeong Kim<sup>1</sup>, Yu Jeong Yeom<sup>1</sup>, Hae Rang Lee<sup>1</sup>, KwangSoo Jung<sup>1</sup>, Seong-Bo Kim<sup>2</sup>, Yong-Jik Lee<sup>3</sup>, Dong-Woo Lee<sup>4</sup>, Sang-Jae Lee<sup>1</sup>, and Mi-Hwa Park<sup>5\*</sup>

<sup>1</sup>Department of Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University, <sup>2</sup>Department of Bio-Living Engineering Major, Global Leaders College, Yonsei University, <sup>3</sup>Department of Biocosmetics, Seowon University, <sup>4</sup>Department of Biotechnology, Yonsei University, <sup>5</sup>Department of Food and Nutrition, Silla University

A *Abelmoschus Manihot* L. (AM) was fermented by *Bacillus licheniformis* CP6 at 37°C for 1 days (CP6-1D). And we investigated the anti-inflammatory effects on LPS - stimulated RAW 264.7 macrophages and the effects on osteoblast differentiation in MC3T3-E1 cells. According to our findings, the CP6-1D suppressed ROS, and the expression of iNOS and COX-2. Also, CP6-1D showed inhibitory effect on the production of pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α. Moreover, nuclear translocation of NF-κB and phosphorylation of MAP kinase were strongly suppressed by CP6-1D in LPS-stimulated RAW 264.7 cells. Furthermore, CP6-1D increased cell proliferation in MC3T3-E1 osteoblasts. Compared to incubated AM treatment, CP6-1D markedly promoted alkaline phosphatase activity and mineralization. Alizarin Red S staining demonstrated that CP6-1D treatment tended to increase extracellular matrix calcium accumulation. Taken together, our data suggests that fermentation may be a useful strategy for improving the biological properties of raw materials including their anti-inflammatory and anti-osteoporosis properties. Thus, CP6-1D is suggested to be a preventive medicinal food against inflammatory bone disorders.

[This work was supported by National Research Foundation of Korea (NRF) grant (2020R1F1A1076624), the Technology Innovation Program (20015807) funded by the Ministry of Trade, Industry & Energy, and by grants from the Ministry of Ocean and Fisheries (PM62830)]

### Effects of Ornithine Lipids on Biofilm Architecture

Huiyan Li, Alexandra Winkler, Wen-Xin Niu, Hyeon-Ji Hwang, and Joon-Hee Lee<sup>\*</sup> Department of Pharmacy, College of Pharmacy, Pusan National University

Biofilm formation is a integral part of infection for opportunistic pathogens. Often, *P. aeruginosa* encounters a variety of environmental conditions during infection and adapt itself to these changes. In phosphate-limiting conditions, *P. aeruginosa* replace the typical phospholipids in the membrane with non-phosphorus lipids like ornithine lipids (OLs). We studied the biofilm formation in phosphate-limiting condition and resulting the physiological state producing high-level non-phosphorus lipids in membrane. For this, we addressed two different conditions: one was an artificial condition in which we overexpressed the olsBA operon, the other was a natural condition in which we exposed P. aeruginosa to a low phosphate condition. The results showed more biofilm formation and higher intracellular c-di-GMP levels both in artificial and natural conditions. As OLs increased in the membrane, the overall net charge of the surface of the cell changed. We hypothesized that exopolysaccharide architecture in the biofilm may also change at the same situation. To investigate this, we integrated the GFP gene to PAO1 to examine the overall biomass of the biofilm, and then enhanced OL production in a flow cell system. Finally, we stained the biofilm with Pel- and Psl- specific lectins to examine the biofilm architecture under a confocal microscope.

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## E028

Effect of Dephosphorylated EIIA<sup>NAG</sup> on FakA-FakB Complex in *Faecalibacterium prausnitzii* A2-165 Jimin Lee and Yeong-Jae Seok<sup>\*</sup>

School of Biological Sciences and Institute of Microbiology, Seoul National University

*Faecalibacterium prausnitzii* is a Gram-positive and strictly anaerobic bacterium that has recently been suggested as a major gut microbe due to its prevalence in the healthy human intestine. To understand what a factor allows *F. praustnitzii* to successfully thrive in the human gut, it is necessary to figure out how it affects the physiological function of the cell. Phosphoenolpyruvate (PEP): carbohydrate phosphotransferase system (PTS) is an efficient carbohydrate transport system widely shared in the bacterial species that catalyzes the consecutive phosphorylation. Beside the role in sugar translocation, PTS proteins are known to regulate various cell physiologies through the phosphorylation-dependent protein-protein interactions. Fatty acid kinase (Fak) is required for the activation of exogenous fatty acids (exoFAs) and widely distributed in Gram-positive bacteria. This Fak system consists of a kinase protein (FakA) and the FA-binding protein (FakB). In this study, we revealed that, a FakB in *F. prausnitzii* strain A2-165 (*Fp*FakB) interacts with the dephosphorylated EIIA domain of EII<sup>NAG</sup> (EIIA<sup>NAG</sup>). The present data suggest that the dephosphorylated EIIA<sup>NAG</sup> sequesters *Fp*FakB from *Fp*FakA. We report a novel role for *F. prausnitzii* PTS in that the regulation of the Fak complex formation by EIIA<sup>NAG</sup> specific for N-acetylglucosamine, a PTS sugar, might change the ability to activate external FA from host. [Supported by grants from BK 21 Plus Program from South Korea.]

## Strategies of Two Kinds of Leader Peptides in NiSOD for the Generation of N-Terminal Histidine

Eun-Soo Han, Jang-Wan Son, Hyun-Ji Noh, Hyeon-Ji Lyu, Ian Jeong, and Jin-Won Lee<sup>\*</sup> Department of Life Science and Research Institute for Natural Sciences, Hanyang University

Superoxide dismutase (SOD) is an enzyme which removes superoxide to protect cells from ROS stress. Nickel Superoxide dismutase (NiSOD) contains Nickel in its active site. NiSOD subunits have each 'Nickel hook motif (His-Cys-X-X-Pro-Cys-Gly-X-Tyr)'. NiSOD activity relies on the exposure of the first amino acid of the Nickel hook motif to the N-terminal. However, in *Streptomyces*, where NiSOD was first discovered, NiSOD has a leader peptide before maturation. The leader peptide of NiSOD in *Streptomyces coelicolor* has a similar structure to signal peptide, but it doesn't have H-region of signal peptide. In the case of *S. coelicolor*, the leader peptide is cleaved by SODX, which is a putative signal peptidase. As a result, Histidine of Nickel hook is exposed as the N-terminal's first residue and the matured NiSOD shows activity. Furthermore, as a result of analyzing the NiSOD sequence of other types of bacteria, *Hirschia baltica* has a complete signal peptide as a leader peptide. *H. baltica*'s NiSOD also shows activity only when Histidine is exposed to N-terminal. This was proved by using *Shewanella violacea* and *Coraliomargarita akajimensis* which have the same leader peptide structure as *S. coelicolor* and *H. baltica*, respectively. Therefore, we propose that the leader peptide of NISOD serves as 1. location, 2. solving the N-terminal Methionine problem, and 3. for the Generation of N-terminal histidine in N-end rule pathway.

[Supported by Science Research Center grant and 2019R1F1A1061890.]

#### E030

**Quorum Sensing Protein VqmA Senses Glucose-induced Host Signals through HPr in Vibrio vulnificus** Gahee Park<sup>1</sup>, Jaehun Lee<sup>1</sup>, Kyoo Heo<sup>2</sup>, and Yeong-Jae Seok<sup>1\*</sup> <sup>1</sup>Seoul National University School of Biological Sciences and Institute of Microbiology, <sup>2</sup>Harvard Medical School of Microbiome Metabolomics Lab

Bacteria recognize and respond to a variety of extracellular environments. Quorum sensing is a cell-to-cell communication process that allows bacteria to regulate genes in response to changes in surrounding bacterial cell density and species composition. In *Vibrio cholerae*, VqmA-VqmR quorum sensing pathway regulates pathogenesis and senses host-derived signals. Here, I identify the structural importance of VqmA to VqmA-VqmR pathway in *Vibrio vulnificus*. Transcription factor VqmA activates expression of *vqmR* and affects pathogenicity in the mouse infection model. The expression level of *vqmR* is increased in the presence of glucose because dephosphorylated HPr interacts with VqmA and increases the transcriptional activity of VqmA. The Interaction between HPr and VqmA is highly species-specific. Also in *Vibrio vulnificus*, VqmA PAS domain has some different amino acid residues compared to other Vibrio species, which can be considered to form a specific structural form with HPr. VqmA amino acid mutant in PAS domain loose its binding affinity to HPr. And mutations of other amino acids, expected to interact with HPr histidine residue, loose their repulsion power to phosphoryl residue in HPr. These structural features of VqmA in *Vibrio vulnificus* give advantages to virulence regulation in different environment. I propose that *Vibrio vulnificus* recognizes the infection niche through the interaction between VqmA and HPr and regulates pathogenicity.

[Supported by grants from BK]

### Sir2 Inhibits Mitophagy during the Stationary Phase in Yeast

Joohye Jung, Ji In Ryu, and Jeong Yoon Kim\*

Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University

Selective autophagy that degrades organelles to control their quality and mass is activated by various external stimuli, such as oxidative stress or nutrient starvation conditions. In this study, we investigated whether Sir2, an NAD-dependent histone deacetylase, plays a role in selective autophagy during the stationary phase in yeast. Here, we report that selective autophagy, especially mitophagy which selectively eliminates damaged or dysfunctional mitochondria, was significantly increased in yeast cells lacking Sir2, compared to wild-type cells. Mitochondrial membrane potential and ROS level were increased in the sir2 $\Delta$  strain in the stationary phase. We analyzed the expression of key autophagy/mitophagy regulators, and found that protein level and phosphorylation of Atg32 were increased in the sir2 $\Delta$  strain compared to wild-type cells, although the mRNA level of ATG32 was not increased. This suggests the possibility that Sir2 may affect mitophagy through the regulation of Atg32 at post-transcriptional or post-translational stage.

[This work was supported by grants from the National Research Foundation of Korea (NRF-2020R1A2C10102541331482092640103).]

## E032

## The Role of 267 Heliorhodopsin to Related Proteins in the Same Operon

Sang-ji Lee, Chenda Hour, and Kwang-Hwan Jung<sup>\*</sup> Department of Life Science and Institute of Biological Interfaces, Sogang University

Heliorhodopsin (HeR), a retinal-binding membrane protein, has been recently discovered by metagenomic analysis, however its function remains unknown. HeR has all-*trans* retinal chromophore like microbial rhodopsin. It showed less than 15 % sequence identities with microbial rhodopsin and N-terminal side is located on the intracellular side opposite to other microbial rhodopsin. HeRs are widespread in bacteria, archaea, algae, and some viruses, and we chose one of those HeRs, herein named 267 HeR. We characterized the 267 HeR using its pumping property and pKa measurement. We checked the DNA sequences around 267 HeR and found three more proteins, named protein A, B and C in the same operon. For protein A and B, we assumed that those are involved in catalyzing glycosyl transfer. Protein C is predicted as a nucleobase permease. We successfully expressed and purified two soluble proteins, protein A and B, one membrane protein, protein C, and 267 HeR. After the purification, the interaction between 267 HeR and each of those three proteins were confirmed by isothermal titration calorimetry (ITC) respectively. We also tried to figure out the functions of those three related proteins. And for now, we are conducting the enzyme assay of protein B. We are planning to test how the 267 HeR regulates other proteins near the HeR gene.

[Supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF-2018R1A6A1A03024940)]

## Characterization of Heliorhodopsin to Interact with the Other Enzyme in Same Operon

Seo-Hyun You, Keo Eng, and Kwang-Hwan Jung<sup>\*</sup> Department of Life Science and Institute of Biological Interfaces, Sogang University

Microbial rhodopsin has an all-*trans* retinal which is attached to the Lys of the opsin to form a Schiff base, and it is converted to 13-*cis* retinal by light activation, resulting in protein structural changes to function. Recently, heliorhodopsin (HeR) has been studied and is known to be type-I rhodopsin. The structural difference from other rhodopsins is that HeR located in an N-terminus on the cytoplasmic side and a C-terminus in the outer membrane. To reveal the function of HeR, we investigated HeR found in Gram positive bacteria. The HeR is known to be located close to the other gene (protein A) of the bacteria. The HeR gene was isolated for characterization of functional roles. We determined HeR binds to protein A via lsothermal Titration Calorimetry analysis. Since the protein A nearby the HeR gene, we were looking for the promoter region for protein A which has been predicted to the transcriptional regulator. When the promoter prediction is completed, the interaction between protein A and promoter region will be tested by selecting the target gene group of the transcriptional regulator. We would like to conduct how to control the interaction between those proteins in the presence or absence of light. It would be able to find out whether the transcriptional regulator is affected by the conformational changes of HeR. [Supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF-2018R1A6A1A03024940).]

## E034

**Discovery of a Novel Function of Photoreceptor in the Regulation of Glutamine Synthetase Activity** Shin-Gyu Cho<sup>1,2</sup>, Myungchul Song<sup>1</sup>, and Kwang-Hwan Jung<sup>1\*</sup> <sup>1</sup>Department of Life Science and Institute of Biological Interfaces, Sogang University, <sup>2</sup>Research Institute for Basic Science, Sogang University

Most rhodopsin, one of the groups of photoreceptors that respond to light and relay signals into the cells, is lightdriven seven-transmembrane retinal-binding protein found in prokaryotic and eukaryotic organisms. The rhodopsins participate in visual and non-visual phototransduction in animals and transport of ions and signal transduction in microbes. Unlike the known rhodopsins, heliorhodopsin, which is invertedly embedded, has been recently discovered; however, its function remains unknown. Herein, we investigated the relationship between *Actinobacteria bacterium* IMCC26103 heliorhodopsin (AbHeR) and an adjacent glutamine synthetase (AbGS) in the same operon. We demonstrate that AbHeR binds to AbGS and regulates AbGS activity. We also confirm that AbHeR upregulates the biosynthetic enzyme activity of AbGS both *in vitro* and *in vivo* in the presence of light. GS is a key enzyme involved in nitrogen assimilation that catalyzes the conversion of glutamate and ammonia to glutamine. Hence, the interaction between AbHeR and AbGS may be critical for nitrogen assimilation as the bacterium survives in low-nutrient environments. Overall, our findings describe, for the first time, a novel function of heliorhodopsin as a regulatory rhodopsin with the capacity to bind and regulate enzyme activity required for nitrogen assimilation. [Supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF-2018R1A6A1A03024940).]

## Methylglyoxal-Detoxifying Enzyme Activities Regulate GSH-independent Antioxidative Enzymes in Glutathionedepleted Yeast

Jaeyoung Son and Min-Kyu Kwak<sup>\*</sup>

Laboratory of Microbiological Physiology and Biotechnology, Department of Food and Nutrition, College of Bio-Convergence, Institute of Food and Nutrition Science, Eulji University

We previously demonstrated that NAD(H)-linked methylglyoxal oxidoreductase (Mgd1/Grp2) and alcohol dehydrogenase (Adh1) are induced by glutathione depletion in  $\Delta gcs1$  ( $\gamma$ -glutamylcysteine synthetase). However, experiments of reciprocal relationships between methylglyoxal-scavenging and reactive oxygen species-detoxifying enzyme activities of Mgd1/Grp2 and Adh1 exerted by glutathione reductase (Glr1) activity on glutathione-independent enzyme activities/metabolites remain unclear. Based on our experiments on methylglyoxal accumulation in ( $\Delta mgd1/grp2$ )/ $\Delta gcs1$  and  $\Delta adh1/\Delta gcs1$ , we modeled an MGD1/GRP2 and ADH1 triple disruptant, (( $\Delta mgd1/grp2$ )/ $\Delta adh1/\Delta gcs1$ ), derived from non-hyphae-inducing and glutathione-depleted  $\Delta gcs1$ . Contrary to  $\Delta gcs1$ , ( $\Delta mgd1/grp2$ )/ $\Delta adh1/\Delta gcs1$ , respectively. Unexpectedly, glutathione-independent antioxidative enzyme activities, those of erythroascorbate peroxidase and cytochrome *c* peroxidase, were remarkably higher in ( $\Delta mgd1/grp2$ )/ $\Delta adh1/\Delta gcs1$  than in double gene-disrupted mutants. The observed Glr1 activity almost disappeared entirely in all disruptants. Our data demonstrate the reciprocal use of Eapx1 and Ccp1 in the absence of both Mgd1/Grp2 and Adh1 activities, pivotal for viability in non-filamentous budding yeast.

#### E036

# Two-component Response Regulator BldM in *Streptomyces coelicolor* A3 (2) Triggers Phenotypic Switching Accompanied by Two-component Regulatory Proteins

Yeonju Hong and Min-Kyu Kwak<sup>\*</sup>

Laboratory of Microbiological Physiology and Biotechnology, Department of Food and Nutrition, College of Bio-Convergence, Institute of Food and Nutrition Science, Eulji University

A putative two-component regulator, BldM protein, is required for aerial mycelium formation in *Streptomyces coelicolor*. However, experimental evidence is scarce for bldM gene disruption and/or overexpression effects on phenotypic/physiological alterations. The mutagenesis strain via point mutation at D54 of SCO4768 (BldM) was examined to assess pseudo-phosphorylation and phenotypic/functional roles of BldM. The *bldM* disruption on phenotypic switching in *bldM* gene-disrupted SDM1 showed typical phenotypic switching presumably due to reciprocal/non-reciprocal *bldM* expression regulated by the dependence on its regulating genes. Through combinations of two-dimensional protein gel electrophoresis with a matrix-assisted laser desorption/ionization time of flight mass spectrometry, genes regulated by BldM were identified/characterized. Among the identified genes, we confirmed the reciprocal expression of genes regulated by *bldM* using northern analysis, which allowed identification of proteins whose expression was different between the wild-type and *bldM* disruptants. The absence of *bldM* stimulated two-component regulator), SCO2343 (acetyltransferase), and SCO5656 (transcription regulator). Our findings suggest that BldM participates in phenotypic switching accompanied by two-component regulatory proteins in *S. coelicolor*.

# Interplay between Alcohol Dehydrogenase 1 and NAD(H)-linked Methylglyoxal Oxidoreductase Activities in *Candida albicans*

Ji-Yoon Yeo and Min-Kyu Kwak<sup>\*</sup>

Laboratory of Microbiological Physiology and Biotechnology, Department of Food and Nutrition, College of Bio-Convergence, Institute of Food and Nutrition Science, Eulji University

We previously demonstrated two methylglyoxal scavengers, including NAD(H)-linked methylglyoxal oxidoreductase [Mgd1(Grp2)] and alcohol dehydrogenase 1 (Adh1), in glutathione-depleted  $\gamma$ -glutamyl cysteinyl synthetase (*GCS1*)-disrupted *Candida albicans*. However, evidence for *C. albicans* pathophysiology lacking the enzyme activities of Mgd1(Grp2) and Adh1 on glutathione-dependent redox regulation remains unclear. (*Amgd1/grp2*)/*Aadh1* showed severe growth defects and G1-phase cell cycle arrest. Supraphysiological accumulation of methylglyoxal and pyruvate was observed in (*Amgd1/grp2*)/*Aadh1* but not in *Amgd1/grp2* and *Aadh1*. Cellular H<sub>2</sub>O<sub>2</sub> and superoxide were significantly increased in *Amgd1/grp2* and *Aadh1*, contrary to the decrease in (*Amgd1/grp2*)/*Aadh1*. Despite the experimental findings underlining the importance of the undergoing unbalanced redox state of (*Amgd1/grp2*)/*Aadh1*, some glutathione-independent antioxidative enzyme activities did not change significantly during proliferation and filamentation. Interestingly, contrary to the significantly lowered glutathione level and Glr1 enzyme activity, the activity staining-based glutathione peroxidase activities were concomitantly increased in this mutant. This suggests that glutathione-dependent redox regulation is evidently associated with *C. albicans* pathogenicity under the control of methylglyoxal-scavenging activities.

#### E038

**Characterization of 23 Heliorhodospin and Binding Interaction with Its Related Soluble Transducer Protein** In-Jung Choi and Kwang-Hwan Jung<sup>\*</sup>

Department of Life Science and Institute of Biological Interfaces, Sogang University

Photoreceptor proteins are used to sense and respond to light in living organisms. Rhodopsin is the pigmentcontaining retinal that converts light into an electrical signal. Microbial rhodopsin (type I) is photoreceptive membrane protein containing a retinal chromophore and Animal rhodopsin (type II) acts as photoreceptor for vision. Heliorhodopsin (HeR) has been recently discovered as novel rhodopsin family. Especially, unlike other two types of rhodopsins, N-terminal of the HeR faces the cytoplasm. However, the function of HeR remains unknown. Here in this study, the 23 HeR was isolated from bacteria and its biological studies were performed to reveal the physiological roles in the cell. In results, we characterized the 23 HeR by measuring pumping activity and pKa titration. In addition, several genes nearby 23 HeR gene was found in the same operon with "Protein E". As a result of testing the binding interaction between 23 HeR and protein E by isothermal titration calorimetry (ITC), we assumed 23 HeR was expected to interact and work with protein E. We are currently conducting an enzyme assay to check whether protein E has enzymatic activity or not. Moreover, we are planning to figure out that there will be an effect on protein E's function under light illumination.

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## Heliorhodopsin in Marine Microbe Works with Cooperative Partners and Regulated Their Function by Light Ji-eun Lee, Brian H. Kim, and Kwang-Hwan Jung<sup>\*</sup>

Department of Life Science and Institute of Biological Interfaces, Sogang University

Microbial rhodopsin is a retinal binding protein which absorb light and conduct several photochemical reactions. Bacteriorhodopsin (BR) which is firstly discovered in 1971 act as a light-driven outward proton pump. Retinal molecule inside BR makes a conformational change from all-trans to 13-cis when it absorbs photons and causes proton movement from inside to outside. Heliorhodopsins (HeRs) are recently found in rhodopsin family and have high sequence similarity with microbial rhodopsins. However, the direction of N-terminal is reversed which is facing cytoplasmic side compared with other rhodopsins. Most of microbial rhodopsins have their function as an ion pump, ion channel, and light sensor but HeRs is still unclear. One of the heliorhodopsin, 79-HeR is a protein from marine microbe. Characterization of 79-HeR were done by measuring absorption maxima, pumping activity and pKa. We demonstrated the unknown function of 79-HeR by investigating the interaction between 79-HeR and 'protein A' in the same operon. Their binding was confirmed by ITC and functional regulation under light was verified by protein analysis techniques *in vitro*.

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#### E040

#### How Can Antibiotic Resistance Factor Protein (ErmN) Acquire High Susceptibility to Chymotrypsin?

Hyung Jong Jin<sup>\*</sup>, Lanke Lin, Xiaofan Liu, Jinrong Chen, Ke Chen, Jae Hyuk Lee, and Tae Suk Jung *Department of Bioscience and Biotechnology, The University of Suwon* 

ErmN, one of the MLS<sub>B</sub> antibiotic resistance factor proteins which methylate a specific adenine residue (A2058 in *E. coli* coordinate) to reduce the affinity of antibiotics thereby conferring the resistance on the microorganisms, appeared to be highly susceptible to chymotrypsin activity. In this report, the underlying mechanism for this observation was pursued. ErmN was found to contain a highly susceptible recognition site (F45) as evidenced by a digestion band (band 1) that represented greater than 80% of the total band intensity after 30 second treatment with chymotrypsin. The exposure rate of hydrophobic core by the action of chymotrypsin was more than 67-fold and 104-fold faster than in ErmN than those in ErmS and ErmE, respectively. After cleavage at F45, some of the hydrophobic interactions were disrupted and further digestion by chymotrypsin could occur. Further digestion of band 1 occurred through the exposed F163 with a half-life of 3.18 min. After 30 min, less than 1% of ErmN remained. On the basis of the structure of ErmC', the location of F was presumed to be in an  $\alpha$  helix at the bottom of a cavity. Both substitution of most common amino acids such as isoleucine, valine, or leucine with phenylalanine (ErmH, ErmI, ErmN, and ErmZ out of 44 known Erms) and the apparent added flexibility, which could be provided by the additional loop region attached to the phenylalanine that is four to nine amino acids longer (ErmI, ErmN, and ErmZ, which form one cluster in the phylogenetic tree) than other Erm proteins, could cause unusually high susceptibility.

### Engineering RNA Phage Coat Protein Based on Insertion-tolerance Regions

Shin-Yae Choi, Hee-Won Bae, and You-Hee Cho<sup>\*</sup> Department of Pharmacy, College of Pharmacy and Institute of Pharmaceutical Sciences, CHA University

Bacteriophages (phages) have been regarded as valuable platforms for virus-based biopharmaceuticals. Despite the utility as biological nanoparticles, RNA phages have been underappreciated most likely due to difficulties in genetic engineering. To overcome this issue, we created the reverse genetic systems for the well-known fiersphages, PP7 and MS2. These systems are based on the cDNA of the RNA phages, whose transcripts derived from bacterial RNA polymerases act not only as the primary mRNA for phage protein synthesis, but also as the template for phage RNA replicases. To further harness the RNA phage nanoparticles for engineering purposes, we have identified the insertion-tolerance regions (ITRs) on the phage genomes, by virtue of *in vitro* transposon mutagenesis using MuA transposase. We have been focusing the ITRs identified at the coat protein (CP) to have the phage particles tagged with heterologous proteins and/or peptides. Topics discussed will include our data on characterization of the CP ITRs to generate the recombinant RNA phage virions with functional modalities.

## F002

**The Tol-Pal System Plays an Important Role in Maintaining Cell Intergrity During Elongation in** *Escherichia coli* Sohee Park and Hongbaek Cho<sup>\*</sup>

Department of Biological Sciences, College of Natural Sciences, Sungkyunkwan University

The Tol-Pal system is a transenvelope complex widely conserved among Gram-negative bacteria. It is recruited to the septal ring during cytokinesis, and its inactivation causes pleiotropic phenotypes mainly associated with the division process. From our genetic screen to identify factors required for delaying lysis upon treatment of beta lactams, we discovered that the tol-pal mutant shares similar defects with mutants of the major class A PBP system in terms of lysis prevention. Inactivation of the Tol-Pal system and the PBP1b-LpoB system leads to lysis during cell elongation as well as during division. Moreover, production of the Lpo activator-bypass PBP1b, but not wild-type PBP1b, partially suppressed the Tol-Pal defect in maintaining cell integrity upon treatment of mecillinam specific for the Rod system, suggesting that the Tol-Pal system plays an important role in maintaining cell wall integrity during elongation in addition to its multifaceted roles during cytokinesis.

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## **Mechanism of** *Candida albicans* **Apoptotic Factor CaNma111 and CaYbh3 in Filamentation and Pathogenicity** Suyoung Kim and Jinmi Kim<sup>\*</sup>

Department of Microbiology and Molecular biology, College of Bioscience and Biotechnology, Chungnam National University

The yeast *Candida albicans* is an opportunistic fungal pathogens. The ability to switch from yeast to hyphae form is a critical virulence determinant. Hyphae form is invasive and proper to tissue penetration and survival. Previous reports has confirmed that apoptosis and pathogenicity are closely associated in *C. albicans*. CaNma111 and CaYbh3 were shown to function as pro-apoptotic regulators. And also CaNma111 and CaYbh3 are involved in the filamentous growth or virulence of *C. albicans*. The hyphal growths of *Canma111/Canma111* and *Caybh3/Caybh3* strains were examined in hyphal inducing medium. *Canma111/Canma111* and *Caybh3/Caybh3* deletion strains showed hyperfilamentation phenotypes than wild-type strain. Next I investigate whether CaNma111 and Caybh3 interact functionally with Nrg1, Tup1, a negative regulator for hyphae formation, and Cph1, a positive regulator. The protein level of Nrg1 was decreased in *Caybh3/Caybh3* deletion strain as compared with the wild type strain. Tup1 protein level was decreased in *Canma111/Canma111* and *Caybh3/Caybh3* deletion strains. But Cph1 protein level was similar with wild type strain. These results suggest that CaNma111 and Caybh3 interact with Nrg1 and Tup1, negative regulator for hyphae formation.

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#### F004

## Role of Puf Protein Families during Yeast Filamentous Growth

Dohyeon Kim and Jinmi Kim<sup>\*</sup>

Department of Microbiology and Molecular biology, College of Bioscience and Biotechnology, Chungnam National University

The *PUF* family of RNA-binding proteins are posttranscriptional regulators. Pufs bind to specific recognition sequences in the 3' untranslated regions of mRNAs to control the stability, translation and localization. Several studies have revealed that Puf protein is involved in the mating switch. In this study, experiments were conducted to examine whether *PUF* protein could affect the filamentation of diploid, which shares the core protein with the mating process of haploids. In a previous study using haploid strains, it was found that Puf6 negatively regulates protein levels of *Ste12*, a transcription factor for mating/filamentous genes. In this experiment, diploid strains were constructed in a  $\Sigma$ 1278b strain capable of filament growth. As a result of examining the protein expression and mRNA localization of Ste12 and Tec1 transcription factors of filamentous genes, Puf6 and Puf5 suppressed the expression of STE12 and TEC1.

[This work was supported by a research grant from the National Research Foundation of Korea]

## Anaerobic Expression of the *Vibrio vulnificus* Nrf, Periplasmic Nitrite Reductase, is Activated by the Iron-Fur Complex

Young-Jin Song, Jeong-A Kim, Dae-Keun Kwon, Hyun-Jung Lee, and Kyu-Ho Lee<sup>\*</sup> Molecular Microbiology and Biofilm Lab., Department of Life Science, Sogang University

Reactive nitrogen species (RNS) including nitrite are generated by the macrophages exposed to *Vibrio vulnificus*, which would be highly detrimental to the survival of this pathogen. *V. vulnificus*, however, could reduce the extracellular concentrations of nitrite through its reduction to ammonia using the periplasmic Nrf system. Thus, installation of the Nrf system is an essential prerequisite for *in-vivo* survival and further colonization of *V. vulnificus* in front of the host-derived RNS. To characterize the expressional regulation of the genes encoding the Nrf system in *V. vulnificus*, its multiple transcription units were examined. Under the anaerobic conditions, the transcription of these genes by Fur, it was required the presence of cellular ferrous irons available to form a holo-Fur that was able to directly bind the upstream regions of both loci. Further activation of the transcription of both *nrfA* and *nrfBCDEF* occurred in *V. vulnificus* cells upon an exposure to nitrite. This induction was mediated via coactivation by both an anaerobic condition-specific FNR and a nitrite-sensing NarQP. Therefore, this study showed that *V. vulnificus* could elaborately and simultaneously sense various parameters in the host environments via utilizing multiple transcription regulators to respond for efficient detoxification of RNS.

#### F006

## Elucidating the Roles of the Casein Kinase 2 Complex in the Growth, Differentiation, Stress Responses, and Pathogenicity of *Cryptococcus neoformans*

Yeseul Choi<sup>1</sup>, Seong-Ryong Yu<sup>1</sup>, Ann-Yae Na<sup>2</sup>, Sangkyu Lee<sup>2</sup>, and Yong-Sun Bahn<sup>1\*</sup> <sup>1</sup>Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, <sup>2</sup>College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University

The basidiomycete human fungal pathogen *Cryptococcus neoformans* causes fatal meningoencephalitis. However, the therapeutic options for treatment are currently highly limited. As a potential antifungal drug target, kinases have been considered to be good candidates as some of them play critical roles in virulence of pathogens. In our previous studies, we demonstrated that Cka1, which is a serine/threonine kinase and the catalytic subunit of the casein kinase 2 (CK2) complex. In this study, we aim to figure out the regulatory mechanism of the CK2 complex in *C. neoformans*. The cryptococcal CK2 complex consists of Cka1 and two regulatory subunits, Ckb1 and Ckb2. The *ckb1* $\Delta$ , *ckb2* $\Delta$ , and *ckb1* $\Delta$  *ckb2* $\Delta$  mutants exhibited increased susceptibility to antifungal drugs, oxidative stress, and DNA damaging agents. Notably, however, the *cka1* $\Delta$  *ckb1* $\Delta$  *ckb2* $\Delta$  mutants showed more severe growth defects and greater stress susceptibility than the *cka1* $\Delta$  mutants, indicating that the regulatory subunits may have Cka1-independent functions. Supporting this, we found that the CK2 complex is required for maintaining normal cell cycle and morphology. Considering pleiotropic roles of the CK2 complex in *C. neoformans*, we elucidated its downstream effector genes and proteins through transcriptomics and phosphoproteomics analyses, respectively. In conclusion, this study provides a comprehensive insight into the function and regulatory mechanism of the fungal CK2 complex.

[Supported by grants from BK21 and NRF]

## A New Approach Using the SYBR Green-based Real-Time PCR Method for Detection of Soft Rot *Pectobacterium odoriferum* Associated with Kimchi Cabbage

Yong Ju Jin, Dawon Jo, Yu-na Jeon, Jeong-Seon Kim, Young-Joon Ko, Jegadeesh Raman, and Soo-Jin Kim<sup>\*</sup> Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration

*Pectobacterium odoriferum* is the primary causative agent in Kimchi cabbage soft rot diseases. The bacterial pathogens *Pectobacterium* genera are responsible for significant yield losses in crops. However, *P. odoriferum* possesses a narrow host range and causes soft-rot disease in storage vegetables. Therefore, the diagnosis process and management of soft rot are complex because developing an accurate diagnostic method is essential. In present study, identification novel species-specific genes for detecting and quantifying targeted *P. odoriferum* via comparative genomic approaches and designed a specific primer set targeting a HAC family hydrolase. This species-specific primer set formed a specific amplicon with a 360bp only in the DNA of *P. odoriferum*. In addition, SYBR green-based real-time qPCR using the primer set enabled estimating the population density of *P. odoriferum*. Consequently, the newly developed diagnostic method allows rapid and accurate diagnosis and continuous monitoring of soft rot disease in Kimchi cabbage.

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#### F008

**Microbial Community Analysis Using MinION Sequencing of Home-made Kimchi in Korea** Hwa Yong An, Chi Hwan Kim, So Yeon Kang, and Gyoungju Nah<sup>\*</sup> Genome Analysis Center, National Instrumentation Center for Environmental Management (NICEM), College of Agriculture and Life Sciences, Seoul National University

We analyzed bacterial community composition of 21 kimchi samples based on full-length sequences of 16S rRNA gene using the MinION. Genomic DNA extracted from the 21 liquids of kimchi was used as a template of the first PCR to amplify the16S rRNA gene, followed by library construction using barcoding PCR kit for MinION sequencing. As a result, the number of observed species of each 21 kimchi samples was counted to 305–783. In kimchi 18 samples except for three (No.9, 11, and 13) showed that phylum Firmicutes occupied more than 90%. In the No. 9 kimchi sample, Firmicutes and Proteobacteria were dominated more than 90% by 69.9% and 21.6%, respectively. In the No. 11 kimchi sample, Firmicutes and Proteobacteria were dominated by 75.7% and 24.1%, respectively. Phylogenetic analysis based on 16S rRNA sequences from the metagenome indicated that the kimchi microbiome was dominated by members of nine genera: *Weissella. Leuconostoc, Lactobacillus, Pediococcus, Pseudomonas, Bacillus, Lactococcus, Bradyrhizobium,* and *Delftia.* The result of bacterial community analysis showed that ten species were dominated in kimchi: *Weissella koreensis, Weissella cibaria, Leuconostoc gelidum, Leuconostoc mesenteroides, Leuconostoc citreum, Leuconostoc carnosum, Lactobacillus sakei, Lactobacillus plantarum, Lactobacillus brevis, and Pediococcus damnosus. In addition, four species, <i>Lactobacillus curvatus, Lactobacillus koreensis, Pseudomonas versuta,* were detected in kimchi.

# Research on the Genomic Functionality Using Industrial Animal-derived Microbial Genome Analysis and Bioinformatics

Sun-Min Lee<sup>1</sup>, Ji-Hye Yang<sup>1</sup>, Soo-Hyun Jung<sup>1</sup>, Ki-Nam Yoon<sup>2</sup>, Jae-Kyung Kim<sup>2</sup>, and Ju-Hoon Lee<sup>1\*</sup> <sup>1</sup>Department of Agricultural Biotechnology, Department of Food and Animal Biotechnology, Center for Food and Bioconvergence, Research Institute of Agriculture and Life Science, Seoul National University, <sup>2</sup>Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute

This study is the contents of genome analysis after receiving 102 intestinal microbial strains from the Korea Atomic Energy Research Institute and performing sequencing using a nanopore based long-read sequencing platform. In the case of microorganisms isolated from the intestine, it is difficult to identify the strain using only the 16S rRNA nucleotide sequence, so identification using comparative genomic analysis is essential. Therefore, 6 strains selected as excellent strains for antibacterial, anti-inflammatory, and enzyme production among 102 strains were identified through comparative genomic analysis with genomes registered in NCBI based on ANI (Average Nucleotide Identity) analysis. In addition, it is necessary to identify strain characteristics through safety evaluation at the genome level (antibiotic resistance gene and virulent factor search) and functional gene search.

As a result, virulent factors and antibiotic resistance genes were not found in the genome, and the existence of genes with the corresponding function was confirmed. Therefore, it is expected that the microbial genome analysis will contribute to the development of formulations by securing safety at the genome level and analyzing the characteristics of the strain.

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#### F010

## **Evolutionarily Conserved Roles of Histone Complex in DNA Damage Stress in** *Cryptococcus neoformans* Sunhak Kwon<sup>1,2</sup>, Jinhyung Park<sup>1</sup>, Jong-Hyun Jung<sup>1</sup>, Yong-Sun Bahn<sup>2</sup>, and Kwang-Woo Jung<sup>1\*</sup>

<sup>1</sup>Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, <sup>2</sup>Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

Genome stability is always threatened by external stresses and intracellular processes. Therefore, living organisms have several sophisticated regulation systems to protect their genome information. Cells activate their DNA repair machineries depending on the types of DNA damage. Before the completion of DNA repair, the cell cycle should be halted not to provide incorrect information to offspring. During DNA repair process, histone production is tightly coupled to ongoing DNA synthesis. Previous studies reveal that the Rad53-Bdr1 pathway regulates DNA damage responses by controlling expression levels of DNA repair genes in *Cryptococcus neoformans*. However, the relationship between the Rad53-Bdr1 pathway and histone production remains elusive. In this study, we found *HTA1* and *HTB1*, encoding H2A and H2B respectively, are required for growth. The deletion of any one histone H3 or H4 locus does not cause lethality because H3 and H4 are encoded by *HHT1/HHT2* and *HHF1/HHF2*, respectively. Expression levels of genes encoding core histone proteins were regulated by Rad53, not but Bdr1. Notably, expression of histone variant *HTZ1* was in a Rad53-independent manner during DNA damage stress and *htz1*Δ mutant was sensitive to DNA damage stress. Among core histones H3 and H4, perturbation of *HHT2* renders cells intrinsic growth defect and susceptible to diverse types of DNA damage insults. Taken together, core histone production is also required for DNA damage stress in *C. neoformans*.

## A Genome-wide Screening of Target Genes against Doxorubicin in Fission Yeast

Min Jung Kim and Kwang-Lae Hoe\*

Department of New Drug Discovery and Development, Chungnam National University

Doxorubicin (DOX)-based chemotherapy is one of the most effective methods for the treatment of human cancer. However, clinical use is limited due to serious side effects. Lansoprazole (LANSO) is a proton pump inhibitor. In this study, the synergistic effect of making the target more sensitive to doxorubicin was confirmed using lansoprazole based on the results of previous studies that discovered the target of doxorubicin in fission yeast. DOX treatment induced apoptosis in cells, reducing the viability of target cells in a dose and time dependent manner. Combination treatment with lansoprazole enhanced the cytotoxicity of dox, shortened the time to penetrate into the cell, and improved the accumulation in the cell. Therefore, these results suggest that the strategy of using dox and lanso together can be a very efficient method for achieving anticancer synergy against human cancer cells in yeast. [This research was supported by the National Research Foundation (NRF) grants from the Korea government, Ministry of Science, and ICT (NRF-2017M3A9B5060880)].

## F012

## Cultivation and Complete Genome Sequencing of Orange-pigmented Bacteria *Chryseobacterium oranimense* 33-M-3

Jegadeesh Raman, Jeong-Seon Kim, Young-Joon Ko, and Soo-Jin Kim<sup>\*</sup> Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration

*Chryseobacterium oranimense* 33-M-3 is isolated from forest soil, Suwon-si, Republic of Korea. The strain earlier decline phase was observed at 39 h incubation in R2B broth, the fresh and dry weights were1.19 and 0.0321 g/L, respectively. 16S rRNA sequence (1505 bp) was extracted from the whole-genome sequences. The genome size of 33-M-3 is 4,373,612 bp. 3,947 protein-coding genes (CDS) were annotated, and 82 and 18 were annotated as tRNA and rRNA, respectively. The DNA G + C filtered dataset content was 37.49% in one contig. Based on the functional categories specified using the EggNOG database, 3947 genes were annotated with amino acid transport and other annotations were discovered. The genome contains carotenoid biosynthesis genes coding for proteins, 15-cis-phytoene synthase and zeta-carotene-forming phytoene desaturase. HPLC profiling of the 33-M-3 exhibited Zeaxanthin, the retention time 14.450, and the yield was 17.95 ppm. Besides, *Chryseobacterium* spp. synthesis of more than 95% of the carotenoids, among them zeaxanthin is produced in large amounts. However, the complete genome sequence data is helpful for comparative genome research, metabolic engineering of carotenoid biosynthesis and exploring the metabolism of *Chryseobacterium* species.

[This research was supported by the National Institute of Agricultural Sciences (Project No. PJ01550601) provided by the Rural Development Administration, South Korea.]

### Role of Signal Peptidase in Aspergillus fumigatus

Ki-Hwan Kim, Suzie Kang, and Cheol-Won Yun<sup>\*</sup> School of Life Sciences and Biotechnology, Korea University

Signal peptidase targets secretory & membrane proteins containing a signal peptide and cleaves the signal peptide in the ER compartment. In *Saccharomyces cerevisiae*, SEC11 which encodes signal peptidase is an essential gene and Sec11 participate in signal peptide processing. Signal peptidase is thought to play the same role in *Aspergillus fumigatus*. We found that the expression of putative signal peptidase in *A. fumigatus* increased during infection of human macrophages. We constructed promoter deletion mutant of signal peptidase encoding gene in *A. fumigatus*. We found that the growth rates, cell wall glucan contents, virulence, and antifungal drug sensitivity of promoter deletion mutant were lower than wild type. These findings suggest that signal peptidase plays an important role in *A. fumigatus*.

#### F014

Nascent Peptides of HspA are Preferentially Bound to I-Ribosomes in *V. vulnificus* CMCP6 Younkyung Choi, Minju Joo, Wooseok Song, Hana Hyun, Ji-Hyun Yeom, Eunkyoung Shin, and Kangseok Lee<sup>\*</sup> Department of Life Science, Chung-Ang University

Ribosomes composed of genome-encoded heterogeneous rRNAs are implicated in rapid adaptation of bacterial cells to environmental changes. Our previous study showed that ribosomes bearing the most heterogeneous rRNAs expressed from the *rrn*I operon (I-ribosomes) can preferentially translate a subset of mRNAs including *hspA* and *tpiA* mRNAs in *Vibrio vulnificus* CMCP6. Here, we show that I-ribosomes are predominantly bound to nascent peptides of HspA protein. Specifically, I-ribosomes occupied ~75% in ribosomes pulled-down by immunoprecipitation of HspA whereas ~35% of ribosomes were I-ribosomes when RNA polymerase ß subunit was immunoprecipitated. Other methods utilizing incorporation of an affinity tag in 23S rRNA or chimeric rRNA tethering 16S and 23S rRNAs, which generated specialized functional ribosomes in *E. coli*, did not result in functional I-ribosomes in *V. vulnificus* CMCP6. Our study provides direct evidence for preferential translation of *hspA* mRNA by I-ribosomes.

## Unprecedented Insights into the Link between Ramadan and Intermittent Fasting via Gut Microbiome Analysis YoungJae Jo<sup>1</sup>, GyuDae Lee<sup>1</sup>, Min-Ji Kim<sup>1</sup>, and Jae-Ho Shin<sup>1,2\*</sup>

<sup>1</sup>Department of Applied Biosciences, Kyungpook National University, <sup>2</sup>NGS Core Facility, Kyungpook National University

Background : Intermittent fasting regimen is widely perceived that leads to various health beneficial effects including weight loss and reduction of insulin resistance by improving blood glucose concentrations. In addition, numerous studies that gut microbiome is improved within intermittent fasting period have been reported. Ramadan fasting is considered nearly the same dietary strategy as intermittent fasting, in that it has something in common with dietary intervention, but the existence of obvious differences are occasionally overlooked. Discussion : During Ramadan, except the total calories, all nutritive components comprising carbohydrate, protein and Fat did now show the significant difference between mid and post Ramadan season. However, fecal microbial diversity in the group after Ramadan was significantly higher than the on Ramadan, and large of bacteria which produce short chain fatty acid. This study is the first cohort study that tracked the alteration of gut microbiome during and post-Ramadan to verify if this dietary routine induces comparable beneficial effects to normal intermittent fasting. In view of our microbial and metabolic findings, Ramadan fasting must be considered scrupulously to be perceived as beneficial dietary strategy for entire Muslims.

[This research was supported by a project to train professional personnel in biological materials by the Ministry of Environment. In addition, sequencing was performed at the KNU NGS core facility.]

#### F016

#### Identification of Novel Virulence-associated Genes of Erwinia amylovora

Seung Yeup Lee<sup>1</sup>, Hyun Gi Kong<sup>2</sup>, In Jeong Kang<sup>3</sup>, Hee-Jong Woo<sup>4</sup>, Kuom Hwa Jeon<sup>1</sup>, and Hyeonseok Oh<sup>1\*</sup> <sup>1</sup>Crop Protection Division, National Institute of Agricultural sciences, <sup>2</sup>College of Agriculture, Life and Environment Sciences, Chungbuk National University, <sup>3</sup>Department of Central Area Crop Science, National Institute of Crop Sciences, <sup>4</sup>National Agrobiodiversity Center, National Institute of Agricultural Sciences

Fire-blight disease in apple and pear trees is devastating disease caused by *Erwinia amylovora*. The antibiotics such as streptomycin, kasugamycin and oxytetracycline are the most effective control method for the fire blight disease, so far. In addition, chemical pesticides such as copper compound also used to control the *E. amylovora*. However, abuse of these chemicals will definitely induce more dangerous troubles in the near future. Therefore, in order to develop novel disease control methods based on genetic traits of *E. amylovora*, we generated the random mutants by Tn5 transposon insertion to screen avirulent strains. Pathogenicity of the mutants was assessed using small apple fruitlets. Total 17 avirulent mutant strains were found through screening of the 960 random mutants. Among them, 14 mutants were already reported as non-pathogenic strains, while 3 mutants were novel mutant strains. Further study of the association between *E. amylovora* pathogenicity and these 3 novel genes might give new insight in development of control method of the fire blight disease.

### The Analysis of Human Microbiome as a Cause of Breast Cancer at a Young Age

Byung-In Moon<sup>1</sup> and Jeongshin An<sup>1,2\*</sup>

<sup>1</sup>Department of Surgery, Ewha Womans University Mokdong Hospital, College of Medicine, Ewha Womans University, <sup>2</sup>Institute of Convergence Medicine Research, Ewha Womans University Mokdong Hospital, College of Medicine, Ewha Womans University

Breast cancer is the most common cancer in women. In particular, breast cancer in young women progresses rapidly and the prognosis is poor. This study was conducted because the microbiome might be one of the triggers of breast cancer in young patients (<40 years). We recruited 10 young patients with breast cancer (< 40 years), 86 elderly patients with breast cancer (> 40 years), 11 young healthy controls (< 40 years), and 181 elderly healthy controls for microbiome studies. Blood samples were collected and NGS sequencing was performed. Tumor markers (CEA, CA15-3) and serologic test results of volunteers were analyzed. Through microbiome data, we were able to obtain microbiome data specific to young patients with breast cancer. This result was different from elderly patients. Compared with the results of the healthy control group, it was confirmed that young patients with breast cancer had a specific target microbiome, excluding the age variable. Results included microbiome previously identified as being involved in metabolic diseases. The significance of this study is that it confirmed the role of the microbiome as one of the causes of young patients with breast cancer. It is necessary to elucidate the effect of these target microbiome on the development of young patients with breast cancer through more studies in the future.

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#### F018

**Novel Roles of Condensin for Iron Regulation in Schizosaccharomyces pombe** Seong Ho An, Seon Ju Park, and Kyoung Dong Kim<sup>\*</sup> Department of Systems Biotechnology, Chung Ang University

Condensin is one of structural maintenance of chromosomes (SMC) complexes, which is highly conserved from yeast to higher eukaryotes and functions in hierarchical genome organization. Condensin has roles in long-range genomic associations among highly expressed genes, Pol III genes, and Ace2/Ams2 targeting genes in fission yeast. In this study, we have explored a novel target of condensin through transcriptome analyses in condensin depleted condition, *cut3-477* and *cut14-208*. In our RNA-seq and RT-qPCR analyses, a group of genes involved in Fep1 regulon are significantly upregulated in *cut3-477* and *cut14-208*. In addition, we employed auxin-inducible degron (AID) system to effectively deplete Cut3 and Cut14 protein and confirmed mis-regulation of Fep1 regulon. We expected condensin depletion may affect to iron regulation directly, but total iron concentration and the pattern of iron responsive transcription was not changed. To uncover the mechanism of up-regulation of Fep1 regulon, we performed FISH (Fluorescence *In Situ* Hybridization) and ChIP-qPCR. We found the decreased proximal association between Fep1 target genes and reduction of Fep1 binding affinity on target genes in condensin depleted condition. This work suggests that condensin depletion causes the decrease of proximal association between Fep1 regulon

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## Role of Mediator-head Module in Gene Expression in Fission Yeast Schizosaccharomyces pombe

Ji Hyun Kim and Kyoung Dong Kim<sup>\*</sup>

Department of Systems Biotechnology, Chung Ang University

Mediator is highly conserved multi-protein complex from yeast to human that acts as co-activator of RNA Polymerase II. Mediator complex consists of head, middle, tail and kinase domains. Mediator complex has a role in global transcriptional regulation, but the role of each domain has not been uncovered. The mediator head module, which includes Med8-Med18-Med20 and other core subunits, contacts pol II and general transcription factors. Here, we employed the auxin inducible degron (AID) system to rapidly deplete mediator components Med8, Med14, Med17, and Med20 in fission yeast to elucidate the role of head module and confirmed that the proteins were effectively degraded within 30 min by using AID system. Next, we investigated genome-wide transcriptional changes under the mediator depleted condition. We found that loss of Med14 or Med17 reduced global gene expression but in the case of other head components, Med8 and Med20 affected to some category of genes such as ribosomal proteins, rRNA, tRNA, and the Ace2 target genes. In summary, we revealed the role of mediator-head module through transcriptome analyses with AID system.

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## F020

## **The Role of Chaperones Encoded in the Transmissible Locus for Protein Quality Control** Soojeong Ham, Hyunhee Kim, and Changhan Lee<sup>\*</sup> Department of Biological Sciences, Ajou University

Maintaining accurate function of proteins is vital to survive, therefore, *Pseudomonas aeruginosa*, an opportunistic pathogen in the cystic fibrosis lung, has developed survival strategies, such as sustaining protein homeostasis by molecular chaperones. Particularly, *P. aeruginosa* clone C strains, which are successfully spread in worldwide, have transmissible locus for protein quality control (TLPQC) encoding various molecular chaperones and proteases. ORF7 and ORF8 of TLPQC encode homologous to an acid-responsive periplasmic chaperone in *Escherichia coli* and *Salmonella typhi*. However, their physiological role has not been explored yet. In this study, the expression pattern in both various media and growth stages was examined, and the in-frame those two genes deletion was constructed. In addition, we found that deletion of each gene affects the protein level of the other gene, suggesting that they may stabilize each other. Interestingly, it was found that ORF7 and ORF8 have different cellular localization, periplasm and cytoplasm, respectively. Furthermore, the overexpression of those two proteins can confer heat resistant phenotype in *E. coli*. By this study, we characterized the role of chaperones in TLPQC which may contribute to stress tolerance and potentially virulence of *P. aeruginosa*.

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# Complete Genome Sequencing and Comparative Genomic Analysis of *Bifidobacterium animalis* subsp. *lactis* as a Potential Cat Probiotic

Hana Kim<sup>1</sup>, Seungwoo Son<sup>2</sup>, Jihye Baek<sup>2</sup>, and Donghyun Shin<sup>2\*</sup>

<sup>1</sup>Department of Animal Biotechnology, Jeonbuk National University, <sup>2</sup>Agricultural Convergence Technology, Jeonbuk National University

*Bifidobacterium animalis* is a Gram-positive, anaerobic, rod-shaped bacterium of the *Bifidobacterium* genus which can be found in the large intestines of most mammals, but strains found in the cat gut have not been studied yet. Our study helps in understanding the genetic features of the *Bifidobacterium animalis* subsp. *lactis* CACC858 strain found in the feline gut, determining its adaptive features evolved to survive in the cat gut environment, and in elucidating its probiotic functions. To examine the feline *Bifidobacterium animalis* genome, we isolated the CACC858 strain from a Korean short hair cat and sequenced it using PacBio SMRT sequencing technology. A comparative genomic approach was used to assess genetic relationships between CACC858 and other strains. We found that number of genes in CACC858 genome was 1,628 and amino acid transport and metabolism related genes were identified the most (147 genes). Additionally, we performed the pan-genome analysis to find the core gene families and phylogenetic tree based on the core genes to compare the genomes according to the host. This study provides insights into genetic features and adaptations of the *Bifidobacterium animalis* subsp. *lactis* CACC858 strain to survive the feline intestinal environment. It also suggests that the evolution of the CACC858 genome is closely related to the host's evolutionary adaptation process.

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#### F022

#### Genetic Characterization of High Pathogenicity Avian Influenza Virus in Vietnam during 2020-2021

Min Ji Park<sup>1</sup>, Ra Mi Cha<sup>1</sup>, Yu-Na Lee<sup>1</sup>, Yoon-Gi Beak<sup>1</sup>, Dang Nguyen Tho<sup>2</sup>, Ngo Van Bac<sup>2</sup>, Youn-Jeong Lee<sup>1</sup>, and Eun-Kyoung Lee<sup>1\*</sup>

<sup>1</sup>Avian Influenza Research & Diagnostic Division, Animal and Plant Quarantine Agency, <sup>2</sup>National Center for Veterinary Diagnosis, Department of Animal Health, Tan Trung Chua village, Hien Ninh commune, Soc Son Dist., Hanoi, Vietnam

Since 2004, High Pathogenicity Avian Influenza (HPAI) have caused huge economic losses in the poultry industry of Southeast Asia including Vietnam. We obtained HPAI suspected samples from Vietnam in 2020-2021 as a part of Korea-Vietnam international co-project. In this study, we investigated genetic characteristics of high pathogenicity avian influenza virus (HPAIV) in Vietnam. Thirty-one viruses isolated from tissue homogenates from HPAI cases in Vietnam. Sequencing and phylogenetic analysis were conducted to genetically characterize these viruses. Phylogenetic analysis showed that subtype of viruses were H5N6 and hemagglutinin (HA) genes were belonged to clade 2.3.4.4h. In addition, viruses were classified into 4 genotypes (VN13, VN14, VN15, VN16) according to multiple distinct genetic lineages of internal genes. Genotype VN15 might be a genetic backbone of the other genotype except genotype VN14, which were major genotype in 2021 and possessed three different gene segment (PB2, PB1, PA). Our results indicate that the continuous monitoring of HPAIV is needed for the effective HPAIV control in Vietnam.

## Whole Genome Sequencing of Cellulase Producing *Bacillus halotolerans* Strain MBH1 Isolated from the Rabbit Feces

Hyunok Doo, Eun Sol Kim, Gi Beom Keum, Jinok Kwak, Sriniwas Pandey, Sumin Ryu, Yejin Choi, Seungjin Yun, Juyoun Kang, Sheena Kim, and Hyeun Bum Kim<sup>\*</sup>

Department of Animal Resources Science, Dankook University

Cellulose is a complex carbohydrate, which in herbivores is broken down by cellulose digesting bacteria into simpler forms like glucose that is used as an energy source. In this study, we performed whole genome sequencing of cellulase producing bacteria isolated from the rabbit feces. The *Bacillus halotolerans* strain MBH1 was isolated from the collected feces. For genome analysis, *B. halotolerans* strain MBH1 was sequenced using the PacBio RS II (Pacific Biosciences, USA). The BLASTn approach was used to determine potential virulence factors and antibiotic resistance genes based on the Virulence Factor Database (VFDB) and the Comprehensive Antibiotic Resistance Database (CARD). The complete genome of *B. halotolerans* strain MBH1 contains one circular chromosome (4,298,464 bp) with a guanine + cytosine (GC) content of 43.5%. Moreover, based on annotation results, 4,427 protein-coding sequences, 30 rRNA and 86 tRNA were discovered. The analysis result of complete genome showed that *B. halotolerans* strain MBH1 has beta-glucosidase encoding gene (EC 3.2.1.21 BG), which catalyzes the final step of hydrolysis of cellulose by converting cellobiose to glucose. In addition, no virulence factors and antibiotic resistant genes were found in the genome. This strain also passed acid tolerance, bile salt tolerance and carboxymethylcellulose (CMC) assay. Collectively, our results suggest that the *B. halotolerans* strain MBH1 can be safely used as synbiotics in swine.

#### F024

## Genome Sequencing of *Hymenobacter tibetensis* Type Strain KACC 21982<sup>T</sup> and *H. monticola* Type Strain KACC 22596<sup>T</sup>

Jun Heo, Jisu Lee, HyoRim Choi, Daseul Lee, Byeong-Hak Han, Seung-Beom Hong, Soon-Wo Kwon, and Miyoung Won<sup>\*</sup>

#### National Institute of Agricultural Sciences, Rural Development Administration

The genus *Hymenobacter* is a Gram-negative and non-motile bacterial genus isolated from various soil such as fish pond, freshwater reservoir, regoliths in Antarctica and mountain and beach soil in South Korea. Strain KACC 21982<sup>T</sup> and KACC 22596<sup>T</sup> were isolated from soils in China. Despite *Hymenobacter* distributed in various soil environments around the world, many strains, including type strains, have not been analyzed genome sequencing. As part of the genome DB construction of bacterial type strains deposited in KACC, we conducted genome sequencing and comparative genomics of *H. tibetensis* KACC 21982<sup>T</sup> and *H. monticola* KACC 22596<sup>T</sup>. The genome of KACC 21982<sup>T</sup> contains a circular chromosome (5,736,710 bp) consists of 4,984 protein coding genes and 63 RNA coding genes and six plasmids. The genome of KACC 22596<sup>T</sup> contains a circular chromosome (5,608,015 bp) which consists of 5,109 protein coding genes and 57 RNA coding genes and six plasmids. The G+C content of strain KACC 21982 and KACC 22596 was 55.6% and 61.9%, respectively.

[This study was carried out with the support (PJ016776) of National Institute of Agricultural Sciences, Rural Department Administration, Republic of Korea.]

### Complete Genome Sequencing of *Mucilaginibacter daejeonensis* Type Strain KACC 14964<sup>T</sup>

Jun Heo, Daseul Lee, Byeong-Hak Han, Seung-Beom Hong, Soon-Wo Kwon, and Miyoung Won<sup>\*</sup> National Institute of Agricultural Sciences, Rural Development Administration

The genus *Mucilaginibacter*, a member of the family *Sphingobacteriaceae*, was first reported in 2007. However, the number of reported species increased rapidly to 74 species so far. As part of the genome DB construction of bacterial type strains deposited in KACC, we conducted genome sequencing of *M. daejeonensis* KACC 14964<sup>T</sup> which was isolated from dried rice straw in Daejeon, South Korea described by An *et al.* (2009). Strain KACC 14964<sup>T</sup> was closely clustered with *M. aquatilis* HME9299<sup>T</sup>. The genome of KACC 14964<sup>T</sup> contains a circular chromosome (4,810,254 bp) with 48.2% of DNA G+C content. The orthologous average nucleotide identity and digital DNA-DNA hybridization values of strain KACC 14964<sup>T</sup> to *M. aquatilis* HME9299<sup>T</sup> were 72.7% and 19.5%. The genome consists of 4,071 protein coding genes and 62 RNA coding genes. Genome analysis using RAST annotation was confirmed 1,134 subsystems.

[This study was carried out with the support (PJ013549) of National Institute of Agricultural Sciences, Rural Department Administration, Republic of Korea.]

## F026

**Complete Genome Sequencing of Ferruginibacter lapsinanis Type Strain KACC 15035<sup>T</sup>** JiSeon Kim, Anbazhagan Mageswari, Yunheoo Choi, Miyoung Won, Daseul Lee, Byeong-Hak Han, Seung-Beom Hong, Soon-Wo Kwon, and Jun Heo<sup>\*</sup> *National Institute of Agricultural Sciences, Rural Development Administration* 

The genus *Ferruginibacter*, a member of the family *Chitinophagaceae*, is Gram-negative, strictly aerobic, non-gliding and single rods which was first reported in 2009. This genus is actively being studied in activated sludge system. *F. lapsinanis* KACC 15035<sup>T</sup> was isolated from freshwater sediment of littoral zone of the hakha lake in Daejeon, South Korea described by Lim *et al.* (2009). As part of the genome DB construction of bacterial type strains deposited in KACC, we conducted genome sequencing of *F. lapsinanis* KACC 15035<sup>T</sup>. KACC 15035<sup>T</sup> was grouped with *F. albus* KIS38-8<sup>T</sup>. The genome of KACC 15035 contains a circular chromosome (3,455,339 bp), and G+C content was 37.0%. The orthologous average nucleotide identity and digital DNA-DNA hybridization values of strain KACC 15035 to *Ferruginibacter albus* KIS38-8<sup>T</sup> were 71.6% and 18.8%. The genome consists of 2,854 protein coding genes and 50 RNA coding genes. Genome analysis using RAST annotation was confirmed 950 subsystems.

Department Administration, Republic of Korea.]

# Determining a Novel Crosstalk between Yap1-Mediated ROS Signaling and Caffeine Tolerance in *Saccharomyces cerevisiae*

Ji Eun Choi<sup>1,2</sup>, Seo-Hee Heo<sup>1,2</sup>, and Woo-Hyun Chung<sup>1,2\*</sup> <sup>1</sup>College of Pharmacy, Duksung Women's University, <sup>2</sup>Innovative Drug Center, Duksung Women's University

Caffeine, a methylxanthine derivative, affects various physiological conditions such as cell growth, proliferation, and energy metabolism. A genome-wide screen for genes required for caffeine resistance in *Schizosaccharomyces pombe* revealed several candidates, including Pap1 and downstream target genes involved in caffeine efflux. We found that Yap1, a budding yeast AP-1 homolog required for oxidative stress tolerance, has a caffeine tolerance function. Although the *yap1* mutant is not sensitive to caffeine, overexpression of Yap1 renders cells resistant to high concentrations of caffeine. Caffeine sensitivity in mutants lacking two multidrug transporters, Pdr5 or Snq2, is completely recovered by Yap1 overexpression. Among Yap1-dependent target genes, *FLR1*, a fluconazole-resistant gene, is necessary but not sufficient for caffeine tolerance. Low concentrations of hydrogen peroxide induce the activation of Yap1, which restores cell viability against caffeine toxicity. Intriguingly, oxidative stress-mediated cellular adaptation to caffeine toxicity requires Yap1, but not Flr1. Moreover, caffeine is involved in the reduction of intracellular ROS level, mutation rate and Rad52 foci formation. Altogether, we identified novel crosstalk between ROS signaling and caffeine resistance.

### Phylogenetic Analysis of H5N1 High Pathogenicity Avian Influenza Virus Isolated in Laos, 2019-2020

Jae-In Shin<sup>1</sup>, Yu-Na Lee<sup>1</sup>, Ra Mi Cha<sup>1</sup>, Phetlamphone Meeduangchanh<sup>2</sup>, Watthana Theppangna<sup>2</sup>, Youn-Jeong Lee<sup>1</sup>, and Eun-Kyoung Lee<sup>1\*</sup>

<sup>1</sup>Avian Influenza Research & Diagnostic Division, Animal and Plant Quarantine Agency, <sup>2</sup>National Animal Health Laboratory, Vientiane, Lao PDR

Since 2004, there have been multiple outbreaks of H5 high pathogenicity avian influenza (HPAI) in Laos. Since 2015, H5N1 viruses belonged to clade 2.3.2.1c identified in Laos. Here, we detected eighteen H5N1 HPAI viruses from swab samples in backyard and live poultry market (LPM) in Laos from March 2019 to November 2020 and performed phylogenetic analysis. The whole genome sequences from H5N1 HPAI viruses were sequenced using the Illumina MiSeq Next Generation Sequencing platform. All viruses belonged to clade 2.3.2.1c and had multi-basic amino acid sequence (PQRERRRKR/GLF) at the HA cleavage site. They had a 20-amino acid deletion in the stalk region of NA, which has been associated with adaptation of influenza viruses to poultry. Phylogenetic analysis revealed that H5N1 viruses were divided into two genotypes (Laos G1 and Cambodia G1) by gene constellation. In previous study, the Laos G1 genotype. All segments of H5N1 viruses in 2020 clustered together with Cambodia G1 genotype, which circulated in Cambodia since 2018, related to Vietnamese H5N1 viruses. Given that H5N1 viruses were classified as the same genotype of viruses identified in Vietnam and Cambodia, the HPAI outbreaks in Laos were probably affected by neighboring countries. Thus, our study emphasizes the needs for enhanced surveillance and consideration on effective border management.

#### F030

## A Novel Spore-specific Transcription Factor is Essential for Conidial Maturation and Dormancy in *Aspergillus* Species

Ye-Eun Son<sup>1</sup> and Hee-Soo Park<sup>1,2\*</sup>

<sup>1</sup>School of Food Science and Biotechnology, Kyungpook National University, <sup>2</sup>Department of Integrative Biology, Kyungpook National University

Aspergillus, a filamentous fungus that makes up the majority of airborne fungi, mainly propagates by forming asexual spores called conidia. Conidia formation is regulated by various transcription factors (TFs). We studied putative spore-specific TFs in three representatives *Aspergillus* based on transcriptomic analysis. As a result, twenty-two spore-specific TFs were identified, and each deletion mutant was phenotypically analyzed in *A. nidulans*. Among them, we characterized one of the **s**pore-**s**pecific-**C**<sub>2</sub>H<sub>2</sub> zinc finger A SscA. The *ΔsscA* mutant showed defective conidiation, conidial viability, and reduced stress tolerance in *A. nidulans*. The amount of trehalose in the *ΔsscA* mutant was decreased compared to that of the WT and deletion of *sscA* caused induced germ tube formation. Furthermore, transcriptome data showed that deletion of *sscA* was involved in the response of conidia to stimuli and stress. The mRNA levels of the β-glucan biosynthesis gene and the sterigmatocystin gene cluster were upregulated in *sscA* mutant conidia. These were validated by the phenotypic analyses. In addition, we confirmed that the roles of SscA in conidia were conserved in *A. flavus* and *A. fumgiatus*. Taken together, these results suggest that SscA is a novel spore-specific transcription factor, essential for proper conidia formation, conidia dormancy and secondary metabolites in *A. nidulans*. And the functions of SscA in conidia are conserved in three representative *Aspergillus* spp.

# A GAL4-like Zinc-finger Transcription Factor CsgA is Essential for Proper Conidiogenesis and Fungal Development in *Aspergillus nidulans*

He Jin Cho<sup>1</sup> and Hee Soo Park<sup>1,2\*</sup>

<sup>1</sup>School of Food Science and Biotechnology, Kyungpook National University, <sup>2</sup>Department of Integrative Biology, Kyungpook National University

Aspergillus spp. mainly reproduce through asexual reproduction, producing the asexual spore called conidia. The process of conidia formation (conidiogenesis) is controlled by various transcription factors. Our previous transcriptomic analysis identified nine novel conidia-specific transcription factors. In this study, we characterized one of the conidia-specific transcription factors CsgA, the Zn<sub>2</sub>Cys<sub>6</sub> transcription factor containing the GAL4-like zinc-finger domain. The roles of CsgA were investigated in the model organism *Aspergillus nidulans*. In *A. nidulans*, the  $\Delta csgA$  strain showed an increase in conidia production. The expression levels of *brlA* in the  $\Delta csgA$  strain increased in the early stage of conidiogenesis. Deletion of *csgA* exhibited a defect in sexual growth and lower *mutA* expression levels, suggesting that CsgA is essential for proper asexual and sexual development in *A. nidulans*. In conidia, deletion of *csgA* resulted in increased trehalose content, higher spore viability and stress tolerance to thermal and oxidative stresses. The  $\Delta csgA$  strain showed delay in conidial germination rate and abnormal germ tube length. The production of sterigmatocystin increased in the  $\Delta csgA$  conidia compared to control. Overall, these results suggest that CsgA is crucial for proper conidiogenesis, fungal development, and secondary metabolism in *A. nidulans*.

#### F032

## Regulation of Asexual Development by Light in Aspergillus flavus

Hye-Min Park<sup>1</sup>, Ye-Eun Son<sup>1</sup>, and Hee-Soo Park<sup>1,2\*</sup>

<sup>1</sup>School of Food Science and Biotechnology, Kyungpook National University, <sup>2</sup>Department of Integrative Biology, Kyungpook National University

Aspergillus flavus is one of the potent pathogenic fungi that produce carcinogenic secondary metabolites, commonly known as aflatoxins. *A. flavus* mainly spread through airborne asexual spores, which produced by asexual development under the influence of environmental factors such as light, temperature, aeration and so on. Light is a general environmental factor that regulates asexual development, stress resistance, and even mycotoxins in several fungi. When light is served as an external signal, photoreceptor proteins sense through a chromophore. Previous studies showed that FphA is a phytochrome for red-light detection, and LreA and LreB are white collar homologs for blue-light detection in *Aspergillus nidulans*. And it was proved that they are essential for asexual development in *A. nidulans*. But it has not yet been studied in *A. flavus*. So, we have studied the roles of three light sensors in asexual development and mycotoxin production in *A. flavus*. First, we produced each deletion mutant and analyzed asexual development in *A. flavus*. In results, the number of asexual spores was decreased in the deletion of *lreA* and *lreB*, while there was not affected in the  $\Delta fphA$  mutant. Next, we checked the ability of aflatoxins in each deletion strain. As a result, light sensors were found to affect the production of aflatoxins. Taken together, these results suggest that three photosensors of *A. nidulans* are present in *A. flavus* and regulate asexual development and aflatoxin production

## Strategies to Isolate Drug-resistant Strains and to Identify New Drug Resistance Mechanisms

Kyung-Tae Lee

Korea Zoonosis Research Institute, Jeonbuk National University

In the concept of One-Health, the most important medium that connects our surroundings and the natural environment is wild animals, through which many zoonotic diseases can spread. In particular, it has been reported that drug resistance of microorganisms can be developed by anti-microbial agents sprayed in large quantities on farmland and can be spread by the wild animals. The opportunistic infections by microorganisms with drug resistance may increase as these strains are introduced into cities. In this study, the medium connecting the environment, animals, and humans was assumed to be animals with relatively high body temperature. The experiment is to compare and analyze the degree of drug resistance of these strains with reference strains and clinical strains by collecting high-temperature-resistant fungal strains from hospital patients or from animal or bird feces in the environment. In the samples, it was possible to selectively classify fungi under the acidic and high-temperature culture conditions, where bacteria are difficult to reproduce. It is possible to isolate and identify fungi, and to measure susceptibility to commercially available antifungal agents. The obtained strains can be comparatively analyzed according to the collection location and can be provided as a database that can comprehensively predict the distribution and migration route of antifungal resistance-related genes.

## F034

**FeS Cluster Stabilization Function in a Cytoplasmic Membrane Protein of** *Escherichia coli* through RseC Joon-Hee Lee<sup>1</sup>, Jung-Hye Roe<sup>2</sup>, and Kang-Lok Lee<sup>3\*</sup>

<sup>1</sup>College of Pharmacy, Pusan National University, <sup>2</sup>Laboratory of Molecular Microbiology, School of Biological Sciences, and Institute of Microbiology, Seoul National University, <sup>3</sup>Department of Biology Education, College of Education, IALS, Gyeongsang National University

A reducing system of SoxR consists of a putative electron transfer system encoded by the *rsxPABCDGE* operon, RseC encoded from the unlinked *rpoE-rseABC* operon, and ApbE. *rseC* is homologous to the N-terminal half of the *rnfF* gene and ApbE is homologous to the C-terminus. ApbE is known to act on Fe-S maintenance in Salmonella while stabilizing the flavin structure of membrane proteins. SoxR, RsxB and RsxC contain redox-active iron-sulfur clusters. In particular, RsxB and RsxC play an important role in electron transport while exposed to the cytoplasm in the membrane structure, so maintaining the iron-sulfur structure is essential for their function. RseC has four cysteines, three of which allow the SoxR reducer complex to function.

# Characterization of Genetic Features between Psychrotolerant and Mesophilic *Bacillus cereus* Group Isolated from Food Industry by Whole Genome Sequencing

Mi Seon Kang<sup>1,2</sup> and Hyun Jung Kim<sup>2,3\*</sup>

<sup>1</sup>Food Safety and Distribution Research Group, Korea Food Research Institute, <sup>2</sup>Department of Food Biotechnology, University of Science and Technology, <sup>3</sup>Food Convergence Research Division, Korea Food Research Institute

*B. cereus sensu lato* (*s.l.*) comprises numerous genetically related species. Some of the strains causing human health risk and showing psychrotolerance thus considered as public health and food safety issue. In this study, we conducted whole genome sequencing for 20 *B. cereus s.l.* isolated from various food industries and characterized these genomes based on *in silico* analyses, (*i*) pan-genome analysis, (*ii*) construction of phylogenetic tree, (*iii*) 16s rRNA-based and average nucleotide identity (ANI)-based genomespecies assignments, (*iv*) presence or absence of psychrotolerant signature gene sequences, ( $\vee$ ) virulence and antibiotic resistance factor detection. For pan-genome and phylogeny, the recently proposed 8 genomespecies and had a set of 2,492 core gene clusters of 16,994 gene clusters. Some genomespecies assigned by 16s rRNA were different from the ANI result, suggesting that it is hard for these group to distinguish taxonomy only by the ribosomal sequence. The detection of nine signature gene sequences (16s rRNA, *cspA*, *glpF*, *gmk*, *tpi*, *purH*, *pycA*) revealed that the psychrotolerant MG-BCG9 assigned to *B. weihenstephanensis* had all of signature sequences, but other isolates had several or no signature sequences. These results highlighted that the need of robust biomarker encompassing *B. cereus s.l.* regardless of genomespecies. [Supported by grant from KFRI (E0210702-01).]

#### F036

**Discovery of Anti-protein Aggregation Factors in Bacteria** Hyunhee Kim and Changhan Lee<sup>\*</sup> Department of Biological Sciences, Ajou University

Protein have their own structure and it is associated with its function and mechanism. Proteins can be denatured by various stresses such as heat, but certain proteins are naturally prone to be aggregated. Of note, the aggregation of proteins is related to the diseases in human, for example the aggregation of  $\alpha$ -synuclein and A $\beta$  is associated with Parkinson's and Alzheimer's diseases, respectively. In this study, we tried to discover known anti-protein aggregation factors in Escherichia coli. Tripartite protein folding sensors fused with either  $\alpha$ -synuclein or A $\beta$  were applied and transposon sequencing methodology was used. Through the screening, we were able to find that OPE1 protein exhibits potent anti-aggregation activity towards both  $\alpha$ -synuclein or A $\beta$ .

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### Transmembrane Helix Stability of Lysis Protein in RNA Phage Lifecycle

So-Yeon Kim, Hee-Won Bae, Eun-Sook Kim, and You-Hee Cho<sup>\*</sup> Department of Pharmacy, College of Pharmacy and Institute of Pharmaceutical Sciences, CHA University

PP7 is an RNA phage that infects *Pseudomonas aeruginosa*. Lysis protein (LP), one of the four phage proteins, is critical for the proper release of the phage progenies, but the actual lysis mechanism of LP remains elusive. As an initial attempt to elucidate the LP-mediated lysis mechanism, we divided the 55-aa LP into 3 regions, based on the sequence characteristics: the N-terminal hydrophilic region (1-20 aa), the transmembrane (TM) domain (21-43 aa), and the C-terminal hydrophobic region (44-55 aa). Considering the critical roles of the TM domain or the TM helix stability, in the lysis function, we analyzed the helical propensity of the individual amino acids in the TM domain and introduced the mutations that change the helical propensity of the amino acids. Topics discussed will include our results on the characterization of the LP muteins with altered TM helix stability in regards to membrane association and lysis function as well as phage production.

[Supported by grants from NRF]

#### F038

**Novel D-Glucose Transporters in** *E. coli* **Identified through Adaptive Evolution** Hyun Ju Kim and Sang Jun Lee<sup>\*</sup>

Department of Systems Biotechnology, Chung-Ang University

The sugar phosphotransferase system (PTS) is an energy-saving sugar transporter in microbial cells, particularly under anaerobic conditions. Since the PTS consumes equimolar phosphoenolpyruvate to phosphorylate each molecule of glucose, and simultaneously generate pyruvate, the absence of PTS system can affect the profile of mixed acid fermentation and anaerobic cellular growth. In this study, we observed that the *ptsG* gene (encoding a glucose-specific PTS component)-deficient *E. coli* K-12 and C strain cells suffered from poor growth and inefficient glucose utilization in fermentation medium containing D-glucose. Growth-coupled experimental adaptation was performed to evolve glucose PTS-deficient strains, and adapted cells were characterized through genome sequencing, genetic complementation, and fermentation. In adapted K-12 and C cells, we found that novel D-glucose transporters are activated by disruption of different transcriptional repressors. Our study provided insight that the different genetic backgrounds of microorganisms lead to the discovery of novel glucose transporters. [This study was supported by the National Research Foundation of Korea (2021R1A2C1013606, 2022R111A1A01053895), Republic of Korea.]

### NGS-based Active Intestinal Microorganism Analysis Using Human Fecal DNA, RNA

Gyochang Hong, Jaewon Lee, HyunJi Sim, Hyeri Kim, Kwang-Moon Cho, Hyunmi Kim, and Sunjae Kwon<sup>\*</sup> Accugene, Inc.

Although the existing intestinal microorganism research is mostly performed using DNA, the DNA-based approach poses limitations in identifying the microorganisms that are alive and checking for high activity levels of the microorganisms because the analysis does not take into consideration the microorganism's activity rate and survival status. The stability of RNA is relatively lower than that of DNA and is easily disintegrated making it mostly undetectable in probiotics and since copies are rapidly produced in highly active microorganisms, our research study involved taking the DNA and RNA from stool samples, performing microbiome analysis for each type, and comparing the results to identify active microorganisms. Our findings showed that the RNA microbiome analysis results contained microorganisms with high abundance compared to the DNA microbiome analysis results, and when the alpha diversity indices were analyzed, there were no significant differences between the DNA and RNA analysis results for the Simpson index and Shannon index but the observed feature count was higher in the RNA analysis results.

## F040

**Draft Genome Sequence of Mycolicibacterium sp. YH-1<sup>T</sup> Isolated from Agriculture Soil** Oung-Bin Lim<sup>1</sup>, Jae-Hyung Ahn<sup>2</sup>, Hang-Yeon Weon<sup>2</sup>, and Dong-Uk Kim<sup>1\*</sup> <sup>1</sup>Department of Life Environmental Sciences, Sangji University, <sup>2</sup>National Institute of Agricultural Sciences, Rural Development Administration

A Gram-staining-positive, aerobic bacterium, designated  $YH-1^T$ , was isolated from the agriculture soil. The strain  $YH-1^T$  grow optimally at 20°C, pH 7.0 in PTYG medium. In this study, we report the draft genome sequence of a *Mycolicibacterium* sp. obtained using PacBio Sequel System, Illumina platform. The genome comprised of 7,267,399 bp which was assembled in 1 contigs. The N50 value of strain  $YH-1^T$  was 7,267,399 bp with genome coverage of 144.30 X. The G + C content of 66.4%, the genome included 6,789 genes were predicted, among them, 6,444 genes are protein-coding genes. Also, the genome of *Mycolicibacterium* sp.  $YH-1^T$  (= CP092915) contained genes encoding enzymes necessary for phenylalanine, tyrosine, and tryptophan biosynthesis. Moreover, the gene encoding acetamidase acetyl esterase which synthesizes acetate, was found.

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**Big Data Analysis Showed the Characteristic of National-specific Gut Microbiomes in Companion Animals** Chan-Yeong Park, Sung-Seok Lee, Ha Da Jang, and San Kim\* *BRD Laboratory* 

The cohabitant with the animals are common world widely and the canine and feline species are the most commonly housed together with human as a companion animals. Currently, the importance of the gut microbiome in the health conditions have been a topic of interest and recent evidences suggested the gut microbiome of the companion animals plays a role in the host condition. The human gut microbiomes showed different compositions according to genetic heterogenicity and geographical distribution. Compared to the human, there were no studies on the gut microbiomes composition of companion animal between geographical differences. In this study, we estimated the differences of national-specific gut microbiomes of canines and felines with National Center for Biotechnology Information (NCBI) SRA database and 1321 SRA data were obtained with 1.507TB including 13 countries. We analyzed natural differences of canine and feline gut-microbiome and the result showed that some bacterial species such as Veillonellaceae and Atopobiaceae were highly abundant in feline gut compared to the canine. Also, we carried out beta-diversity with distance-based redundancy analysis (dbRDA) analysis. As a result, microbiome also exhibited different compositions according to national-specific sites on feline and canine, respectively. Further studies will be required to analyzed these differences.

#### F042

## Value Evaluation of *Streptomyces* sp. AN091965, Which Produces Spectinabilin, a Nematicidal Active Substance through Genome Sequencing Analysis

Min-Kyoung Kang<sup>1</sup>, Jong-Hoon Kim<sup>1</sup>, Hyeon Ji Jeong<sup>1,2</sup>, Byeong Min Lee<sup>1,3</sup>, Jeong Sang Yi<sup>4</sup>, Dong-Jin Park<sup>1</sup>, Yeo Joon Yoon<sup>4</sup>, and Kwang-Hee Son<sup>1\*</sup>

<sup>1</sup>Microbiome Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>2</sup>Department of College of Pharmacy, Chungnam National University, <sup>3</sup>Department of Bio Environmental Chemistry, Chungnam National University, <sup>4</sup>College of Pharmacy, Natural Products Research Institute, Seoul National University

Spectinabilin is known to have excellent nematicidal activity. We analyzed the spectiabilin-producing genes of Streptomyces spectabilis KCTC9218<sup>T</sup> and *Streptomyces* sp. AN091965. The secondary metabolism of both strains was analyzed to aid in the development of efficient nematicidal drug production strains. *S. spectabilis* KCTC9218<sup>T</sup> and Streptomyces sp. AN091965 was analyzed using the PacBio and Illumina sequencing platforms. For both strains, a total of 8,374 and 8,054 protein-coding genes and 39 and 45 secondary metabolite biosynthetic gene clusters were identified, respectively. The production of spectinabilin was 18.4 ± 6.45 mg/L and 213.89 ± 21.30 mg/L, respectively, and the production of AN091965 was more than 10-fold superior to that of KCTC9218<sup>T</sup>. AN091965 was successfully prevented by injecting spectinabilin at a lower concentration than abamectin, a drug recommended by the manufacturer, against pine wilt disease. Spectinabilin production was observed in both strains using high-resolution liquid chromatography-mass spectrometry (LC-MS) analysis. In addition, whole genome sequencing of both strains revealed multi-nematicidal production. In particular, AN091965 showed high production levels of spectinabilin, and this study provides information for the development of potential nematicide drug-producing strains.

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## In Vitro Screening Study to Identify the Potential of Millet Powder as a Prebiotic Source

Jiwoon Kim and Hakdong Shin<sup>\*</sup>

Department of Food Science & Biotechnology, and Carbohydrate Bioproduct Research Center, Sejong University

Dietary fiber-rich millets are known to have beneficial effects on health, such as increased HDL cholesterol, and reduced CVD. This study aimed to confirm the effects of millet on the gut microbiota using *in vitro* fecal microbiome incubation system. The millet was subjected to oral, gastric, and small intestinal incubation. Incubation conditions were based on the consensus digestion protocol. To evaluate the digestion rate, the total starch was measured with the Total Starch Assay Kit from Megazyme. Fecal samples were collected from healthy Korean donors (n=6) and cultured with millets treated under anaerobic conditions for 24 h. Subsequently, a 16S *rRNA* gene-based sequencing technique (V4 region, Illumina MiSeq) was applied with QIIME2 pipeline to analyze microbial changes in this study. Digestive simulations showed that the amount of starch digested was 16.4 g per 100 g of millet, and the amount of starch that was not digested was 35.7 g per 100 g of millet. As a result of the alpha diversity analysis, we found that the millet group was significantly reduced compared to the control group. The relative abundance of *Lachnospiraceae* genus, which hydrolyzed starch to produce SCFA, increased in the millet group. As a result of the functional profile of the microbial community predicted by PICRUISt2, metabolic differences between groups were identified. This study suggests that the consumption of millet can have beneficial prebiotic effects to the gut microbiome.

## F044

## **Prebiotic Effect Screening Study of Root Vegetables Based on** *In Vitro* **Fecal Incubation Model** Yebeen Yoo and Hakdong Shin<sup>\*</sup>

Department of Food Science & Biotechnology, and Carbohydrate Bioproduct Research Center, Sejong University

The root vegetable is a generic term for root-eating vegetables. The purpose of this study is to confirm the effect of root vegetables on gut microbiota. As root vegetables, we used beet, carrot, sweet potato, purple sweet potato, lotus root, and radish in powder form. We obtained feces (n=6) from healthy Korean donors, then the basal media inoculated with fecal samples was incubated with root vegetable groups at 37°C under an anaerobic condition for 24 h. The microbiota analysis was carried out by 16S *rRNA* gene-based amplicon sequencing (V4 region, MiSeq platform). As a result of the analysis, there are differences in the microbial composition according to root vegetables. Most of the root vegetable groups increased the relative abundance of *Lachnospira*, *Faecalibacterium*, which are known to produce short chain fatty acids, when compared to the control group. In particular, the sweet potato and lotus root groups increased the relative abundance of *Bifidobacterium* genus. These results showed the ability of root vegetables to increase bacteria which has beneficial effects. In conclusion, we can suggest the potential of some root vegetables to induce changes of gut microbiota with beneficial effects.

Genomic Characterization of *Bifidobacterium longum* subsp. *Infantis* Strains Which Can Utilize Different Human Milk Oligosaccharide

Hana Jo<sup>1,2</sup>, Haneol Yang<sup>1</sup>, Yeong Rak Son<sup>1</sup>, Seung Hyun Kim<sup>1</sup>, Chan-Seok Yun<sup>1</sup>, Yu-Ri Lee<sup>1</sup>, Young-Min Kim<sup>2</sup>, and Doo-Sang Park<sup>1\*</sup>

<sup>1</sup>Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>2</sup>Department of Food Science & Biotechnology, Chonnam National University Gwangju

Bifidobacterium Isolated from breastfed infants have often catabolism of human milk oligosaccharides (HMOs) genes. *Bifidobacterium longum* subsp. *infantis* (BI) is a representative intracellular human milk oligosaccharides (HMOs) utilizer and beneficial bacterium that colonizes in the early intestinal microbiota of breast-fed newborns. HMO catabolic abilities significantly vary among different Bifidobacterium species and strains, BI strain was isolated using respectively 2'-fucosyllactose, 3'-sialyllactose and 6'-sialyllactose (2-FL, 3-SL and 6-SL) as a sole carbon sources in the vitro through previous studies. The purpose of this study was to find out the genetic differences of each strain by analyzing whole-genome sequence (WGS) and HMO gene clusters (H1~H5). GH (glycosyl hydrolases) was the enzyme that all had in H1, and differences in transport-related genes had different numbers for each strain. Understanding the catabolism of HMO may contribute to modulating the gut environment. Further studies many samples are required. These studies characterize BI strain by means of a comparative analysis of the dielectric properties and paves the way to understanding HMO metabolism.

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### Molecular Imaging Development Consisting of a Specific Peptide Probe Pancreatic Cancer

Yoonjin Roh

Department of Dermatology, Chung-Ang University Hospital

Purpose: Pancreatic adenocarcinoma is a highly aggressive disease usually diagnosed in an advanced state and for which there are little or no effective therapies. Curative surgical removal is the best therapeutic option; however, more than 80% of patients are not candidates for surgical treatment. Therefore, improved approaches for detecting pre-cancer lesion are needs. We aim to develope probe possible to detect and treat the small lesion at the same time.

Methods: To isolate the peptide specific to pancreatic cancer, we screened phage display peptide libraries against with fluorescence. Immunocytochemistry staining using FITC showed high level of binding affinity to pancreatic cancer cell lines. We also saw no binding the peptides to normal colon cell line.

Results: The result from this study suggest that targeted peptide probe may be a promising candidate drug in the development of a useful pancreatic cancer diagnosis and treatment.

Conclusion: A novel peptide is discovered to have a specific binding activity to pancreatic cancer and can be used to distinguish neoplastic from normal tissue, demonstrating the potential for early cancer detection.

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## G002

## A Microcurrent Technology for Biofilm Infection Management

Young Wook Kim *ProxiHealthcare Inc.* 

Bacterial biofilms cause severe infection in biomedical field. According to the National Institute of Health in the United States, biofilm infection contributes approximately 80% of infection and costs \$94 billion USD only in USA. The biofilm comprises of multispecies bacteria with extracellular matrix that prevents antibiotic penetration. Thus, it typically requires 500–5000 times more concentration of antibiotic than the planktonic bacterial infection treatment. As a result, an effective treatment of biofilm infection includes an invasive surgery. In this work, we have developed a microcurrent technology for biofilm infection management. We utilize a bioelectric current (~100 uA), that is safe for human application, and applied for a toothbrush to demonstrate effective management of oral biofilm reduction. The toothbrush has been successfully fabricated and tested through clinical trials. The results demonstrate 1.75 times more reduction of gingivitis and 598% enhanced dental biofilm decrease than the non-microcurrent toothbrush.

Based on this work, we are going to further develop for biomedical device focused on non-invasive biofilm infection management.

# Production of Rainbow Colorants in *Escherichia coli* Through Systems Metabolic Engineering and Vesicle Engineering Strategies

Hengrui Zhou<sup>1</sup>, Dongsoo Yang<sup>1,2,3</sup>, Seon Young Park<sup>1,2,3</sup>, and Sang Yup Lee<sup>1,2,3\*</sup>

<sup>1</sup>Department of Chemical and Biomolecular Engineering (BK21 Plus Program), Korea Advanced Institute of Science and Technology (KAIST), <sup>2</sup>Systems Metabolic Engineering and Systems Healthcare Cross-Generation Collaborative Laboratory, KAIST, <sup>3</sup>BioProcess Engineering Research Center and BioInformatics Research Center, KAIST

Natural colorants has gained more and more attention due to the health concerns of synthetic colorants. In this study, various metabolic engineering tools has been applied in *Escherichia coli* for the production of natural colorants. Considerable amounts of natural colorants are are either accumulated inside or on the cell membrane because of its hydrophobic property. These may cause cell growth limitation and eventually reduces production of target chemicals. In this study, membranes of *Escherichia coli* were engineered for enhanced production of rainbow colorants, including three carotenoids and four violacein derivatives, which are hydrophobic. Here, various systems metabolic engineering strategies including cell morphology engineering, inner-membrane and outer-membrane vesicle formation were conducted. As a result, by fermentation optimization, 322.47 mg/L of astaxanthin (red), 343.25 mg/L of β-carotene (orange), 218.17 mg/L of zeaxanthin (yellow), 1.42 g/L of proviolacein (green), 0.844 g/L of prodeoxyviolacein (blue), 6.19 g/L of violacein (navy), and 11.26 g/L of deoxyviolacein (purple) were produced. The membrane engineering strategies applied in this study will be useful for the production of a broader range of hydrophobic natural products in microorganisms.

#### G004

## Production of Carminic Acid through Metabolic Engineering of Escherichia coli

Hengrui Zhou<sup>1,2</sup>, Dongsoo Yang<sup>1,2,3</sup>, Woo Dae Jang<sup>1,2,3</sup>, and Sang Yup Lee<sup>1,2,3\*</sup> <sup>1</sup>Metabolic and Biomolecular Engineering National Research Laboratory and Systems Metabolic Engineering and Systems Healthcare Cross-Generation Collaborative Laboratory (BK21 four), KAIST, <sup>2</sup>KAIST Institute for the BioCentury and KAIST Institute for Artificial Intelligence, KAIST, <sup>3</sup>BioProcess Engineering Research Center and BioInformatics Research Center, KAIST

Carminic acid is an aromatic red polyketide found in scale insects that are widely used as colorants for cosmetics applications. Due to the laborious farming of insects and multistep extraction processes, producing carminic acid by engineered microorganism has gained more and more attention. In this research, various metabolic engineering tools have been applied to *Escherichia coli* for the biosynthesis of carminic acid from glucose. Type II polyketide synthase machinery derived from *Photorahbdus luminescens* were first engineered, and the introduction of cyclases ZhuI and ZhuJ from *Streptomyces* sp. R1128 was followed to secure the production of the precursor flavokermesic acid. For downstream pathway, remaining two reactions were unknown. In that case, various enzymes were screened based on literature mining. Among them, the aklavinone 12-hydroxylase (DnrF) and *C*-glucosyltransferase (GtCGT) were found to be capable of performing hydroxylation and *C*-glycosylation reactions. Then, homology modeling and docking simulations were performed on the two enzymes to generate more advantageous mutants with enhanced conversion efficiencies. As a result, fed-batch fermentation was conducted to produce  $0.63 \pm 0.02 \text{ mg/L}$  of carminic acid from glucose. This is the first carminic acid production from *E. coli* reported to date, and the engineering strategies described here will be helpful for natural products synthesis of which its biosynthesis pathway are undiscovered.

### **Development of Gram-negative Bacterial Target Peptidomimetic Treatment**

Bomi Park, Suhyun Hwangbo, Hyejin Hyun, and Kangchang Kim<sup>\*</sup> WellPep Co., LTD

As the incidence of antibiotic-resistant strains increases around the world, it is becoming a health and social issue. Antibacterial peptides can be an alternative to overcome the problems of existing antibiotics. Our company secured new peptides through design and synthesis. Bacteria was diluted with Mueller-Hinton broth (MHB) (Difco, USA) and added to a microtiter plate. A two-fold serial dilution of samples was subsequently added, and the plate was incubated for 16 h at 37°C. In multidrug-resistant *E. coli* (MDREC), AMP and melitin showed antibacterial activity with an MIC value of 4 µg/ml to 16 µg/ml. AMP showed effective antibacterial activity compared to melitin in *E. coli*. We investigated the synergistic effect of AMP combined with colistin using checkerboard assays (Wu *et al.*, 2017; Qu *et al.*, 2020). The FICI value for MDREC was 0.15625, showing a distinct synergy effect with AMP and colistin. These results demonstrate that the combination of AMP and existing antibiotics is an approach to overcoming multidrug-resistant Gram-negative bacteria in clinical practice. By using peptides in parallel treatment, it is expected that infections of multidrug-resistant strains can be treated more safely for the human body by reducing the amount of antibiotics used with side effects.

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## G006

## An Extremely Thermostable and Halophilic β-Class Carbonic Anhydrase from *Thermovibrio ammonificans* as a Biocatalyst for CO<sub>2</sub> Capture

Joo Hyun Kim<sup>1</sup>, Do Young An<sup>1</sup>, and Byung Hoon Jo<sup>1,2\*</sup>

<sup>1</sup>Division of Applied Life Science, Gyeongsang National University, <sup>2</sup>Division of Life Science, Research Institute of Life Science and ABC-RLRC, Gyeongsang National University

Carbonic anhydrase (CA) is an enzyme that catalyzes the reversible hydration of carbon dioxide (CO<sub>2</sub>) with an ultrafast kinetics. CA has been studied as a potential biocatalyst for CO<sub>2</sub> capture and utilization. However, the application of CA to CO<sub>2</sub> capture has been hampered by low thermostability and halotolerance of CA. Herein, a novel  $\beta$ -class CA from *Thermovibiro ammonificans* ( $\beta$ -TaCA) was studied as a highly thermostable and halophilic CA for CO<sub>2</sub> capture applications. The solubility of purified  $\beta$ -TaCA was dependent on salt supplementation, requiring high salinities for conformational stability. The CO<sub>2</sub> hydration activity of  $\beta$ -TaCA dramatically increased with increasing salt concentration. The enzyme showed an exceptional thermostability under high-salt conditions. The practical applicability of  $\beta$ -TaCA to CO<sub>2</sub> capture system was examined by testing the enzyme thermostability in aqueous potassium carbonate (K<sub>2</sub>CO<sub>3</sub>), a widely used CO<sub>2</sub> absorbent. In 20 wt% K<sub>2</sub>CO<sub>3</sub>, the  $\beta$ -TaCA showed a superior kinetic stability to DvCA10.0, one of the most thermostable CAs known to date. Collectively, these results demonstrate that  $\beta$ -TaCA is an exceptionally thermostable and halophilic CA, which can be applied to CO<sub>2</sub> capturing systems in the future.

### **Characterization of Oligochitosan-nanoceria Biocomposites Synthesized Using Solution Plasma Process** Su-Jin Kang<sup>1</sup>, MubarakAli Davoodbasha <sup>2,3</sup>, Sang-Yul Lee<sup>4</sup>, and Jung-Wan Kim<sup>1,3\*</sup>

<sup>1</sup>Department of Bioengineering and NanoBio Engineering, Graduate School of Incheon National University, <sup>2</sup>School of Life Sciences, B.S.Abdur Rahman Crescent Institute of Science and Technology, Chennai, 600048, India, <sup>3</sup>Research Center for Bio Material and Process Development, Incheon National University, <sup>4</sup>Department of Material Engineering, Korea Aerospace University

Nanoceria (nCe) has potential in the biomedical applications due to its distinct low redox potential of the Ce<sup>4+</sup>/Ce<sup>3+</sup> redox couple. Oligochitosan (OC) prepared by solution plasma process (SPP) had good antioxidant activity. In this study, OC/nCe biocomposites were synthesized via SPP to enhance the antioxidant activities of each component. Plasma was discharged in the OC solutions (1.0-2.0%) with 1-5 mM CeNO<sub>3</sub> at 800 V, 30 kHz for 15 min using a unipolar power supply. The color of the solution turned to brown and UV-Vis spectroscopy showed two peaks ~260 nm and ~290 nm, indicating the synthesis of nCe. No change in the chemical structure of OCs was detected by FTIR. Prevalence of nCe as Ce<sup>3+</sup>/Ce<sup>4+</sup> was detected in the 2.0% OC/5 mM nCe biocomposite by the XPS analysis. The nCe particles were well-dispersed in the OC matrix when observed by SEM/EDS and TEM. Average size of the 2.0% OC/5 mM nCe biocomposite was 79.88±0.0 nm; zeta-potential 32.64±0.2 mV. The DPPH,  $\cdot$ OH,  $\bigcirc O^{2-}$ , and H<sub>2</sub>O<sub>2</sub> radical scavenging activity of the biocomposite was 91.1%, 100%, 86.2% and 100% when 11.1, 4.4, 5.5, and 2.2 mg/ml was used. Biofilm formation of *V. vulnificus* decreased by ~ 93.7±5.4% when 0.8 mg/ml of 2.0% OC/5 mM nCe was added. No cytotoxicity of the biocomposite was detected against the HEK 293 cells and the LD<sub>50</sub> value was 1737  $\mu$ g/ml. Therefore, more effective and safe antioxidant and antibiofilm biocomposite of OC/nCe was synthesized readily via SPP that can be utilized as a quencher of ROS.

## G008

## **Redesign of an Intrinsically Disordered Peptide Tag for Enhanced Soluble Expression of Recombinant Proteins** Gyun Taek Lim<sup>1</sup> and Byung Hoon Jo<sup>1,2\*</sup>

<sup>1</sup>Division of Applied Life Science, Gyeongsang National University, <sup>2</sup>Division of Life Science, Research Institute of Life Science and ABC-RLRC, Gyeongsang National University

Fusion protein tagging is a method that can improve the solubility and expression level of recombinant proteins that are prone to aggregation. The NEXT tag, an intrinsically disordered protein (IDP) consisting of 53 amino acids, has been recently developed as a solubility tag. Although the NEXT tag has a powerful effect, it was found that the host cell growth was significantly impaired when the NEXT tag fused protein was expressed. In this study, we tried to tackle this problem by redesigning the NEXT tag. We hypothesized that the NEXT tag has an antimicrobial peptide (AMP) activity, and the cell growth was retarded due to this toxicity. To eliminate AMP activity while retaining the intrinsic disorder propensity, we created a sequence shuffling library of NEXT tag and screened the library to select sequences that are predicted to be lack of AMP activity. Four candidate sequences (NEXTV1~V4) were selected and compared the cell growth was restored to some extents by using the NEXT variants, and notably, the protein expression level was remarkably increased up to ~200%. Sequence analysis showed that there is a negative correlation between the AMP activity and the intrinsic disorder propensity. The redesigned NEXT tags can be used as a more powerful tool for soluble expression of recombinant proteins.

## Engineering of Lysin by Fusion of Antimicrobial Peptide (Cecropin A) Enhances Its Antibacterial Properties against Multidrug-resistant *Acinetobacter baumannii*

Md. Maidul Islam, Dooyoung Kim, Kyeongmin Kim, Samia Akter, Seunghyeok Bang, and Minsang Shin<sup>\*</sup> Department of Microbiology, School of Medicine, Kyungpook National University

Most clinical isolates of *Acinetobacter baumannii*, are multidrug-resistant (MDR), fueling the search for alternative therapies. Bacteriophage-derived endolysins have potent antibacterial activities and are considered as alternatives to antibiotics against *A. baumannii* infection. Gram-negative bacteria possess an outer peptidoglycan layer that prevents direct contact between the endolysins and the cell wall. We hypothesized that the fusion of antimicrobial peptide (AMP) with endolysin could help to reduce bacterial endolysin resistance and increase antimicrobial activity by membrane permeabilizing action. Accordingly, we fused cecropin A, a commonly used AMP, with the N-terminus of AbEndolysin, which enhances the bactericidal activity of the chimeric endolysin. The bactericidal activity of cecropin A-fused AbEndolysin increased by at least 2–8 fold against various MDR clinical *A. baumannii* strains. The *in vitro* bactericidal activity results also showed higher bacterial lysis by the chimeric endolysin than that by the parental lysin. The engineered AbEndolysin (eAbEndolysin) showed synergistic effects with the beta-lactam antibiotics cefotaxime, ceftazidime, and aztreonam, and an additive effect with meropenem and imipenem. eAbEndolysin had no cytotoxic effect on A549 cell line and rescued mice (40% survival rate) from systemic *A. baumannii* infection. Together, these findings suggest the potential of lysin therapy and may prompt its use as an alternative to antibiotics.

## **Optimization of a Fermentation Process to Improve Microbial Production of Heme**

Kyeong Rok Choi<sup>1,2</sup>, Hye Eun Yu<sup>1</sup>, Hoseong Lee<sup>1</sup>, and Sang Yup Lee<sup>1,2,3\*</sup>

<sup>1</sup>Metabolic and Biomolecular Engineering National Research Laboratory, Dept. Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), <sup>2</sup>BioProcess Engineering Research Center, KAIST, <sup>3</sup>BioInformatics Research Center, KAIST Institute for the BioCentury, KAIST Institute for Artificial Intelligence, KAIST

Heme has recently gained attention owing to its wide applications in healthcare and food industries. Metabolic engineering of microorganisms has been exploited to devise sustainable fermentation process for heme production. Here we report optimization of a fermentation process for the HAEM7 strain, a metabolically engineered *Escherichia coli* strain we reported in a previous study, to improve production of heme. First, different carbon sources, induction points, pH control strategies, and iron concentration in the medium/feeding solution were screened to find optimal conditions and improve heme production. Then, increasing the cell density, supplementing iron, and supplying excess feeding solution have been explored. Applying the optimized conditions during fermentation of the HAEM7 strain, production of 1.0 g/L heme in 48 h was achieved. The fermentation strategies/processes developed and reported in this study will help establish industry-level production of heme. [This work was supported by the "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01577901)" from Rural Development Administration, Republic of Korea.]

## G012

Repurposing a Heme-producing Escherichia coli Strain to a Zinc Protoporphyrin IX Producer

Kyeong Rok Choi<sup>1,2</sup>, Hye Eun Yu<sup>1</sup>, and Sang Yup Lee<sup>1,2,3\*</sup>

<sup>1</sup>Metabolic and Biomolecular Engineering National Research Laboratory, Dept. Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), <sup>2</sup>BioProcess Engineering Research Center, KAIST, <sup>3</sup>BioInformatics Research Center, KAIST Institute for the BioCentury, KAIST Institute for Artificial Intelligence, KAIST

Zinc protoporphyrin IX (ZnPPIX) is a promising red colorant for meat products and is a potential anti-cancer drug. However, biological production of ZnPPIX has not been devised yet. In this study, the metabolically engineered *E. coli* HAEM7 strain originally developed for heme production is repurposed to a ZnPPIX production strain by optimizing parameters of a fermentation process. First, the iron/zinc concentrations of the culture medium were rebalanced, and the zinc concentration of the feeding solution was optimized to improve the production of ZnPPIX and reduce the formation of heme. Next, conditions and strategies optimized for high-level production of heme, such as pH control, induction, and the increasing the cell density, were integrated together to further enhance ZnPPIX production. As a result, a fermentation process applying the optimized conditions and strategies enhabled production of 2 g/L ZnPPIX by the HAEM7 strain. The fermentation strategies reported in this study will expedite establishing industry-level production of ZnPPIX.

[This work was supported by the "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01577901)" from Rural Development Administration, Republic of Korea.]

## Evaluation of Feeding Strategy for the Production of Polyhydroxyalkanoate (PHA) by Ralstonia eutropha

Jong-Min Jeon<sup>1</sup>, Yung-Hun Yang<sup>2</sup>, and Jeong-Jun Yoon<sup>1\*</sup>

<sup>1</sup>Green & Sustainable Materials R&D Department, Korea Institute of Industrial Technology (KITECH), <sup>2</sup>Department of Biological Engineering, College of Engineering, Konkuk University

Polyhydroxyalkanoate (PHA) is bio-degradable materials which has similar properties of petroleum based plastics. Considering that PHA accumulation is regulated in opposite with cell growth, optimized fed-batch strategy is required to maximize cell growth and PHA production both. In this study, one-pot fed-batch based on C/N ratio balance regulation was used throughout 10 L scales for PHA production. *Ralstonia eutropha* was cultured to produce PHA with the fructose and frying oil as major carbon source to increase of biomass and PHA production to investigate of fed-batch condition for the PHA production, ammonia solution was supplied as nitrogen source to held C/N ratio balance, it has been controlled by pH maintaining. Then 10 (v/v)% of frying oil was added to maximize PHA production. As the result, 56 g/L of biomass were obtained, the PHA content is 60.2%. Considering that C/N ratio regulation by pH control is effective to increase cell mass and amounts of produced PHA, these results suggest that application of C/N ratio control based feeding strategy could enhance the productivity of PHA. [This study was supported by National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT, MSIT) (NRF-2022M3J4A109144111)]

## G014

## Isolation and Characterization of a Marine Microalgae, *Nannochloropsis oceanica* for the EPA Production Using Cheap Substrates

Hye Ran Lee and Bongsoo Lee<sup>\*</sup> Department of Microbiology and Biotechnology, Mokwon University

Microalgae have been spotlighted and studied as sustainable producers for the various value added chemicals as well as biofuels. In this study, we isolated the various marine microalgae and screened microalgae that have superior traits in EPA production compared with others. Based on 18S rRNA analysis, the isolated strain was identified as *Nannochloropsis oceanica*, and named BSL-005. To evaluate the industrial potential of BSL-005 for the eicosapentaenoic acid (EPA) production, we first analyzed the auxotrophy of this strain, and tried to replace with cheap fertilizer, Eco-sol. When used the Eco-sol, biomass (cell numbers and dry cell weight) generally increased compared to that in f/2 which have been known as optimal media for *Nannochloropsis* sp., and 2-folds increased biomass was finally obtained in media containing 0.25 g/L of Eco-sol. In an effort to increase the productivity of EPA, we next screened and optimized additional substrates which are able to affect the growth and EPA contents. The results showed that the EPA productivity increased up to 173% compared to the control under condition containing 1 g/L of NaNO<sub>3</sub> and 4 g/L of MgSO<sub>4</sub> in optimized Eco-sol. These all results suggest that Eco-sol can be employed for industrial production of EPA using *Nannochloropsis* sp.

## Comparative Study of Responses in *Nannochloropsis gaditana* to Nitrogen and Phosphorus Limitation Based on Transcriptome Analysis

Sun Young Kim and Jaoon Young Hwan Kim<sup>\*</sup> Department of Microbial Resources, National Marine Biodiversity Institute of Korea

*Nannochloropsis gaditana* belonging to the Eustigmatophyceae class is a marine microalga of great interest for industrial applications due to its high content in PUFAs and pigments. Nitrogen and phosphorus are essential macronutrients for the optimal growth of microalgae. Nutrient limited conditions can induce physiological and metabolic responses to trigger biosynthesis of secondary metabolites. Although various transcriptome-based studies have been performed to understand the responses in *Nannochloropsis* species to nitrogen and phosphorus limitation, most of them mainly focused on the lipid synthesis. In the present study, we analyzed the content of pigments and lipids, and photosynthetic activity in response to the limitation of nitrogen and phosphorus. In addition, we investigated whole gene expression by RNA-seq to understand the underlying mechanisms of cellular responses to phosphorus limitation. We found that the content of pigments and photosynthetic activity in response to nitrogen limitation. We also found correlation between such cellular responses and transcriptome data. Our results of integrated analysis can provide clues to understand mechanisms of cellular responses to nutrient stress and engineering of *Nannochloropsis gaditana* for biotechnological exploitation.

### G016

## Isolation of *Aurantiochytrium* sp. for the Docosahexaenoic Acid (DHA) Production and Optimization of Fermentation Condition

Do Young Kim, Jin Kyeong Yoo, and Bongsoo Lee<sup>\*</sup> Department of Microbiology and Biotechnology, Mokwon University

Microalgae-derived docosahexaenoic acid (DHA) production has been considered an alternative technology to overcome several disadvantages of fish oil-based DHA production. To secure the strains for an industrial purpose, we have isolated several *Aurantiochytrium* sp. which has been known as the superior DHA producer in Korea. Among 58 strains, we first chose 15 strains based on their growth, total lipid contents, DHA contents and glucose consumption in 500 mL of flask culture condition. Then finally 4 strains whose traits for DHA production enhanced were selected and named M19, M21, M29 and M30, respectively. To further optimize culture condition and enhance the DHA productivity, 7 L-scale fermentation equipped with microbubble sparger was performed. The results showed that the four strains produced more amount of DHA than ABC101, which is known for producing a lot of DHA. M19 produced DCW of 30.4 g/L, total Lipid of 16.2 g/L, Yield 1 of 0.46 g DCW/g Glucose, Yield 2 of 0.24 g Lipid/ g Glucose which was higher than ABC 101's 28.2 g/L, 12.9 g/L, 0.42 g DCW/ g Glucose and 0.19 g Lipid/ g Glucose. M21 produced low DCW and total lipid compared to ABC 101. However, M21 produced Yield 1 of 0.56 and Yield 2 of 0.25 which was higher than M19. M29 produced low DCW and total lipid compared to ABC 101. However, M21 produced to ABC101, however M29 produced Yield 1 of 0.57 and Yield 2 of 0.33 which showed the highest level among the 4 strains. M30 produced PUFA of 51.8% which showed the highest level among the 4 strains.

## Validation of Screening Method for Personalized Probiotics Using Intestinal Environmental Simulation Fermentation

Ji Eun Kim, Jae Hyuk Chung, Ik Hoon Oh, Tae Yoon Kim, and Jin Seok Moon<sup>\*</sup> *Research Laboratories, ILDONG Pharmaceutical Co., Ltd.* 

Modulation of the gut microbiota using probiotics has been widely used to treat or prevent several intestinal diseases. However, inconsistent results have compromised the efficacy of this approach, especially in functional foods for gut health. The purpose of our study was to verify a strategy for probiotics and assess their efficacy in the antibiotic-treated mice model. Gut dysbiosis was successfully induced in mice by treatment with an antibiotic cocktail. High-performance liquid chromatography and 16S rRNA high-throughput sequencing techniques were used to investigate short-chain fatty acid content and gut microbial diversity and composition. The results showed that probiotic supplementation significantly improved the diversity of the gut bacterial community in antibiotic-treated mice. The group that received the responding probiotic showed a reduction of *Escherichia coli* group susceptibility to antibiotic-indued as compared to a non-responding probiotics treatment group. Moreover, the personalized probiotic was more effective in modulating the host metabolites. In conclusion, our study suggests that personalized probiotics may possess an advantage over commercial probiotics in treating dysbiosis-related conditions.

## G018

## Analysis of Human Gut Microbiome: Culturomics and Sequencing Approaches

Piyapat Rintarhat<sup>1</sup>, Yong-Joon Cho<sup>2</sup>, and Won Hee Jung<sup>1\*</sup>

<sup>1</sup>Department of Systems Biotechnology, Chung-Ang University, <sup>2</sup>Department of Molecular Bioscience, Kangwon National University

The human gut is colonized by diverse microorganisms, including bacteria, viruses, protozoa, and fungi. Several studies have suggested that the gut fungal microbiome (mycobiome) impacts host immunity and the development and progression of human diseases. However, most gut microbiome studies have focused exclusively on bacteria, and the mycobiome in the organ has largely been unexplored. Here, we developed a culturomics platform to isolate the fungal strains from fecal samples of a cohort of patients with ulcerative colitis (UC) and compared them with those of healthy subjects (HT). Moreover, we optimized the methodology for amplicon sequencing analysis to compare the fungal community structure between the UC and HT subjects. Our culturomics analysis showed that, overall, most identified fungal colonies belonged to the phylum Ascomycota followed by Basidiomycota both in HT and UC. The total number of the colonies from the fecal samples of UC was significantly higher than that of HT, suggesting that more fungal strains may persist in the intestine of UC compared to that of HT. Finally, we will present the results of the comparisons between different methodologies for amplicon sequencing analysis for the fungal community analysis. Our study emphasizes the importance of the gut mycobiome and provides useful information on human mycobiome analysis.

[This work was supported by the National Research Foundation of Korea (NRF)]

## Complete Genome Sequencing and Comparative Genomic Analysis of *Clostridium butyricium* IDCC1301, a Promising Probiotic

Ye Ji Jang, Han Sol Choi, Da Young Kim, Tae Yoon Kim, and Jin Seok Moon<sup>\*</sup> Research Laboratories, ILDONG Pharmaceutical Co., Ltd.

*Clostridium butyricum* is a butyric acid-producing Gram-positive anaerobic bacterium that is commonly present as a member of the gut microbiota in humans. This species has been used as a probiotic for the prevention of diarrhea in humans. In this study, the whole genome of the *C. butyricium* IDCC1301 strain used in probiotic products was sequenced, and the genetic features related to its safety and functionality were determined. The result of the assembly was two contigs with an N50 value of 3,816,806 bp. Based on the assembly information, this genome consists of 4,611,195 bp divided into 1 closed circular chromosome of 3,816,806 bp (G+C content, 28.86%) and 1 circular plasmid of 794,389 bp (G+C content, 28.32%). We sequenced the IDCC1301 strain isolated from infant feces and compared it with the available genomes of *C. butyricum* on a public database. Genes related to various oligosaccharides utilization were detected in the genome of strain IDCC1301 and compared with other genera. We found that this strain can metabolize a wide range of carbohydrates in comparative genome results. These findings indicate that the *C. butyricium* IDCC1301 strain genetically has beneficial genetic features for human health to use in healthy functional foods.

## G020

## Overexpression of RuBisCO Activase for Efficient CO<sub>2</sub>-Fixation in Yeast

Sujeong Park, Deokyeol Jeong, Jongbeom Park, Suhyeung Kim, and Soo Rin Kim<sup>\*</sup> School of Food Science and Biotechnology, Kyungpook National University

Industrial biotechnology based on yeast fermentation is promising strategy to alleviate global warming and climate change. However, *Saccharomyces cerevisiae*, widely used in bioprocesses, emits a lot of carbon dioxide (CO<sub>2</sub>) during fermentation. Therefore, strategies for reducing CO<sub>2</sub> emission of *S. cerevisiae* should be developed. In our previous study, we successfully constructed CO<sub>2</sub>-fixation pathway in xylose utilizing *S. cerevisiae* by introducing heterologous ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) and phosphoribulokinase (PRK). To improve CO<sub>2</sub>-fixation efficiency, delta-integration strategy was conducted and the RuBisCO copy number was increased to 10 copies. CO<sub>2</sub>-fixation pathway related genes were also overexpressed by additional genome editing and CO<sub>2</sub>-fixing yeast SJ03 was developed. In this study, we overexpressed RuBisCO activase cbbX in SJ03 to further increase the CO<sub>2</sub>-fixation efficiency. The final strain showed highest RuBisCO activity. Moreover, ethanol production was improved by CO<sub>2</sub>-fixation during fermentation. These results can suggest that the overexpression of CO<sub>2</sub>-fixation pathway and RuBisCO activase in *S. cerevisiae* could be a strategy to reduce CO<sub>2</sub> emission in bioprocess. [This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01577003)" Rural Development Administration, Republic of Korea.]

# **Chlorella Transformation System Using a Salt Inducible Promoter (SIP) Derived from** *Chlorella vulgaris* **PKVL7422** Min-Jeong Kim, Su-Hyun Kim, and Tae-Jin Choi<sup>\*</sup>

Department of Microbiology, Pukyong National University

*Chlorella* is a freshwater green alga, which has been widely used in aquaculture and variety of feed products. Also, several proteins have been expressed by using transformed microalgae and evaluated for a vaccine. A new promoter, isolated increases the expression efficiency of recombinant protein in *chlorella vulgaris* by using the promoter of Salt Inducible Promoter (SIP) from *C. vulgaris* PKVL7422. The SIP analyzed factors that respond to salt or stress based on RNA seq data. The expression level of the target gene was analyzed in the *Chlorella* Nitrate Reductase (NR) transformation system. The target gene, the VHSV glycoprotein, with the SIP2 promoter and *Chlamydomonas* rbcs gene terminator was flanked by DNA fragments from the NR gene of *C. vulgaris* PKVL7422. After introduction into *C. vulgaris* PKVL7422 by electroporation, the target DNA can be integrated into the chromosome of *C. vulgaris* PKVL7422 by double homologous recombination. The expression of the recombinant protein encoded by the inserted gene was confirmed by a polymerase chain reaction and western blot. The identified transformed *Chlorella* was treated with salt by concentration to confirm the expression level. The salt concentration in 250m M NaCl was the most effective for the high expression rate of the foreign gene. This suggests that protein accumulation can be efficiently induced by culturing by adding salt.

### G022

Application of Indole-3-Acetic Acid (IAA) derived from *Sphingomonas* sp. CV7422 in the Cauliflower Mosaic Virus (CaMV) 35S Promoter Transgenic Microalga

Seonghyun Kim and Tae-Jin Choi\* Department of Microbiology, Pukyong National University

Sphingomonas sp. CV7422, microalga growth-promoting bacterium, was isolated from the freshwater microalga, *Chlorella vulgaris* PKVL7422 culture. According to the 16S rRNA gene sequencing result, it was confirmed that this bacterium is a member of the *Sphingomonas* genus and is most closely related to *Sphingomonas taxi* ATCC 55669, which is a plant growth-promoting rhizobacterium via plant growth hormone, IAA production. Similar to other plant growth-promoting *Sphingomonas* spp., the genes involved in the biosynthesis of tryptophan (trp), a precursor of IAA, were found in the genome of *Sphingomonas* sp. CV7422. However, the genes involved in the trp-dependent biosynthesis pathway revealed to date were not found. Instead, genes involved in the trp-independent IAA biosynthesis pathway were found in the genome of *Sphingomonas* sp. CV7422. Furthermore, expression level of the downstream gene, which is regulated by the CaMV 35S promoter, was increased when CaMV 35S promoter transgenic *C. vulgaris* was co-cultured with *Sphingomonas* sp. CV7422. The reason for this phenomenon is presumed to be the putative Aux/IAA-responsiveness cis-regulatory element (CRE), TGA-box present in the CaMV 35S promoter. In this study, it was confirmed that the activity of the β-glucuronidase (GUS) reporter gene increased when the CaMV 35S::GUS transgenic *C. vulgaris*, which was transformed *C. vulgaris* PKVL7422, was co-cultured with *Sphingomonas* sp. CV7422 and when IAA was treated.

## Lycopene Production Using Methylorubrum extorquens AM1 from Formate as a Sole Carbon Source

Woo Young Kim<sup>1</sup>, Eun Jee Kim<sup>1</sup>, Seung-Jin Kim<sup>1</sup>, Yoonyong Yang<sup>2</sup>, Sungho Choi<sup>2</sup>, Moonsuk Hur<sup>2</sup>, Byounghee Lee<sup>2</sup>, and Min-Kyu Oh<sup>1\*</sup>

<sup>1</sup>Department of Chemical and Biological Engineering, Korea University, <sup>2</sup>Biological and Genetic Resources Assessment Division, National Institute of Biological Resources

Formate is a C1 compound that can be converted from carbon dioxide, the main chemical causing global warming. In this study, we produced lycopene from formate using *Methylorubrum extorquens* AM1 by engineering nonmevalonoate pathway. Through metabolic engineering, lycopene production of the strain increased by 229.3% in the medium using methanol as a carbon source. Then, to utilize formate as a sole carbon source effectively, formate assimilation protein, FtfL was overexpressed. As a result of formate-based fed-batch fermentation, the lycopene production increased by 157%. Lycopene production using formate as a sole carbon source has not been reported yet and this study showed that engineered *M. extorquens* AM1 can be a candidate strain that produces carotenoids from formate.

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## G024

**Molecular Cloning and Characterization of NAD-dependent Formate Dehydrogenase from Wood Rot Fungus** Su-Yeon Lee, Seokyoon Jang, Soo-Kyeoug Jang, Ji-Yoon Yang, Mi-Jin Park, and Soo-Min Lee<sup>\*</sup> *National Institute of Forest Science* 

Enzymatic conversion of CO<sub>2</sub> has been recognized important technology. NAD-dependent formate dehydrogenase(fdh) have been used for conversion of carbon dioxide to useful products, formate. In this study, we demonstrated CO<sub>2</sub> reducing property of fdh from wood rot fungus, *Phanerochate sordida* for production of formate from CO<sub>2</sub> gas. To produce the recombinant protein, Psfdh, pQE-30 vector (Qiagene, Germany) and M15 *Escherichia coli* were used. Plasmid was isolated from teh transformant, and the inserted gene was partially sequenced. Psfdh contains 358 amino acid with theoretical molecular weight of 39.27 kDa. protein expression of Psfdh was identified by His-trap affinity chromatography column. Enzyme activities of Psfdh were calculated using NADH absorbance. The highest specific activities were observed in the pH 6 conditions. In the oxidation activities (formate to CO<sub>2</sub>), the specific activities were shown as 3.25–3.65 U/mg. In the reduction activities (CO<sub>2</sub> to formate), the specific activities were shown as 2.5–0.5 mU/mg.

## A Reference Data Approach to 16S rRNA Metagenomics for Korean Children's Oral and Gut Microbiota

Younseo Choi, Junhyung Lee, and Joshua SungWoo Yang<sup>\*</sup> Division of Healthcare Research and Development, Research Center, NgeneBio

It is prominent that the oral and gut microbiome and the host's oral and gut health are closely related. Nevertheless, Korean children's oral and gut microbiomes are rarely reported. Thus, this study investigated Korean children's oral and gut microbiomes across different ages. Also, we aimed to investigate the connection between the reference data and the microbial changes throughout the age using 16S rRNA sequencing. In total, 72 Korean children aged 0, 2, 4, and 6 years were collected for buccal swabs and fecal samples, resulting in 144 samples. During the investigation, Microbial diversity and proportion sequence changes were analyzed through 16S rRNA gene sequencing. Beta-diversity analysis indicated that the microbial community clusters changed from age 0 to 4 in Korean children. However, after age 4, there was no change in the microbial community. In the enterotype analysis, the difference in cluster formation was confirmed at the average age of 1.3, 4.2, and 4.9. These results suggest that differences in diet and tooth formation stages, especially between 0 to 4 years of age and after four years, influence oral and intestinal microbiota changes. In conclusion, it can be inferred that the growth environment, dietary composition, and weaning experience are decisive factors in developing oral and intestinal microflora. After, the research will access a reference database of oral and gut microbiota from children after age 6. [Supported by the NgeneBio Corp. grant.]

## G026

## Functional Characterization of 4- $\alpha$ -Glucanotransferase and $\alpha$ -Amylolytic Enzyme from Hyperthermophilic *Fervidobacterium islandicum* AW-1

Sondor Ganbat<sup>1</sup>, Jae-Hong Park<sup>1</sup>, Bo Gyoung Choi<sup>1</sup>, Dariimaa Ganbat<sup>1</sup>, Dong-Woo Lee<sup>2</sup>, Seong-Bo Kim<sup>3</sup>, Han-Seung Lee<sup>1</sup>, and Sang-Jae Lee<sup>1\*</sup>

<sup>1</sup>Department of Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University, <sup>2</sup>Department of Biotechnology, Yonsei University, <sup>3</sup>Bio-Living Engineering Major, Global Leaders College, Yonsei University

The combination of starch-active enzymes in the glucanohydrolase and glucanotransferase families has been widely studied to identify enzymes that successfully combined to produce novel and functional isomaltooligosaccharides. In this research, functional characterization of  $\alpha$ -amylase and 4- $\alpha$ -glucanotransferase from hyperthermophilic *Fervidobacterium islandicum* AW-1, which can be key enzymes for innovative glucan structures was conducted. As a result of the CAZy (Carbohydrate-Active enZYmes) analysis, three genes annotated as  $\alpha$ -amylase in *F. islandicum* AW-1 genome. Among them, we selected two genes (NA23\_09645 and NA23\_09780). The genes were cloned and expressed in *E. coli* BL21 using the pET system. The recombinant enzyme NA23\_09645 (49 kDa) could not hydrolyze starch and amylose; however, it reacted with hydrolyzed small maltooligosaccharides such as maltotriose to form various maltooligosaccharides. It showed the enzyme was 4- $\alpha$ -glucanotransferase (FIGTase). The enzyme NA23\_09780 (81 kDa) (FIAmyA) showed high hydrolysis activity not only with maltooligosaccharides or maltodextrin but also cyclodextrins. The recombinant FIGTase and FIAmyA enzymes exhibited maximal activity at 80°C and 65°C, respectively, and pH 6.0.

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## Antimicrobial Spectrum and Characterization of Purified Recombinant Micro Halocin HB384 Derived from Halophiles

Bo Hyeon Park<sup>1</sup>, Ga Eul Jeong<sup>1</sup>, Dariimaa Ganbat<sup>1</sup>, Ji U Im<sup>1</sup>, Hae Rang Lee<sup>1</sup>, Dong-Woo Lee<sup>2</sup>, Seong-Bo Kim<sup>3</sup>, Yong-Jik Lee<sup>4</sup>, and Sang-Jae Lee<sup>1\*</sup>

<sup>1</sup>Department of Food Biotechnology and Research Center for Extremophiles & Marine Microbiology, Silla University, <sup>2</sup>Department of Biotechnology, Yonsei University, <sup>3</sup>Bio-Living Engineering Major, Global Leaders College, Yonsei University, <sup>4</sup>Department of Bio-Cosmetics, Seowon University

Excessive misuse of conventional antibiotics leads to antibiotic resistance in bacteria, which causes super bacteria, multi-drug resistant organisms. To solve this problem, several alternative strategies have been proposed, among which antimicrobial peptides (AMPs) are the most promising due to they do not reveal the resistance problem. In this study, AMPs derived from halophiles were obtained based on the gene information of halocin peptides, which are bacteriocin-like substances produced from halophiles. It was named HB384, and the gene encoding HB384 was cloned into pGST vector and the recombinant HB384 was expressed in *E. coli* BL21. The recombinant protein was purified by GST affinity chromatography, and the molecular weight of HB384 was stable at 99°C for 8 h, and antimicrobial activity against pathogen increased with the increase in the concentration used. As a result, purified HB384 is expected to be used as a substitute for antibiotics. In future research, we plan to conduct a study to analyze the antiviral potential of HB384.

[This work was supported by National Research Foundation of Korea (NRF) grant (2020R1F1A1076624), the Technology Innovation Program (20015807) funded by the Ministry of Trade, Industry & Energy, and by grants from the Ministry of Ocean and Fisheries (PM62830)]

### G028

## **Keratinase Production Using Non-food Source** Jongbeom Park and Soo Rin Kim<sup>\*</sup> *School of Food Science and Biotechnology, Kyungpook National University*

*Pichia pastoris* is an important host strain for the production of industrially and medically important enzymes. This methylotrophic yeast produces recombinant protein in high yields with rapid cell growth, a strongly regulated promoter and target protein secretion system, and post-translational modification (PTM) similar to mammalian cells is suitable for the production of therapeutic glycoproteins. For industrial enzyme production using non-food sources such as lignocellulosic biomass, it is important for the yeast to use xylose as a carbon source. Xylose-utilizing *P. pastoris* was developed by introducing a xylose-metabolizing pathway consisting of xylose reductase, xylitol dehydrogenase, and xylulose kinase derived from *Pichia stipitis*. To improve xylose metabolism, the expression levels of the three genes were optimized by promoter library approach. The most optimized strain was selected and used as a host to produce keratinase, which is one of industrially important enzyme. As a result, we were able to develop a host strain efficiently producing keratinase using xylose derived from lignocellulosic biomass.

## Evaluation of the Cosmeceutical Potential of *Nannochloropsis* sp. Extract

Sun Young Kim, Yong Min Kwon, and Jaoon Young Hwan Kim<sup>\*</sup> Department of Microbial Resources, National Marine Biodiversity Institute of Korea

Microalgae are a diverse group of photosynthetic microorganisms with an estimated number of 72,500 species. As one of the oldest living organisms on Earth, they survived and evolved by adaptation to a broad range of harsh environmental conditions. In order to protect themselves against stress conditions, microalgae have developed chemical defense systems by producing bioactive compounds including a number of secondary metabolites with the potential for cosmeceutical applications. In the present work, we evaluated the cosmeceutical functions of an ethanol extract from *Nannochloropsis* sp. obtained from the West Sea of the Republic of Korea. The extract contained diverse compounds including PUFAs, carotenoids, and phenolic compounds, which are known for various skin protective activities. We confirmed that the extract had various skin protective functions of anti-melanogenic, antioxidant, skin hydration, anti-inflammatory, anti-wrinkling, as well as UV protective function at the concentration of low cytotoxicity. These results indicated that the ethanol extract of *Nannochloropsis* sp. has can be a source of potential cosmeceutical active ingredients.

### G030

**The Study of Anti-aging and Anti-allergic Activitys of Green Tea Fermented with** *Aspergillus chevalieri* Jayoung Kim, Jiyoung Choi, Jumi Lee, Gi Yeon Han, Youngkeun Song, and Joonseok Cha<sup>\*</sup> *Research Center, The Garden of Naturalsolution Co., Ltd.* 

Advanced glycation end-products (AGEs) are formed from the nonenzymatic reaction between sugars and proteins. In the skin, glycation induces the formation of cross-links in the extracellular matrix of the dermis. The accumulation of free radicals and modifications induced by AGEs play critical roles in skin aging. Green tea is well known for its powerful antioxidant and anti-aging effects, due to the high content of vitamins and polyphenolic compounds, such as flavonoids and catechins in tea leaves. Greentium is a fermented green tea using *Aspergillus chevalieri* a called "golden mold". Greentium showed an antioxidant effect with DPPH radical scavenging and intracellular ROS generation inhibitor. It also displayed potent inhibitory effect on porcine pancreatic elastase and D-glucose-induced AGEs formation. Additionally, it exerted anti-allergic and skin brightening effects on skin cells. These results suggest that Greentium may act as a cosmesutrical ingredient with anti-aging property to prevent oxidative stress and other complications like AGEs formation.

## Identification of Bacteria Isolated from Barley Rhizosphere Soil and Their Antifungal Activity

Hye Ji Yang, Soonju Kwon, Myungbo Shim, Jung Wook Yang, and In-Jeong Kang<sup>\*</sup> Division of Crop Cultivation and Environment Research, National Institute of Crop Science

To screen for antifungal activity against *Fusarium graminearum* and *Fusarium asiaticum*, the causal agent of Fusarium head blight, more than three hundred bacteria were isolated from rhizospheric soil and rhizoplane of barley plants. Six of the tested bacteria inhibited the hyphal growth of *Fusarium graminearum* and *Fusarium asiaticum*, both. All six strains showed ACC deamination activity and produced IAA, iron-chelating siderophore. Also, five strains can produce hydrolytic enzymes, such as cellulase and chitinase. The strain HNW 3-5 can produce phosphatase and showed the highest siderophore production. Especially, among the strains, GJB 1-11 showed the highest inhibition rate against *Fusarium graminearum* and *Fusarium asiaticum*. GJB 1-11 was identified as *Streptomyces rishiriensis* based on the 16S rRNA gene sequence. These results showed that selected bacteria could have potential as a biocontrol agent and a plant growth-promoting agent.

## G032

**Highly Efficient Secretion System of Recombinant Protein by DsRed-Intein Mediated Secretion in** *Escherichia coli* Eon Ji Jo<sup>1</sup> and Byung Hoon Jo<sup>1,2\*</sup>

<sup>1</sup>Division of Applied Life Science, Gyeongsang National University, <sup>2</sup>Division of Life Science, Research Institute of Life Science and ABC-RLRC, Gyeongsang National University

Extracellular production of recombinant proteins in bacterial hosts has advantages over intracellular production: protein purification can be simplified with a low cost, and the oxidizing environment ensures disulfide bond formation. In addition, extracellular secretion of protein is essential particularly when the protein must be outside the cell for proper functioning. Based on the recent observation of self-secretory ability of the monomeric red fluorescent protein DsRed, we developed a novel secretion system in *E. coli* comprising the DsRed and the intein Mtu ΔI-CM, which were sequentially fused to the N terminus of a model protein (bovine carbonic anhydrase; bCA). This system was originally designed for the autocleavage of DsRed from the fusion protein after the secretion. In contrast to the expectation, the cleavage of DsRed-intein::bCA occurred intracellularly. Surprisingly, both DsRedintein and bCA were successfully secreted into the extracellular medium, and the secretion was much faster than the DsRed-bCA without the intein. The system was compared to the well-known fusion partner YebF, showing its superiority in terms of both the speed and the quantity of protein secretion. This novel system offers a facile and efficient tool for the extracellular production of recombinant protein in *E. coli*, requiring no post-production cleavage of fusion partner.

## Enhancement of the Solubility of Recombinant Proteins by Fusion with a Short-disordered Peptide

Jun Ren, Suhee Hwang, Hyang-Mi Lee, Thi Duc Thai, Kha Mong Tran, Wonseop Lim, and Dokyun Na<sup>\*</sup> School of Integrative Engineering, Chung-Ang University

Bacterial cells, especially *Escherichia coli*, are widely used as a host to produce recombinant proteins. However, heterologous proteins often become insoluble and thus lose their activity. To solve this problem, in protein biotechnology, large soluble fusion partners are widely utilized for increased yield and solubility of recombinant proteins. Nevertheless, the production of additional large fusion partners poses an additional burden to the host, leading to a decreased protein yield. In this study, we identified two highly disordered short peptides that were able to increase the solubility of an artificially engineered aggreagation-prone protein, GFP-GFIL4, from 0.6% to 61% (D3-DP00592) and 46% (D4-DP01038) selected from DisPort database. For further confirmation, the peptides were applied to two insoluble E. coli proteins (YagA and YdiU). The peptides also enhanced solubility from 52% to 90% (YagA) and from 27% to 93% (YdiU). Their ability to solubilize recombinant proteins was comparable with strong solubilizing tags, maltose-binding protein (40 kDa) and TrxA (12 kDa), but much smaller (< 7 kDa) in size. For practical application, the two peptides were fused with a restriction enzyme, I-SceI, and they increased I-SceI solubility from 24% up to 75%. The highly disordered peptides did not affect the activity of I-Scel while I-Scel fused with MBP or TrxA displayed no restriction activity. Despite the small size, the highly disordered peptides were able to solubilize recombinant proteins as efficiently as conventional fusion tags and did not interfere with the function of recombinant proteins. Consequently, the identified two highly disordered peptides would have practical utility in protein biotechnology and industry.

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## G035

## **Multiplex Transcriptional Characterizations across Diverse Bacterial Species Using Cell-free Systems** Sung Sun Yim

Department of Biological Sciences, KAIST

Precise tuning of gene expression levels is crucial for engineering predictably behaving genetic circuits. Our current understanding of how regulatory sequences control gene expression levels remains limited for most bacterial species. Cell-free expression systems greatly simplify prototyping of genetic designs *in vitro*. However, the small number of simultaneous measurements that can be made using fluorescence or color as readouts limits the scale at which biological parts can be characterized. Here we devised a method to measure expression levels from thousands of regulatory sequences in single cell-free reactions using oligo library DNA synthesis and targeted deep sequencing of RNA and DNA. This multiplexed approach was highly robust and corresponded well with *in vivo* measurements in *E. coli*. We further applied this approach in active cell-free transcription systems developed from ten diverse bacterial species, enabling comparison of sequence-function relationships across hosts and predictive modeling of transcriptional activation. We anticipate that this multiplexed approach using cell-free expression systems will expand the capacity for genetic circuit prototyping in new bacterial chassis.

## Antimicrobial Activities of Engineered Endolysin LNT103 and Its Synergetic Effect with Colistin against Acinetobactor baumannii

Yu Mi Won<sup>1</sup>, JungKum Park<sup>1</sup>, Hye Soo Oh<sup>1</sup>, Hye-Won Hong<sup>1</sup>, Min Soo Kim<sup>1</sup>, Hojin Park<sup>1</sup>, Yoonjung Hwang<sup>1</sup>, Yurim Kim<sup>1</sup>, and Heejoon Myung<sup>1,2,3\*</sup>

<sup>1</sup>LyseNTech, Inc., <sup>2</sup>Bacteriophage Bank of Korea, <sup>3</sup>Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

Few antibiotics against Gram-negative pathogens have been newly introduced over the last two decades. The rapid development of antimicrobial resistance to existing antibiotics is threatening human health, and thus, new antibiotics are urgently needed. Phage endolysins are a potential candidate, but the presence of an outer membrane in Gram-negative bacteria hinders the attack of recombinant endolysin from outside. Here, we sought to mine an endolysin effective against Gram-negative pathogens from a bacteriophage bank and improve its efficacy by protein engineering. An endolysin encoded by phage PBPA90 was selected and its antibacterial activity was improved by substituting 15 amino acids and by fusing cecropin A to its N-terminus. The resulting engineered endolysin, LNT103, showed a strong antibacterial activity against various Gram-negative pathogens, particularly *Acinetobacter baumannii* with minimum inhibitory concentrations as low as 8 µg/ml. The engineered endolysin rendered bacterial membrane permeable and combination use with colistin showed a synergistic effect in antibacterial efficacy. Minimal cytotoxic effect and hemolytic activity were observed from LNT103. Its *in vivo* efficacy was verified in a mouse infection model.

[This work was supported by the R&D program on Bio Industrial Technology (20018377) funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea)]

### G037

## High Throughput Screening Platform for Discovering Microbiome-based Therapeutics in Inflammatory Bowel Disease

Ki-Jin Kwon, Jae-Young Kim, Jaehyun Kim, Hye-min Yu, Hye-Jin An, So-hee Kim, Ju-Hyeon Lim, and Soo-Jin Kim<sup>\*</sup> Biome Research Lab, Kolmar Korea Holdings

The new drug development process needs a lot of cost and time. For this reason, today, many researchers are becoming interested in inventing new drug faster by adopting high throughput screening (HTS) methods. In the last decade, increasing evidence designed the gut microbiota is closely related to the Inflammatory bowel disease (IBD) by modulating multiple physiological functions including metabolism, inflammation and immunity response. Therefore, nowadays, a lot of microbiome-based therapeutics for IBD are being developed.

In this study, we have established an HTS platform to detect the new drug candidates of microbiome therapeutics in the mouse model of IBD, which is dinitrobenzene sulfonic acid (DNBS) induced colitis models. We analyzed clinical data and molecular data to discover a colitis-related biomarker in mice. As results, we found that certain biomarkers were changed in the mouse model of IBD when three different *Lactobacillus* strains were administered. Therefore, we suggest that our HTS platform could be used as a strong analysis tool to discover the hit-to-lead drug candidates, and, furthermore, to understand the mechanism underlying IBD.

[This work was supported by Kolmar Korea Holdings]

## Induction of Ginsenosides Using Fungal Endophytes Isolated from Mountain-simulated Ginseng

Salsabila Salsabila, Jake Adolf Montecillo, and Hanhong Bae<sup>\*</sup> Department of Biotechnology, Yeungnam University

*Background:* Ginseng (*Panax ginseng*) has been used as a medicinal plant for thousands of years, owing to its pharmacological activities. The main active ingredients are ginsenosides, which contain various bioactive metabolites. Endophytes colonize plant tissues without causing diseases and can induce and/or produce bioactive compounds.

*Methods:* Fungal endophytes isolated from mountain-simulated *ginseng* (MSG) were evaluated for their ability to increase ginsenoside biosynthesis after inoculation into ginseng plants. We also analyzed the plant hormones and the expression of the genes involved in the ginsenoside biosynthesis pathway.

*Results:* Three fungal endophytes (*Fusarium proliferatum, Diaporthe* sp., and *Trichoderma koningii*) were selected for ginsenoside production. Correspondingly, we found a significant upregulation of the genes involved in ginsenoside biosynthesis in endophyte-inoculated plants. Two endophytes (*F. proliferatum and T. koningii*) were formulated as granules (GR). Treatment of MSG fields with GR significantly increased ginsenoside production. *Conclusion:* The selected fungal endophytes showed great potential as elicitors of ginsenoside production in ginseng plants.

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## G039

## Phylogenetic Comparison and Biochemical Characterization of a Laccase Belonging to the Multicopper Oxidase Superfamily in an Edible Mushroom *Hericium erinaceus*

Thuat Van La<sup>1,2</sup> and Seonghun Kim<sup>1,2\*</sup>

<sup>1</sup>Jeonbuk Branch Institute, Korea Research Institute of Bioscience and Biotechnology, <sup>2</sup>Department of Biosystems and Bioengineering, KRIBB School of Biotechnology, University of Science and Technology (UST)

A laccase was molecularly identified and functionally characterized using recombinant enzyme expressed in yeast strain from an edible mushroom *Hericium erinaceus*. cDNA encoding laccase (Lac1a and Lac1b) from the mushroom mycelium were amplified by RACE-PCR, cloned and expressed in the culture supernatant of *Pichia pastoris* under the control of the alcohol oxidase 1 promoter. The coding region consisted of 1,536 bp and encodes each protein of 511 amino acids with a signal peptide of 21 amino acids. Phylogenetic comparison analysis showed that the deduced amino acid sequence of the matured Lac1a and Lac1b protein displayed high homology with laccases from Basidomyces rather than Ascomyces. The Lac1a gene was successfully produced extra-cellularly to a high level of 87 unit/L as a glycoprotein form, whereas the lac1b gene was not expressed as a secreted protein due to hyper-glycosylation. Biochemical characterization of the recombinant Lac1a (rLac1a) protein purified by a simple two-step procedure to homogeneity is capability for oxidation activity toward variety of aromatic azo, carboxylic acid, and alcohol substrates. rLac1a was highly specific for the oxidation, with a catalytic efficiency of 1,435 s<sup>-1</sup> mM<sup>-1</sup> and 515 s<sup>-1</sup> mM<sup>-1</sup> toward 2,6-dimethylphenol and ABTS, respectively. In addition, rLac1a displayed the enzyme stability in the presence of organic solvents and enhanced its activity within non-ionic detergent such as Triton X-100 and Tween 80.

## Artificial Intelligence Approaches to Identify Metabolic Reactions Important for Bacterial Cell Growth

Hyun Jae Woo<sup>1</sup>, Youngshin Kim<sup>2</sup>, Dohyeon Kim<sup>2</sup>, and Sung Ho Yoon<sup>1,2\*</sup> <sup>1</sup>Department of Systems Biotechnology, Konkuk University, <sup>2</sup>Department of Bioscience and Biotechnology, Konkuk University

Efficient growth of a microorganism has been a challenge for the biotechnological industry. Identification of metabolic genes involved in promotion and retardation of cell growth under given environmental conditions is relevant to elucidation of the critical steps in metabolic network. In this study, we report machine learning and deep learning approaches to identify metabolic reactions related to growth fitness. Experimental growths of single-gene deletions in *Escherichia coli* were integrated with metabolic fluxes predicted from the metabolic network model. This data was used to train the computational models. Predictions from the machine- and deep-learning models were validated and combined to find metabolic reactions that significantly influence bacterial cell growth with different carbon sources. This work demonstrates how artificial intelligence can be used to find metabolic steps critical to a cellular trait.

## G041

Verification of the Combined Effect of Synbiotics of Carbohydrate Material and *Lacticaseibacillus rhamnosus* GG Gyungcheon Kim and Hakdong Shin<sup>\*</sup>

Dept. of Food Science & Biotechnology, and Carbohydrate Bioproduct Research Center, Sejong University

The aim of this study is to validate the effect of a symbiotic product using a combination of a carbohydrate substance and *Lacticaseibacillus rhamnosus* GG (LGG) on the gut microbiota. Fecal samples of healthy donors were inoculated into a basal medium (2% w/v), and three types of carbohydrates (FOS, Inulin, Sucrose) and an LGG combination group were incubated in anaerobic conditions. And the microbiota analysis was performed based on 16S *rRNA*-based sequencing (V4 region). The bacterial alpha diversity of all carbohydrate groups was reduced, and the gut microbiota structure was separated compared to the control. All groups showed the overrepresented relative abundance of *Bifidobacterium* and *Faecalibacterium* genus, in relation to the control. Also, treatment with LGG on each material increased the relative abundance of the *Lactobacillus* genus. As a result, a synergistic effect was observed in which the beneficial bacteria gut bacteria increased when the carbohydrate material and the combination of LGG were treated.

## Mammalian Cell Culture Process Optimization Using Design of Experiment

Minjoo Kim<sup>1</sup>, Duk Sang Kim<sup>2,3\*</sup>, and Hwa Jeong Lee<sup>2\*</sup>

<sup>1</sup>Major in Industrial Pharmaceutical Sciences The Graduate School of Industrial Pharmaceutical Sciences Ewha Womans University, <sup>2</sup>Graduate School of Pharmaceutical Sciences & College of Pharmacy, <sup>3</sup>Sartorius Korea Biotech Ewha Womans University

This study was intended to establish optimized cell growth conditions in the mammalian cell culture process, and it deduced the results with efficient methods using High-Throughput technology and statistical analysis approaches. For the multiple culture conditions and accurate results, an integrated system Ambr 15 operating culture process in multiple vessels and a metabolic analyzer were used. Data were analyzed specifically with Design of Experiment software (MODDE).

This study was targeted for the optimization of viable cell density (VCD) and cell viability in the culture process with genetically recombined CHO K-1 cell lines targeted for antibody production. A risk assessment from Quality by Design was performed and pH, rpm and inoculation density were selected for the factors. To set up the design spaces with the results obtained from the process and optimized process operational conditions, DoE was used. The highest VCD and viability were verified at 0.3x 10<sup>5</sup>cell/mL inoculation cell density, pH 6.9 and rpm 1,000. It proves that High-Throughput technology and statistical analysis are useful approaches in optimization of the culture process operational conditions in process development. It verified optimized conditions of the factors (pH, rpm, inoculation cell density) impacting on the VCD and viability in culture process statistically. The verified approaches are anticipated to be a guide in the various factor studies required for the cell culture process development.

### G043

## Securing the Biosynthesis Genes of Cinnamaldehyde from Diverse Crops for Producing Cinnamaldehyde in *Escherichia coli*

Jeung-Seon Kim, Young-Joon Ko, Jegadeesh Raman, and Soo-Jin Kim<sup>\*</sup> Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration

Cinnamaldehyde, a volatile, yellow liquid has various application in food and pharmaceuticals and is becoming popular in agricultural area owing to its antimicrobial effect. Cinnamaldehyde is reported for inhibiting the growth of plant pathogen causing soft rot disease, especially. Although chemical synthesis from cinnam bark oil is widely used for producing cinnamaldehyde, the methods have several disadvantages such as difficulty to separate other derivatives and consumption of plants. Therefore the development of eco-friend biological platform is required. Phenylalanine-ammonia lyase (PAL), 4-coumarate-CoA ligase(4CL), and cinnamoyl-CoA reductase (CCR) are key enzymes for biosynthesis of cinnamaldehyde. We searched biosynthesis gene of cinnamaldehyde (PAL,4CL,CCR) from genome data of diverse crops and secured the primers to amplify these genes. We plan to construct plasmid vectors for producing cinnamaldehyde in *E. coli* by introducing three genes. Futhermore, the heterologous expression of these genes will be a background system to increase the yield of cinnamaldehyde in *E. coli* by carrying out genetic, metabolic engineering tools.

[This study is supported by National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea (PJ01679901)]

## Maximal Truncation of Guide RNAs Enabled Efficient CRISPR-mediated Microbial Genome Editing

Ho Joung Lee, Hyun Ju Kim, and Sang Jun Lee<sup>\*</sup> Department of Systems Biotechnology, and Institute of Microbiomics, Chung-Ang University

CRISPR-Cas complex composed of a target-recognizing single molecular guide RNA and a proteinaceous Cas nuclease, can recognize both a specific target sequence and a PAM (protospacer adjacent motif), and subsequently cleave the double stranded target DNAs. However, DNA sequences similar to the target DNA sequence could be cleaved occasionally, which might be the result of mismatch tolerance, which may interfere with CRISPR/Cas-mediated accurate genome editing. We systematically determined the genomic cleavage activities of SpCas9 and FnCpf1 with serially truncated guide RNAs carrying a single-base mismatch in *E. coli*. As a result, single base mismatch intolerance of SpCas9 and FnCpf1 was observed with maximally truncated RNAs. Compared to other truncated guide RNAs, the use of both maximally-truncated guide RNAs showed the highest editing efficiency and accuracy in *galK* and *xylB* of *E. coli*. Furthermore, we successfully introduced single-nucleotide indels with high efficiency by the use of the maximally truncated guide RNAs. Collectively, the maximal truncation of guide RNA enables efficient microbial genome editing independent of Cas9 and Cpf1 at the single-nucleotide resolution. [This study was supported by the National Research Foundation of Korea.]

## G045

## High-quality Genome Sequence Data Which Could be the Possible Biomarker for the Presence of Norovirus in Shellfish in Korea

Geunsoo Jung and Hakdong Shin<sup>\*</sup> Dept. of Food Science & Biotechnology, and Carbohydrate Bioproduct Research Center, Sejong University

The food poisoning caused by norovirus from shellfish is increasing every year in Korea. Prompt detection of norovirus could be the best solution, but due to the difficulty of culturing norovirus, Male-specific coliphages (MSCs) have been suggested as an indicator of foodborne viruses. The purpose of this study is to identify the genome type of MSCs isolated from Korea. We newly isolated a total of 77 bacteriophages from about 500 shellfish samples (*Mytilus edulis*) of the southern sea in Korea. Two types of F+DNA MSC coliphages were selected and additional characteristics were confirmed based on full genome sequencing. Sequencing was performed at the Illumina MiSeq platform and analyzed with the CLC genomics workbench (version 20.0.4). As a result of comparative analysis with the obtained genomes using the NCBI database, Nobel genetic elements were identified. These sequencing data from F+DNA coliphages from Korea could be used as a biomarker to screen the safety of shellfish in Korea.

## Inhibition of Adipogenesis in Mouse 3T3-L1 Pre-adipocytes by an Extract from *Sophora japonica* Fruit Acting via the AMP-activated Protein Kinase Pathway

Young-Kyung Lee, Chul Hwan Kim, Jin-Woo Jeong, and Yong Taek Oh<sup>\*</sup> Nakdonggang National Institute of Biological Resources

The world wide rate of obesity is increasing continuously, representing a serious medical threat since it is associated with a variety of diseases including type2 diabetes, cardiovascular disease, and numerous cancers. *Sophora japonica* is used as a traditional herb for medicinal purposes in eastern Asia. However, the anti-obesity effects of *S. japonica* fruit have not been explored. Here we investigated the inhibition of adipocyte differentiation and adipogenesis by an ethanol extract of *S. japonica* fruit (EESF) in 3T3-L1 pre-adipocytes. Our results demonstrate that EESF suppressed the terminal differentiation of 3T3-L1 pre-adipocytes in a dose-dependent manner, as confirmed by a decrease in lipid droplet number and lipid content throught Oil Red O staining. EESF significantly reduced the accumulation of cellular triglyceride, which was associated with a significant inhibition of the levels of pro-adipogenic transcription factors, downregulated the expression levels of adipocyte-specific proteins. Furthermore, EESF treatment effectively increased the phosphorylation of AMPK and ACC. These results together indicate that EESF has significant effects on the inhibition of adipogenesis and acts by stimulating the AMPK signaling pathway.

### H002

## **Anti-inflammatory and Anti-atopic Effects of** *Rorippa cantoniensis* **Extracts** Minjin Kim, Byeongsu Hwang, and Yeongtaek Oh<sup>\*</sup> *Nakdonggang National Institute of Biological Resources*

This study describes a preliminary evaluation of the anti-inflammatory activity and anti-atopic activity of *Rorippa cantoniensis* extracts (RCE). In order to effectively screen for anti-inflammatory agents, we first investigated the inhibitory effects of RCE on production of pro-inflammatory factors [nitric oxide (NO), prostaglandin E2 (PGE2), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2)] and pro-inflammatory cytokines [tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) and interleukin-1 $\beta$  (IL-1 $\beta$ )] in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. In addition, we also evaluated of their inhibitory effect on the atopic dermatitis-like inflammatory markers such as macrophage-derived chemokine (MDC) and thymus and activation-regulated chemokine (TARC) in Human keratinocyte HaCaT cells. RCE could effectively inhibit the LPS-induced production of pro-inflammatory factors and pro-inflammatory cytokines in a dose dependent manner, respectively. And RCE also showed inhibitory activity for MDC and TARC expression levels in IFN- $\gamma$ -stimulated HaCaT cells, respectively. These results suggest that RCE have significantly effects of anti-inflammatory activity and anti-atopic activity that might be beneficial for the topical treatment of inflammatory skin disorders

## Development of Antibiotics Administration Diagnostic Kit through DNA Methylation Analysis Following Repeated Antibiotic Exposure in Porcine

Hong-Il Kim<sup>1</sup>, Seong-Jin Yoon<sup>1</sup>, Ye-Ryung Lee<sup>1</sup>, Kang-Il Lee<sup>2</sup>, and Yong-Un Jeong<sup>1\*</sup> <sup>1</sup>Strategic Business Division R&D Center, Biosesang Co., Ltd., Incheon, <sup>2</sup>Biosesang Co., Ltd., Gyeonggi-do

Currently, various pig farms are using various antibiotics for the prevention and treatment of diseases caused by microbial infection. However, indiscriminate administration of these antibiotics can cause various health hazards, such as the occurrence of antibiotic-resistant bacteria or human ingestion by remaining in pigs. In this study, we aimed to develop an antibiotic exposure history diagnostic kit through analyzing antibiotic treatment induced genome methylation at the porcine cells level. We performed cell viability analysis and WGBS (whole genome bisulfite sequencing) analysis of PSM (porcine-drived semi-membranous muscle) cells treated with various antibiotics. According to the WGBS analysis result, we confirmed that there were 104,062 and 103,422 significant DMCpG (Differential methylation CpG) in the Amoxicillin-treated group and Sulfathiazole-treated group compared to the non-treated group. For the PCR primer production, the above DMCpG was selected in two ways. The first is a region containing three or more DMCpG within 30 bp, and the second is a region containing consecutive DMCpG. As a result, 32 and 31 bio-marker candidates capable of diagnosing the administration of amoxicillin and sulfathiazole were selected.

### H004

## The Study on Prevalence of Severe Fever with Thrombocytopenia Syndrome Virus among Ticks Collected in Northern Gyeonggi-Do

Seon-Jae Bang, Sun-Mok Kwon, Seo-In Oh, Su-Jin Kim, Bo-Mi Kim, Chae-Eun Kim, Jong-Soo Kim, and Mi-Jung Jang<sup>\*</sup> Gyeonggi Province Research of Health & Environment Nothern Support Institute

This study was carried out to investigate the distribution of ticks in northern Gyeonggi Province where has SFTS positive patients prevalence area and progressed for confirmation epidemiologic relationship between patients and ticks. From May to October 2019, total 2,354 ticks were identified *Haemaphysalis longicornis* (97.5%), *Haemaphysalis flava* (1.8%) and *Ixodes persulcatus* (0.7%). In the first half of the year, mostly nymph were collected, after August, the number of larva increased rapidly.

A total of 129 groups of collected ticks were pooled and nested RT-PCR was performed to detect SFTSV. Among 19 collected area, SFTSV was detected from ticks in the 2 areas. As a result, comparing with serum of SFTS positive patients, each of them showed homologue 96.8–97.0%, 99.2% epidemiologic relationship between SFTS patients and ticks. Additionally, *Borrelia burgdoferi* 16S rRNA causing Lyme disease was identified in *I. persulcatus* collected from one area.

[Supported by grants from Gyeonggi Province]

## Anode Biofilm Maturation Time, Stable Cell Performance Time, and Time-course Electrochemistry in a Singlechamber Microbial Fuel Cell with a Brush-anode

Huong Tran Viet Hoa, Zita Michael, and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

For accurate and reproducible MFC experiments, it is important to know when MFCs produce stable cell performance. Herein, four replicate single-chamber MFCs were tested for 17 weeks by using polarization and cyclic voltammetry (CV) tests. The strong MFCs (#2,4,3) showing continuous performance enhancement initially (3<sup>rd</sup>-9<sup>th</sup> week) produced good subsequent performance (9<sup>th</sup>-17<sup>th</sup> week). The weak MFC-1 experienced a performance drop initially and showed bad subsequent performance. All the MFC performance became stable after 9 weeks. The strong MFCs produced power 2.8–3.6 times higher and anode resistance 7.5–23.9 times lower than the weak. However, their cathode resistances were similar. CV results showed anodic current production increased continuously in all MFCs, indicating anode biofilms kept growing; MFC performance did not increase accordingly. Anodic CVs had a typical S-shape curve, but those of MFC-1 showed straight lines from the 9<sup>th</sup> week. The weak MFC-1 showed smaller CV currents and thinner CV curves than those of the strong MFCs. In MFC-1, at the 17<sup>th</sup> week, the anode resistance was a limiting factor of the weak MFC and cathode resistance was that of the strong MFCs. This result suggests one operating principle: improve anodes in weak MFCs and cathodes in strong MFCs to achieve better MFC performance.

## H006

Improvement of Air Cathode Performance in Microbial Fuel Cells by Using Catalysts Made by Binding Metalorganic Framework and Activated Carbon through Ultrasonication and Solution Precipitation Huong Tran Viet Hoa, Zita Michael, and Sokhee Jung<sup>\*</sup>

Department of Environment and Energy Engineering, Chonnam National University

Activated carbon (AC) is an inexpensive catalyst for the oxygen reduction reaction in the air cathode of microbial fuel cells (MFCs). However, since the electrochemical catalytic activity of AC is poor, it is necessary to improve its performance. The metal–organic framework (MOF) is composed of a metal ion and an organic linker. It has high porosity and high electrochemical catalytic activity. Herein, ZIF-67 was combined with activated carbon through ultrasonication (U) and solution precipitation (H), which was used to make ZIF-67U and ZIF-67H cathodes, respectively. In maximum power density, ZIF-67U cathode produced 4203 mW m<sup>-2</sup>, and ZIF-67H did 3881 mW m<sup>-2</sup>, which is 60% and 48% higher than AC cathode and 160% and 140% higher than Pt cathode. Cobalt and nitrogen contents increased in the ZIF catalysts. In atomic nitrogen contents of catalyst surface, pyridine-N was 28% in ZIF-67U and 38% in ZIF-67H, respectively; pyrrole-N was 56% in ZIF-67U and 25% in ZIF-67H, respectively; no nitrogen was detected in AC. These cobalt-nitrogen increased the active site of the oxygen reduction reaction (ORR), improved the reaction rate, and decreased charge transfer impedance. AC and ZIF-67 were bonded using ultrasonication and tested in the MFC for the first time, producing the highest power ever among the MOFs in the 50-mM phosphate-buffer-saline condition so far.

## Improved Structures of Stainless Steel Current Collector Increase Power Generation of Microbial Fuel Cells by Decreasing Cathodic Charge Transfer Impedance

Huong Tran Viet Hoa, Zita Michael, and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Microbial fuel cell (MFC) is an innovative environmental and energy system that converts organic wastewater into electrical energy. For practical implementation of MFC as a wastewater treatment process, a number of limitations need to be overcome. Improving cathodic performance is one of major challenges, and introduction of a current collector can be an easy and practical solution. In this study, three types of current collectors made of stainless steel (SS) were tested in a single-chamber cubic MFC. The three current collectors had different contact areas to the cathode (P 1.0 cm<sup>2</sup>; PC 4.3 cm<sup>2</sup>; PM 6.5 cm<sup>2</sup>). The results showed that increasing the contacting area enhanced the power and current generations and coulombic and energy recoveries by mainly decreasing cathodic charge transfer impedance. Application of the SS mesh to the cathode (PM) improved maximum power density, optimum current density and maximum current density by 8.8%, 3.6% and 6.7%, respectively, comparing with P of no SS mesh. The SS mesh decreased cathodic polarization resistance by up to 16%, and cathodic charge transfer impedance by up to 39%, possibly because the SS mesh enhanced electron transport and oxygen reduction reaction. However, application of the SS mesh had little effect on ohmic impedance.

### H008

Addition of Reduced Graphene Oxide to an Activated-carbon Cathode Increases Electrical Power Generation of a Microbial Fuel Cell by Enhancing Cathodic Performance

Huong Tran Viet Hoa, Zita Michael, and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Activated carbon (AC) is an inexpensive catalyst for oxygen reduction in an air cathode of microbial fuel cells (MFCs). In the AC-based cathode, carbon black (CB) is used as a conductive supporting material. In this study, it was hypothesized cathodic performance would increase if reduced graphene oxide (rGO) replaces CB in an optimum ratio. rGO replaced CB in the four different weight ratios of rGO to CB: 0:30 (rGO0); 5:25 (rGO5); 15:15 (rGO15); 30:0 (rGO30). Maximum power density was the best in rGO15 (2642 mW/m<sup>2</sup>) followed by rGO5 (2142 mW/m<sup>2</sup>). In the optimum external resistance operation, rGO5 and rGO15 showed similar power (~1060 mW/m<sup>2</sup>), higher than the others. Linear sweep voltammetry, cyclic voltammetry, and impedance spectroscopy also showed that the optimal rGO additions improved cathodic performance and reduced cathodic internal resistance. Due to the flatter and wider shape of rGO and 5 times higher electrical conductivity than CB, the rGO addition improved the cathodic performance due to the increased thickness and the morphological crack. The optimum rGO addition is a simple and effective method for improving cathodic performance.

## Effects of Vertical and Horizontal Configurations of Different Numbers of Brush Anodes on Performance and Electrochemistry of Microbial Fuel Cells

Huong Tran Viet Hoa, Zita Michael, and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

To maximize wastewater treatment and energy production by microbial fuel cells (MFCs), it is important to design the optimal anode arrangement. In this study, four brushes were tested horizontally or vertically to the cathode as the number of the anodes increased from one to four. In the horizontal configuration, adding the anodes greatly reduce electrode resistance and enhanced cell performance, showing four anodes (H4) was the best. In the vertical configuration, two anodes (V2) showed greatest performance and greatest decrease in anode resistance. Compared with one anode, maximum power increased by 59% in H4 and by 18% in V2; anode polarization resistance decreased by 95% in H4 and by 74% in V2; anode impedance decreased by 91% in H2 and by 73% in V2. Cathode resistance was relatively constant, showing adding anodes had negligible effect on it. In this study, adding more anodes vertically decreased cell performance in V3 and V4. However, in a cyclic voltammetry test, current production was substantially increased when the third and the fourth brush anodes were introduced in the both arrangements. Compared with one anode, current production increased by 200% in H4 and by 205% in V4. It shows that the external electrical input relieved diffusion resistance and increased current generation and that installing anodes away from the cathode is a good strategy to increase current production in a system with external power supply such as microbial electrolysis cell.

### H010

## Energy-efficiency Performance Analysis and Maximization Using Wireless Energy Harvesting in Wireless Sensor Networks

Ali Mohammad Mahasin, Huong Tran Viet Hoa, and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Paradigm shift to wireless power transfer provides opportunities for ultra-low-power devices to increase energy storage from electromagnetic (EM) sources. The notable gain occurs when EM sources deliver information as a meaningful signal with power transfer. Thus, energy harvesting (EH) is an active approach to obtain power from surrounding EM sources that transfer energy deliberately. This paper discusses energy efficiency (EE) trade-offs and EE maximization in simultaneous wireless power and information transfer (SWIPT) for wireless sensor networks (WSNs). The power splitting (PS) and time switching (TS) model for SWIPT are investigated in detail, where EE optimization is essential. At present, the study on SWIPT focuses on cornering its basic architectural design and applications in some systems, such as MIMO wireless broadcast systems and cooperative relay network. This work formulates EE maximization problem as non-linear fractional programming and proposes a novel algorithm to solve the maximization problem using Lagrange dual decomposition. By utilizing the SWIPT system model, this work adapts two different receiver's architectures for PS and TS mode. The EE is enhanced notably by modifying PS ratio of power splitter as well as varying time-slots. Numerical results reveal that the proposed algorithm maximizes EE in both PS and TS modes through noteworthy improvements.

## Polymer Film-based Screening and Isolation of Polylactic Acid (PLA)-degrading Microorganisms

Ali Mohammad Mahasin, Huong Tran Viet Hoa, and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Polylactic acid (PLA) has been highlighted as an alternative renewable polymer for the replacement of petroleumbased plastic materials, and is considered to be biodegradable. The biodegradation of PLA by terminal degraders, such as microorganisms, requires a lengthy period in the natural environment, and its mechanism is not completely understood. PLA biodegradation studies have been conducted using mainly undefined mixed cultures, but only a few bacterial strains have been isolated and examined. In this study, the PLA-degrading bacteria from digester sludge were isolated and identified using a polymer film-based screening method. The enrichment of sludge on PLA granules was conducted with the serial transference of a subculture into fresh media for 40 days, and the attached biofilm was inoculated on a PLA film on an agar plate. 3D optical microscopy showed that the isolates physically degraded the PLA film due to bacterial degradation. 16S rRNA gene sequencing identified the microbial colonies to be Pseudomonas sp. MYK1 and Bacillus sp. MYK2. The two isolates exhibited significantly higher specific gas production rates from PLA biodegradation compared with that of the initial sludge inoculum. In this respect, the screening method and isolation of PLA-degrading bacteria might be a significant stepping-stone for the further application and development of environmentally friendly polymers and plastics.

## H012

**Recent Advancements in the Cathodic Catalyst for the Hydrogen Evolution Reaction in Microbial Electrolytic Cells** Ali Mohammad Mahasin, Huong Tran Viet Hoa, and Sokhee Jung<sup>\*</sup> *Department of Environment and Energy Engineering, Chonnam National University* 

The microbial electrochemical technology is a foremost viable technology for hydrogen production from organic matter or wastewater catalyzed by electroactive microorganisms. Generation of hydrogen is emphasized by many researchers due to nonpolluting, safe, and affordable alternative energy sources are being recognized widely as its eco-friendly nature, easy availability, and non-emission of air pollutants after combustion. In this article, the cathode materials and catalysts for hydrogen evolution reaction (HER) in MECs are reviewed due to the microbial electrochemical technology is a foremost viable technology for hydrogen production from organic matter or wastewater catalyzed by electroactive microorganisms. There is an essential requirement of cost-effective HER catalysts for improving MEC performance and as the practical findings fell short of the ideal catalyst's expectations, the density functional theory (DFT) can give essential molecular knowledge and anticipate viable catalysts. Developing a high-efficient and low-cost cathode for hydrogen production is crucial for the practical applications of MEC. Additionally, this article provides an overview of the development of density functional theory (DFT), as well as computer simulations for HER processes using DFT, and also computational designs and virtual screens of novel HER catalysts.

## **Microbial Electrolysis Cells for Electromethanogenesis: Materials, Configurations and Operations** Ali Mohammad Mahasin, Huong Tran Viet Hoa, and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Anaerobic digestion is a traditional method of producing methane-containing biogas by utilizing the methanogenic conversion of organic matter like agricultural waste and animal excreta. Recently, the application of microbial electrolysis cell (MECs) technology to a traditional anaerobic digestion system has been extensively studied to find new opportunities in increasing wastewater treatability and methane yield and producing valuable chemicals. The finding that both anodic and cathodic bacteria can synthesize methane has led to the efforts of optimizing multiple aspects like microbial species, formation of biofilms, substrate sources and electrode surface for higher production of the combustible compound. MECs are very fascinating because of its ability to uptake a wide variety of raw materials including untreated wastewater (and its microbial content as biocatalysts). Methane is generally detected in MECs during the hydrogen production stage due to the growth of methanogens and generation of methane varies with shift in inoculum, substrate, and reactor design. This review is dedicated to explaining the operating principles and mechanism of the MECs for electromethanogenesis using different biochemical pathways. Emphasis on single- and double-chambered MECs along with reactor components is provided for a comprehensive description of the technology. Methane production using hydrogen evolution reaction and nano-catalysts has also been discussed.

### H014

Enhanced Denitrification of Contaminated Groundwater by Novel Bimetallic Catalysts Supported on Kaolinderived Zeolite: Effects of Natural Dissolved Inorganic and Organic Matter

Ali Mohammad Mahasin, Huong Tran Viet Hoa, and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Nitrate (NO<sub>3</sub><sup>-</sup>) contaminated groundwater has increased worldwide owing to anthropogenic activities such as the overuse of nitrogen-based fertilizers and discharge of poorly-treated wastewater. In this study, a novel Pd–In bimetallic catalyst supported on kalinite-induced zeolite (ZK) was developed for highly enhanced nitrate conversion into nitrogen gas. However, only a few studies have investigated the effect of natural substances in real groundwater on catalytic denitrification. Herein, we investigated the effects of natural dissolved inorganic and organic matter on catalytic denitrification of contaminated groundwater using a novel Pd–In bimetallic catalyst supported on kaolin derived zeolite (ZK). The developed Pd–In/ZK catalyst showed a highly enhanced performance for denitrification, resulting in a high turnover frequency ( $18.9 \times 10^{-3} \text{ s} -1$ ) and N<sub>2</sub> selectivity (98%). Its application to the NO<sub>3</sub><sup>-</sup> contaminated groundwater revealed that humic-like and fulvic-like organic substances potentially inhibited the catalytic NO<sub>3</sub><sup>-</sup> reduction and some inorganic ions inhibited the catalytic NO<sub>3</sub><sup>-</sup> reduction via different inhibitory mechanisms, i.e. i) the competitive adsorption of Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> with NO<sub>3</sub><sup>-</sup> and ii) CaCO<sub>3</sub>(s)-induced catalyst fouling.

## Microbially Powered Electrochemical Systems Coupled with Membrane-based Technology for Sustainable Desalination and Efficient Wastewater Treatment

Ali Mohammad Mahasin, Huong Tran Viet Hoa, and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Seawater has a potential for managing the intensive potable drinking water demand. The recent conventional desalinating technologies are environmentally unsustainable and energy intensive. Thus, in the quest to find an alternative to the traditional desalination technologies, microbial desalination cells (MDC) have come into limelight. MDCs are considered the promising technologies for treating wastewater while simultaneously producing electricity, which can be utilized for desalinating seawater along with production of some value-added products. However, some technical limitations associated with the practical usage of MDCs are pH maintenance at the cathodic side, internal resistance along with membrane fouling and its durability. These challenges can be dealt by utilizing various integrated configurations. The objective of the current review is to evaluate all MES-MT on the basis of the latest papers. The specification in terms of theory and implementation of MES-MT are described in detail in terms of design, performance and important operational factors. In addition to discussing the barriers to exploit the full potential of MES-MTs, current problems have also been addressed, including low biodegradability of wastewater, sluggish marine desalination levels, upscaling issues, and membrane-related issues including fouling.

### H016

## Characterization of Impedance and Polarization of Carbon-felt Bioanodes and Activated-carbon Cathodes in a Continuous-flow Microbial Fuel Cell

Bonyoung Koo and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Characterizations of the electrochemistry of a microbial fuel cell (MFC) is very important in developing bioelectrochemical energy producing wastewater treatment process. Compared to the development of MFC technology, however, understanding of its electrochemical characterization is still insufficient. The main reason is that its electrochemical analysis is very difficult due to the complex nature of the anode biofilm, which is a key to generating electricity. In this experiment, the influence of the measurement potential of impedance and the scanning rate for polarization curve on the MFC electrochemistry was investigated. The experiment was performed after stabilizing the system for accurate measurement. Unlike the previous batch tests showing the lowest anodic impedance at -400 mV vs. Ag/AgCl, the anodic impedance decreased and the current production increased as the anode potential increased up to +5.7 mV vs. Ag/AgCl in the continuous flow MFC. However, the cathodic impedance was very little affected by the measuring potential because the activated carbon catalyst is abiotic and much less sensitive to electrical stimulation than the anode biofilm. The polarization curves were produced at two scanning rates (1 and 0.1 mV/s) in a continuous mode, and those electrochemical data were comparatively analyzed. When it is difficult to maintain a steady state for a long time in an MFC, it will be possible to produce polarization curves in a short time with a faster scanning rate.

## Recent Trends of Oxygen Reduction Catalysts in Microbial Fuel Cells

Bonyoung Koo and Sokhee Jung<sup>\*</sup>

Department of Environment and Energy Engineering, Chonnam National University

Microbial fuel cell (MFC) is a system being developed for a next-generation energy-producing wastewater treatment process that treats wastewater and recovers energy. Because an MFC cathode performing oxygen reduction is a bottleneck in performance enhancement, significant improvement of the cathode performance is necessary for the practical implementation of MFC system. The most ideal oxygen reduction catalyst is known as platinum. However, platinum is expensive and not long-lasting, making it difficult to apply to a real application. For this reason, a lot of research has been conducted on development of cathode catalysts in the MFC field. One of the important goals of MFC technology is an energy-independent or energy-supplied wastewater treatment process. Considering the size of the wastewater treatment plant and the characteristics of the wastewater, the ideal cathode catalyst for this should have high durability and high economic efficiency in long-term operation. Summarizing the research results so far, the activated carbon-based catalyst is considered to be the most promising ORR catalyst for the practical use of MFC. The 1,210 mW/\$ achieved by the activated carbon catalyst mixed with CB is the highest cost-performance ratio in the studies reported to date. It is necessary to study electrochemical mechanisms and materials to improve the performance and durability of activated carbon catalysts, and research for mass production of electrodes is needed.

### H018

**Trends of Microbial Electrochemical Technologies for Nitrogen Removal in Wastewater Treatment** Bonyoung Koo and Sokhee Jung<sup>\*</sup>

Department of Environment and Energy Engineering, Chonnam National University

The removal of organic carbon and nutrients (i.e. N and P) from wastewater is essential for the protection of the water environment. Especially, nitrogen compounds cause eutrophication in the water environment, resulting in bad water quality. Conventional nitrogen removal systems require high aeration costs and additional organic carbon. Microbial electrochemical system (MES) is a sustainable environmental system that treats wastewater and produces energy or valuable chemicals by using microbial electrochemical reaction. Innovative and cost-effective nitrogen removal is feasible by using MESs and increasing attention has been given to the MES development. In MEC, nitrogen removal technology through external applied voltage electrochemically oxidizes ammonium under perfect anaerobic conditions and achieves denitrification without additional carbon source. The nitrogen removal efficiency can be increased by improving the adhesion of electroactive microorganisms of the oxidation electrode and the reduction electrode catalyst of the MEC, the activity of the biofilm catalyst, and the electrode electron transfer efficiency. However, in terms of practical use, it is necessary to develop an effective electrode structure to solve the electrode clogging problem caused by various contaminants and suspended matter in wastewater.

## Recent Trends and Prospects of Microbial Fuel Cell Technology for Energy Positive Wastewater Treatment Plants Treating Organic Waste Resources

Bonyoung Koo and Sokhee Jung<sup>\*</sup>

Department of Environment and Energy Engineering, Chonnam National University

Although MFC has many applications, the most promising application is in wastewater treatment. MFC can be grafted into a sewage treatment plant to remove the remaining organic matter and generate energy for the operation of the sewage treatment plant. Many studies have been carried out to improve the performance of these MFCs, and they have shown the potential for commercialization of MFCs, such as showing results of grafting them with actual sewage treatment plants. The current research trend of MFC scale-up is as follows. i) development of electrodes and membranes with high economy and performance to reduce high cost, ii) development of materials resistant to contamination, corrosion and clogging for long-term operation, iii) connection of multiple modules to minimize losses Scale up in the form of a stack. However, there are still a number of obstacles that prevent scale-up. The main obstacles are high cost, low power generation due to internal losses, durability and removal of contaminants. Therefore, it is important to develop efficient electrodes and increase the actual wastewater treatment efficiency in order to reduce the high cost and internal losses. Looking at the results of MFC scale-up studies so far, it has been shown that stack configuration can achieve high performance by reducing internal losses, is cheaper than scaling up a single reactor, and has high pollutant handling capacity.

### H020

## Comparison of Hydrogen Production and System Performance in a Microbial Electrolysis Cell Containing Cathodes Made of Non-platinum Catalysts and Binders

Sunghoon Son and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Microbial electrolysis cell (MEC) is an innovative electrochemical technology that decomposes organic matter in anode and produces hydrogen in cathode. It is imperative to use a high-performance and a low-cost cathode material to make the application of MEC economically viable. In this study, five different cathodes made of low-cost materials were tested in MECs. The materials included activated carbon (AC) and nickel powder (Ni) as a cathode catalyst; polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (PVDF) as a catalyst binder; stainless steel mesh (SSM) as a cathode substrate or a cathode itself. Among the tested cathodes, Ni/AC/PTFE obtained the best performance, followed by Ni/AC/PVDF, AC/PVDF, flamed-oxidized SSM (SSM/F) and SSM. Ni/AC/PTFE exhibited the best performance in hydrogen production rate (HPR, 1.88 L/L d), hydrogen purity (97.5%), coulombic efficiency (124%), energy efficiency (216%), cathodic capacitance (0.9924 F), along with the lowest cathodic impedance (35  $\Omega$ ). The results confirmed that Ni/AC/PTFE had the combined advantage characteristics of nickel with high catalytic activity and PTFE with high cathode capacitance and porosity. This study shows quantitatively the electrochemical and performance transitions of MEC according to the cathode component changes.

## Effects of Wire-type and Mesh-type Anode Current Collectors on Performance and Electrochemistry of Microbial Fuel Cells

Sunghoon Son and Sokhee Jung\*

Department of Environment and Energy Engineering, Chonnam National University

Carbon-based material is commonly used for the anodes in MFCs, but its low conductivity often limits anodic performance. Application of corrosion-resistive current collector to the carbon-based anode can be a promising strategy for increasing the anodic performance. In this study, it was hypothesized increasing the metal current collector improved anodic performance. Two different carbon-felt anodes with titanium wires (CF-W) or stainless steel mesh (CF-M) as a current collector were tested in a single chamber MFC. In the short-term tests such as polarization and impedance tests, CF-M with the larger current collector area ( $21.7 \text{ cm}^2$ ) had 33% higher maximum power (2311 mW/m2), 81% lower anodic resistance ( $3 \Omega$ ), and 92% lower anodic impedance ( $1.1 \Omega$ ). However, in the long-term tests, the CF-W with the smaller current collector area (0.6 cm2) showed higher performance in power and current generation, COD removal, and CE (51%, 10%, 11%, and 5% higher, respectively) and produced 41% higher net current in the cyclic voltagramm (20.0 mA vs. 14.2 mA). The stainless steel mesh used as the current collector of CF-M might inhibit mass transfer. Although stainless steel has more advantages as an anode current collector material over titanium in terms of both current collection and microbial reaction, CF-M showed lower electrochemical performances in the long-term tests than CF-W, implying that mass transfer is more important in a long-term operation.

### H022

## Comparative Evaluation of Performance and Electrochemistry of Microbial Fuel Cells with Different Anode Structures and Materials

Sunghoon Son and Sokhee Jung<sup>\*</sup> Department of Environment and Energy Engineering, Chonnam National University

Various materials and anode structures have been applied to enhance MFC performance. However, their comparative evaluation of performance and electrochemistry has not yet been investigated in detail under a same condition. In this study, a carbon-cloth anode, an anode-cathode assembly, and a brush anode with two different orientations were tested under a same condition for comparative analyses on their performance and electrochemistry, in order to reveal their unique electrochemical characteristics. The brush anode cells exhibited better performance than the carbon cloth cells. The brush anodes showed 41–72% higher maximum power densities, 18–75% higher maximum current density and 24–32% higher optimum current densities than the carbon cloth anodes. The brush anodes showed 25–43  $\Omega$  lower anodic polarization resistance than the carbon cloth anodes. The brush anodes showed 1.6–21.2  $\Omega$  lower ohmic impedance, 7.7–10.6  $\Omega$  lower charge transfer impedance and 9.3–31.8  $\Omega$  lower anodic impedance than the carbon cloth anodes. Anodic ohmic impedance was greatest in the carbon-cloth-anode MFC (21.9  $\Omega$ ), where loose contact between a carbon cloth and a current collector might cause the high ohmic resistance, and large solution resistance in the cell could diminish anode performance due to slow ion transport. In order to improve MFC performance by modifying anode structures.

## Influence of Flowrates to a Reverse Electro-dialysis (RED) Stack on Performance and Electrochemistry of a Microbial Reverse Electrodialysis Cell (MRC)

Sunghoon Son and Sokhee Jung\*

Department of Environment and Energy Engineering, Chonnam National University

Salinity gradient and organic wastewater with simultaneous treatment. Operating an MRC at an optimum flowrate to RED is important because it is closely related with energy production rate and economic feasibility. However, influence of RED flowrates on MRC electrochemistry and power production have not been investigated. For this purpose, four different flowrates of high concentration and low concentration solutions were tested. Maximum power density was highest in 10 ml/min (3.71 W/m<sup>2</sup>) and optimum current density was highest in 7.5 ml/min (5.36 A/m<sup>2</sup>). By mere increasing the flowrate to MRC, maximum power and optimum current densities increased by 17.7% and 16.2%. EIS showed that impedances of anode, cathode and full-cell were decreased by 51%, 31% and 19%, respectively. Anode CV showed that peak current density was increased by 25.7%. COD removal and CE were not affected by RED flowrate. Power generation at 7.5 mL/min and 10 mL/min were not so different, but current production was better at 7.5 ml/min. We tested influence of the RED flowrates on performance, electrochemistry and optimal flowrate for maximum power production in MRCs. For this purpose, different flowrates of high- and low-salinity concentrations were tested. Considering electricity power production, energy efficiency and energy recovery, RED flowrate 7.5 ml/min is the best choice among the tested flowrates in the tested MRC.

### H024

Effects of Brush-anode Configurations on Performance and Electrochemistry of Microbial Fuel Cells Sunghoon Son and Sokhee Jung<sup>\*</sup>

Department of Environment and Energy Engineering, Chonnam National University

For practical implementation of microbial fuel cell, increasing power generation is important because it is closely related with energy production rate and wastewater treatability. However, it is not known which relative arrangement of anode and cathode gives the best performance, and it is necessary to know the electrochemical reference point of the brush anode for this. Five different brush-anode configurations were tested in a single-chambered cubic microbial fuel cell. By merely changing a brush anode configuration, power and current densities were increased by 20% and 30%, respectively. The horizontally-positioned anode configuration (H1) with the closest anode-cathode distance produced the highest power and current. EIS showed that anode impedance and full-cell impedance were decreased by 60% and 49%, respectively. Distance between the brush anode and the cathode appreciably affected microbial fuel cell performance and internal resistance of an anode and a full cell. Coulombic efficiency and energy efficiency were not significantly affected by the anode-cathode distance, but the horizontal type cells showed relatively higher coulombic efficiency and energy efficiency, and COD removal rate and shorter batch time. The center of a titanium current collector and the center of carbon fibers of a brush-anode were found to be statistically-significant reference points for microbial fuel cell electrochemistry.

## Quantitative Analysis of Potyvirid Genome Variants Involving the PIPO Slippage Site

Dongjin Choi, Chaerim Shin, and Yoonsoo Hahn<sup>\*</sup> Department of Life Science, Chung-Ang University

The P3 N-terminal region fused with pretty interesting Potyviridae ORF (P3N-PIPO) is a universally conserved transframe fusion protein in the members of the family *Potyviridae* and is essential for the cell-to-cell movement of viruses. P3N-PIPO is translated as the result of an adenine insertion, produced in a small fraction by RNA polymerase slippage at the conserved slippery sequence of six or seven consecutive A residues in front of PIPO. We analyzed publicly available plant RNA-seq data to identify 19 potyvirid genome contigs from 13 potyvirids and investigated the RNA polymerase slippage efficiencies. The frequency of the A insertion variant was estimated from 11 potyviruses (genus *Potyvirus*) and was between 0.53–4.07%, which was comparable to the previously reported frequency from five potyviruses. In two macluraviruses (genus *Macluravirus*), the frequency of the A insertion variant was 0.72% and 10.96%. The variation of RNA polymerase slippage efficiencies among potyvirids may be genetically modulated or affected by physiological or experimental conditions. Our results demonstrated the usefulness of plant RNA-seq data for the quantitative analysis of potyvirid genome variants involving the PIPO slippage site and expanded our understanding of the RNA polymerase slippage phenomenon in potyvirids. [This research was supported by grants from the National Research Foundation of Korea funded by the Government of Korea (grant Nos. NRF-2018R1A5A1025077 and 2020R1A2C1013403)].

## H027

## Safety Assessment Systems for Microbial Starters Derived from Fermented Foods

Sojeong Heo<sup>1</sup>, Seungeun Oh<sup>1</sup>, Yura Moon<sup>1</sup>, Hee-Jung Park<sup>2</sup>, and Do-Won Jeong<sup>1\*</sup> <sup>1</sup>Department of Food and Nutrition, Dongduk Women's University, <sup>2</sup>Department of Food and Nutrition, Sangmyung University

Microorganisms involved in food fermentation not only improve the aroma and taste of the food, but also enhance its preservation. Thus, they are added as starter cultures to improve the final product quality of commercial fermented foods. Although these microorganisms originate from fermented foods and have a long history of consumption, the European Union recently applied the concept of Qualified Presentation of Safety (QPS), a safety evaluation system for microorganisms used in food or feed in Europe. The QPS system is a species-level safety system and shares results with the European Novel Food System, a strain-level safety evaluation system. In the United States, microorganisms added to fermented foods are judged to be a type of additive, not a food ingredient, and safety evaluation centers on the Generally Recognized as Safe notification system. In Korea, food microbe lists are presented at the species level. A strain-oriented evaluation system has been established by applying a temporary safety evaluation system for food raw materials to new raw materials. However, when applying microorganisms isolated from traditional fermented foods in Korea to fermented foods, there is no definition of the term species, and there is a lack of an evaluation system at the species level. An evaluation system for microbial species used in Korean fermented foods is necessary.

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## Epitope-based Prediction on Structural Proteins of Dengue Virus through *In Silico* Analysis for Novel Vaccine Design

Seokhwan Hyeon, Sundong Jang, Byung Chul Kim, and Yookyoung Lee<sup>\*</sup>

Division of Vaccine Development Coordination, Center for Vaccine Research National Institute of Infectious Diseases, Korea National Institue of Health, Korea Disease Control and Prevention Agency

Dengue virus, the most common flavivirus, is occurring highly endemic infectious diseases of tropical countries and is becoming a serious public health problem. However, immunogenicity on the structural viral protein has not been fully understood yet. Here, we investigated prediction of epitope-based immunogenicity on dengue-viral proteins of 4 serotypes with in silico analysis. We focused analysis on these structural proteins: precursor membrane (prM), envelope (E) which contains domain I (EDI), domain II (EDII) or domain III (EDII). The epitope regions of linear B cell showing the immunogenicity were predicted via BepiPred-2.0 determined by its sensitivity and specificity. In addition, we also analyzed the regions of immunogen that induce T cell-mediated immune response employing the Immune epitope database (IEDB) resource that validates peptide presented on HLA class I. The candidates were selected by scoring whether over than 1.0 or not compared to between epitopes and non-epitopes as indicated reference. Then we found consensus sequences of peptide between predicted linear B cell epitopes and T cell immunogenicity peptides to measure each score following descending. Taken together, our results suggest that in silico data demonstrated many of immunogenic candidates showed high specificity against human B and T lymphocytes. This study will contribute to the developing vaccines against dengue virus and discovering novel targets for a number of infectious diseases research.

### H029

## A Metagenomic Study of the Gut Microbiome in Crohn's Disease and Ulcerative Colitis

Da-Yeon Kang<sup>1,2</sup>, Jong-Lyul Park<sup>3</sup>, Min-Kyung Yeo<sup>4</sup>, Sang-Bum Kang<sup>5</sup>, Ju Seok Kim<sup>5</sup>, and Seon-Young Kim<sup>3\*</sup> <sup>1</sup>Department of New Drug Development, Graduate School of New Drug Discovery and Development, Chung-Nam National University, <sup>2</sup>Disease Target Structure Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>3</sup>Aging Convergence Research Center, Korea Research Institute of Bioscience and Biotechnol, Korea Research Institute of Bioscience and Biotechnology (KRIBB), <sup>4</sup>Department of Pathology, Chung-Nam National University School of Medicine, <sup>5</sup>Division of Gastroenterology, Department of Internal Medicine, Daejeon St. Mary's Hospital, College of Medicine, The Catholic University of Korea

Inflammatory bowel disease is a chronic, progressive inflammatory disease that is multifactorial and polygenic, resulting from the dysregulation of the mucosal immune response and intestinal microbiome. Currently, Crohn's disease (CD) and ulcerative colitis (UC) are challenging to distinguish, so differential diagnosis is essential to establish a long-term treatment plan for patients. In this study, we aimed to identify a biomarker for differential diagnosis of the two diseases. We investigated the microbiome diversity in 499 Korean cohort (50 healthy control, 265 UC, and 184 CD) using the shotgun metagenomics sequencing technology. For data analysis, we used the Kraken2 and Bracken pipeline to identify and quantify the microbiome in fecal samples. Then, diversity, relative abundance and difference abundance analysis were performed. Microbiome difference between CD and UC was estimated by the ANCOM-BC, LEfSe, MaAsLin2 and LinDA. Our result showed that intestinal microbial diversity was decreased in patients with UC and CD compared to healthy controls. We also selected microbiome that were "TRUE" in at least three of the four tools we ran to find differentiated microbiome. After testing various combinations of 18 microbiomes, we tested a logistic regression model after spliting samples into training (70%) and testing sets (30%). The best model predicted with an AUC of 0.84, and an AUC of 0.71 in independent data set using public data (CD: 576, UC: 347, 80% train and 20% test).

## **Evaluation of Zone Disinfection Effect of Cold Plasma Filter**

Ki Ho Baek<sup>1</sup>, Taeyoon Kim<sup>2</sup>, Yoohyun Hwang<sup>2</sup>, Joo Young Park<sup>1</sup>, Jun-Yeong Yang<sup>1</sup>, Sungweon Ryoo<sup>2</sup>, and Seunghun Lee<sup>1\*</sup>

<sup>1</sup>Korea Institute of Materials Science, <sup>2</sup>Masan National Tuberculosis Hospital

Disinfection of shared spaces has become essential to minimize the spread of various diseases. In this study, an airpassable plasma filter (APF) was developed to effectively control microorganisms present in an enclosed space. We also developed a chamber model that simulates real environments to objectively evaluate the capabilities of a potential device that disinfects a given space. When APF was continuously operated for 10 and 30 min, the ozone concentrations in the chamber were approximately 10 and 30 ppm, respectively. The operation of APF in the chamber showed a complete zone disinfection effect for *Escherichia coli* (10 min), *Staphylococcus epidermidis* (10 min), and *Mycobacterium smegmatis* (60 min) present in the air and on the walls at various locations. Overall, APF has the potential to exhibit significant germicidal effects on various microorganisms and can be an effective alternative for disinfection of enclosed spaces.

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## H031

## Antioxidant and Algicidal Potentials of Oligochitosan Generated by Solution Plasma Process

Yu Been Ko<sup>1</sup>, Ji Hae Park<sup>2</sup>, Mubarak Ali Davoodbasha<sup>3,4</sup>, Sang Yul Lee<sup>5</sup>, and Jung Wan Kim<sup>1,4\*</sup> <sup>1</sup>Department of Bioengineering and NanoBio Engineering, Graduate School of Incheon National University, <sup>2</sup>Development and Planning Office, Ghent University Global Campus, <sup>3</sup>School of Life Sciences, B.S.Abdur Rahman Crescent Institute of Science and Technology, Chennai, 600048, India, <sup>4</sup>Research Center for Bio Material and Process Development, Incheon National University, <sup>5</sup>Department of Material Engineering, Korea Aerospace University

Chitosan, deacetylated form of chitin, is non-toxic and biocompatible, but its application is limited due to insolubility and high viscosity. As an effort to solve the problems, oligochitosan (OC) was generated from chitosan solution (0.5%, 1N acetic acid) using solution plasma process (SPP) by discharging plasma at 800 V, 30 kHz for 90 min. Previously, OC showed excellent antimicrobial activity. In this study, potentials of OC as antioxidant and algicidal agents were examined. The molecular weight of OC (4.1 kDa) was 73-folds smaller than chitosan (300 kDa) without change in the chemical structure during SPP. DLS analysis showed that OC had an average particle size of  $39\pm1$  nm and a zeta potential of  $47.5\pm1.5$  mV. The DPPH and hydroxyl radical scavenging activities of OC were higher than those of chitosan by 81 and 2 folds, respectively. OC showed algicidal activity against *Cylindrotheca closterium* and *Ulva pertusa* with EC<sub>50</sub> of 1520 $\pm$ 60 and 570 $\pm$ 200 µg/ml, respectively. *Lemna minor* was sensitive to OC with EC<sub>50</sub> of 102 $\pm3$  µg/ml, while *Daphnia magna* was not affected by OC (EC<sub>50</sub> >20 mg/ml). No cytotoxicity of OC was observed against the HEK293 cells up to 1 mg/ml, and growth of the cells was enhanced by 125% by OC (1 mg/ml). These results suggested that OC had various potentials as a biofunctional material such as a novel algicidal agent.

## Synthesis of Selenium Nanoparticles Using Antioxidant Activity of Oligoalginate Simultaneously Generated by Solution Plasma Process

Ji-Min Kim<sup>1</sup>, Su-Jin Kang<sup>2</sup>, Mubarak Ali Davoodbasha<sup>1,3,4</sup>, Sang-Yul Lee<sup>4,5</sup>, and Jung-Wan Kim<sup>1,2,4\*</sup> <sup>1</sup>Research Center for Bio Materials & Process Development, <sup>2</sup>Department of Bioengineering and NanoBio Engineering, Graduate School of Incheon National University, <sup>3</sup>School of Life Sciences, B.S.Abdur Rahman Crescent Institute of Science and Technology, Chennai, India, <sup>4</sup>Center for Surface Coating and Technology, <sup>5</sup>Department of Material Engineering, Korea Aerospace University

Nanoselenium (SeNPs) is an anticancer, antioxidant, and anti-inflammatory agent. Oligoalginate (OA) has antioxidant and growth promoting activities for plant, animal cells, and bacteria. In this study, OA/SeNP biocomposite was synthesized using OA being synthesized by depolymerization of alginate via solution plasma process (SPP) to generate SeNP. Alginate solution (0.75%) with various concentrations of Na<sub>2</sub>SeO<sub>3</sub> (3-7 mM) was discharged with plasma for 30-90 min at 800 V, 30 kHz. Unique peaks were observed at 260-270 nm in the UV-Vis spectra and the solutions turned to red, indicating the synthesis of OA/SeNP. FTIR analysis showed no change in the chemical structure of OA by SPP. XPS confirmed the elemental states of Se in OA/SeNP. The average size and size distribution of OA/SeNP (7 mM, 90 min) was 188.37±16.0 nm and 108.0±0.0~515.23±45.9 nm, respectively; zeta-potential -39.23 ± 0.95 mV. SEM showed they had multi-layered microfibril structures but turned to porous sheet like structures as the discharge time increased. SeNPs in ununiformed shapes, mostly spherical with irregular edges, were observed by TEM. 70.5% of SeNPs were 31~60 nm in diameter and the average particle size was 45.5±16.6 nm. DPPH and  $\cdot$ O<sup>2-</sup> scavenging activity of OA/SeNP was higher than that of OA by 16% and 13%, respectively. The LC<sub>50</sub> of OA/SeNPs for the HeLa and HEK 293 cells was 277 and 74 µg/ml, respectively. Therefore, more effective antioxidant agent of OA/SeNP was synthesized efficiently via SPP.

## H033

## Effect of Copper Alloy on the Inactivation of Covid-19 Virus

Sun Young Mun<sup>1</sup>, Cheol Min Park<sup>1</sup>, Eun Ha Kim<sup>2</sup>, and Hyo Moon Nam<sup>1\*</sup> <sup>1</sup>Poongsan, <sup>2</sup>ChungBuk University

Copper has been known to have antibacterial and antiviral properties since ancient times, and examples of using copper's antibacterial properties can be found in various historical sources. Around 2000 BC, copper was used in Egypt to sterilize water. As such, in the United States, the number of infectious diseases has greatly decreased since the use of copper pipes as drinking water pipes in the 1930s.

Although the antiviral properties of pure copper are well known, the antiviral effect of copper alloys with low copper content has not yet been fully studied. Copper alloys have higher strength than pure copper and also have high price competitiveness, which is more suitable for industrial purposes, such as places that can be exposed to various virus contamination or devices requiring high hygiene.

In this study, the antiviral results of four types of copper alloys with different pure copper contents, aluminum alloy, and stainless steel material were compared.

In the case of the copper alloys, although there is a time difference depending on the copper content, all viruses were killed in about 2 h. In contrast, the virus remained on the stainless steel and aluminum alloys, leaving the risk of cross-infection.

Therefore, it will be possible to reduce virus cross-infection in hospitals and public facilities by using copper and copper alloys for industrial materials.

## **Comparison of Dimension Reduction Methods for Sample Classification of 16S rRNA Microbial Community Data** Do Hyeon Kim and Keun Wan Park<sup>\*</sup>

Natural Product Informatics Research Center, KIST Gangneung Institute of Natural Products

As the knowledge of microbiome rapidly increases due to recent advances in NGS, it becomes clear that human microbiome plays a key role in human disease and health. For this reason, many studies have been conducted to classify diseases based on microbial community data and find important microbial features directly related to various diseases. However, because of the sparse and high-dimensional characteristics of microbiome data, it is critical to evaluate microbiome classification models with respect to the different data processing methods such as data normalization and dimension reduction (DR). In the present study, we compared 5 different DR methods [feature selection, principal component analysis, non-negative matrix factorization (NMF), autoencoder and linear optimal low rank projection) and 3 data normalization methods (total-sum scaling (TSS), cumulative-sum scaling (CSS) and trimmed mean of the M-values (TMM)] in terms of sample classification performance on 16S rRNA microbial community data. The results showed that, among the 5 DR methods, the classification model built on dimension-reduced data by NMF showed relatively higher performance than the classification model based on raw data. We also found that in most datasets the classification models using CSS showed improved performance compared to other normalization methods.

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### H035

### Computational Design of SARS-CoV-2 Antigens for Protein Stability Enhancement

Mi-ran Yun<sup>1</sup>, Yun-Ho Hwang<sup>1</sup>, Jinah Yeo<sup>1</sup>, Ji Hyang Jeon<sup>1</sup>, You-Jin Kim<sup>1</sup>, Heedo Park<sup>2</sup>, Jong Hyeon Seok<sup>2</sup>, Kisoon Kim<sup>2,3</sup>, Man-Seong Park<sup>2,3,4</sup>, and Dokeun Kim<sup>1\*</sup>

<sup>1</sup>Division of Infectious Disease Vaccine research, National Institute of Health, <sup>2</sup>Department of Microbiology, Institute for Viral Diseases, College of Medicine, Korea University, <sup>3</sup>Vaccine Innovation Center, Korea University College of Medicine(VIC-K), <sup>4</sup>Biosafety Center, College of Medicine, Korea University

Protein stability is one of major factors for the expression, purification and structural studies of protein. The stability regulates the activities and the functions of protein. This study subjects to design a novel vaccine against SARS-CoV-2 responsible pathogen for COVID-19, improved protein stability based on antigen structure, using insilico methods.

We targeted spike protein (S1 domain) and orf3a coding sequence, and the sequences of putative universal antigen protein were obtained from phylogenetically conserved antigen. Using computational methods, firstly structures of the universal sequences were modelled. Through two algorithms used phylogenetic analysis and Rosetta energy calculations, potential suboptimal positions were identified, then five top-ranked designs which made effective combinations of mutations on flagged positions, conforming to empirical thresholds, were chosen.

With constructed designs, codon optimization was performed and RNA secondary structure, cavity area and solubility were predicted. Considered all predictions, each one designs for 2 antigens were finally selected as vaccine candidate, respectively.

This computer-aided protein design result is under experimental validation and evaluation for expression and immunogenicity.

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## Development of Antigen Expression System for the Establish of Vaccine Efficacy Evaluation Method against Emerging Infectious Diseases

YoungSeok Oh, JeongHyun Nam, JuneWoo Lee, Byoungguk Kim, and WonSeok Gwak<sup>\*</sup> Division of Vaccine Clinical Research, Center for Vaccine Research, National Institute of Health

In order to quickly develop a vaccine to respond to emerging infectious disease, it is necessary to secure antigenic substances for efficacy evaluation. Serum or antibodies for various infectious diseases are distributed in Korea, but the supply of antigens is dependent on other countries. Therefore, it is required to develop a mammalian cell expression system capable of quickly supplying high-quality antigen materials. In this study, for the development of an overexpression system (vector) of antigenic substances for emerging infectious disease, a group of overexpression vector candidates was established through a combination of promoters and transcriptional enhancer known for their industrial usefulness. In addition, the expression activity between overexpression vectors was compared and evaluated through green fluorescent protein (EGFP) and COVID-19 antigen protein. Finally, we intend to establish an antigen substance overexpression system through cell line selection and optimization of expression conditions.

## H037

## **RVFV Universal Primers for Whole Genome Amplification and Sequencing**

Kwan Woo Kim<sup>1,2</sup>, Seil Kim<sup>3,4</sup>, and Hana Yi<sup>1,5,6\*</sup>

<sup>1</sup>Interdisciplinary Program in Precision Public Health, Korea University, <sup>2</sup>Department of Public Health Sciences, Graduate School, Korea University, <sup>3</sup>Korea Research Institute of Standards and Science, <sup>4</sup>Center for Convergent Research of Emerging Virus Infection, <sup>5</sup>Integrated Biomedical and Life Science, Graduate School, Korea University, <sup>6</sup>School of Biosystems and Biomedical Sciences, Korea University

Rift Valley Fever Virus (RVFV) is one of the "Disease X" reported by WHO and causes an acute viral hemorrhagic fever. Rift Valley Fever (RVF) which is common zoonotic disease and is mainly transmitted through mosquitoes. In this study, we aimed to develop a whole genome sequencing protocol for RVFV. To amplify the genome, 8 pairs of primers stretched over the three genome segments (segment L of 6 kb, M of 4 kb, and S of 1.6 kb) were designed. The primers were applied to four RNAs of RVFV strains (Lunyo, ZH548, Kenya 56, and BIME 01) and produced amplicons of expected size (1.6-2.2 kb). The amplicons were purified and sequenced using MinION, MiSeq, and Sanger sequencer. The resultant sequencing reads were successfully assembled into complete genome regardless of the sequencing platform. The limit of detection of this amplicon-based sequencing was >100 copies/µl implying that the whole genome sequence of RVFV could be directly obtained from clinical samples. Accordingly, this developed method would contribute to the classification of RVFV which is molecularly identified into 15 clades (A-O) in epidemiological surveillance.

[This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Ministry of Education (No. 2022R1A2C1007966)]

## Comparative Analysis of Interferon Gamma Release Assay and Tuberculin Skin Test of Guinea Pigs Using Purified Protein Derivatives of *Mycobacterium bovis* Produced under Different Conditions

Sungkyun Kim<sup>1,2,3</sup>, Sang-Hun Son<sup>1,2</sup>, Ji-Ae Choi<sup>1,2,4</sup>, Junghwan Lee<sup>1,2,4</sup>, Jaewhan Kim<sup>1,2</sup>, Doan Nguyen Tam<sup>1,2</sup>, and Chang-Hwa Song<sup>1,2,4\*</sup>

<sup>1</sup>Department of Microbiology, <sup>2</sup>Department of Medical Science, College of Medicine, Chungnam National University, <sup>3</sup>Choongang Vaccine Laboratories Co., Ltd., <sup>4</sup>Translational Immunology Institute, Chungnam National University

Bovine tuberculosis (bTB) is one of the zoonotic diseases caused by *Mycobacterium bovis* (*M. bovis*). Purified protein derivatives (PPDs) of *M. bovis* have been used for the diagnosis of tuberculosis infection for more than half a century. It is highly complex and poorly characterized reagents for bTB diagnosis. In this study, we focused on the identification of secreted proteins by *M. bovis* in various culture conditions and analyzed the bTB diagnostic ability of the isolated PPDs, such as the tuberculin skin test (TST) and the interferon-gamma release assay (IGRA). *M. bovis* AN5 was grown in Sauton media and culture supernatant was collected at two different phases of growth (late exponential and stationary phases). Supernatants were heated to  $68^{\circ}$ C and  $100^{\circ}$ C to inactivate the bacilli. The PPDs of *M. bovis* used for each evaluation is as follows. Group 1 (G1), PPDs heated to  $68^{\circ}$ C after 3 weeks incubation, Group 2 (G2), PPDs heated to  $68^{\circ}$ C after incubation for 6 weeks, group 3(G3), PPDs heated to  $100^{\circ}$ C after incubation for 3 weeks, group 4 (G4), PPDs heated to  $100^{\circ}$ C after incubation for 6 weeks. Some specific proteins were found in G2 and G4 comparing to G1 and G3. TST with G2 and G4 can distinguish active TB, while there was no concordance between TST and IGRA. These data suggest that effective PPDs of *M. bovis* for diagnosing bTB should be cultured for at least 6 weeks. The clinical judgment of bTB diagnosis using TST and IGRA remains a fundamental problem in its application to the field.

### H039

**Cellulase and Laccase Activity of Eight Basidiomycetes Isolated from Decayed Quercus variabilis** Hyunjeong Na, Su-Yeon Lee, Soo-Kyeong Jang, Jiyoon Yang, and Mi-Jin Park<sup>\*</sup> *Forest Industrial Materials Division, Forest Products and Industry Department, National Institute of Forest Science* 

*Quercus variabilis* is one of the most abundant broad-leaved trees in Korea and is useful such as making cork and commercial charcoal. But, *Quercus* easily rots at the roots and trunks of growing trees, causing economic loss. The purpose of this study is to find out the causative strains of *Q. variabilis* decay by measuring the enzymatic activity of eight basidiomycetes isolated from decayed *Q. variabilis*. For measuring the cellulase activity, the strains were incubated in modified TYE medium with CMC sodium salt for 19 days at 25°C on a rotary shaker at 100 rpm. The cellulase activity of the enzyme solution was measured using the DNS method. To evaluate the laccase activity, the strains were measured at 420 nm by mixing sodium acetate buffer, ABTS diammonium salt and enzyme solution. As a results, *Pholiota aurivella, Dentipellis rhizomorpha, Phlebia acerina*, and *Emmia lacerata* showed both cellulase and laccase activity. Also, *P. acerina* showed the highest activity at 5.1 IU/ml for cellulase on 15 day and at 19.8 U/L for laccase on 5 days, respectively. These results show that the strains have a significant effect on *Q. variabilis* decay. As such, it is expected that providing information on strains related to *Q. variabilis* decay will enable follow-up studies for its control.

[This work was supported by research grant from National Institute of Forest Science]

## Method for Rapid Detection of Henipavirus Genomic Mutations

Sujeong Eom<sup>1,2</sup>, Kwan Woo Kim<sup>2</sup>, and Hana Yi<sup>1,2,3\*</sup> <sup>1</sup>Integrated Biomedical and Life Science, Graduate School, Korea University, <sup>2</sup>Interdisciplinary Program in Precision Public Health, Korea University, <sup>3</sup>School of Biosystems and Biomedical Sciences, Korea University

To identify genetic mutations in viral pathogens, rapid genome sequencing covering the entire genome is effective. However, it is required to conduct additional reverse-transcription and amplification steps due to the genome structure of RNA viruses and the low biomass of clinical samples. In this study, for rapid and accurate identification of Henipavirus, we designed 6 pairs of universal primers and developed genome amplification and sequencing methods. The primer sets were designed to produce amplicons with a length of 3 kb. We performed library preparation and the samples were sequenced by Sanger's method, Illumina MiSeq platform, and ONT MinION sequencer. The sequencing raw data were assembled using *de novo* assembly and reference mapping methods. As a result, we could acquire complete genome sequences in all sequencing technologies examined in this study and discover genetic mutations by comparing the sequences with reference sequences. We successfully developed a full genome sequencing strategy for Henipavirus. The universal primer sets and following experimental procedures would enable more accurate identification of the mutant virus of the health-threatening Henipavirus in the future. [This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Ministry of Education (No. 2022R1A2C1007966)]

## H041

Antibiotic Resistance Test of Microorganisms Isolated from Agricultural Products and Agricultural Environments in Chungcheongnam-do

Mi Kyung Kwon<sup>1</sup>, Kyung Soon Lee<sup>1</sup>, and Oh Kwang Kyo<sup>2\*</sup>

<sup>1</sup>Chungcheongnam-do Agricultural Research and Extension Services, <sup>2</sup>Microbial Safety Division, National Institute of Agricultural Science, Rural Development Administration

This study was conducted to provide basic data for antibiotic resistance research in the agricultural field through a survey on antibiotic resistant bacteria in agricultural products and agricultural environments in Chungnam in 2021. Various microorganisms, including *Escherichia coli* were isolated from lettuce and red pepper in agricultural products, agricultural water, soil, and compost using selective media. As a result, *Escherichia coli*, *Acinetobater* sp., *Enterococcus* sp., *Klebsiella pneumoniae*, *Pectobacterium carotovorum*, *Pseudomonas* sp. and *Staphylococcus aureus* were isolated. *Salmonella* sp. and *Campylobacter* sp. were not isolated at all. In the case of lettuce crops, *Escherichia coli*, *Enterococcus* sp. and *Staphylococcus aureus* were isolated from lettuce, but not from agricultural water, soil, and compost. *Acinetobacter* sp. and *Pseudomonas* sp. were separated from both agricultural products and agricultural environments. In the case of pepper crops, *Escherichia coli*, *Acinetobater* sp. were isolated from agricultural products as well as agricultural water, soil, and compost. As a result of antibiotic resistance studies against ampicillin, tetracycin, etc. for isolated strains, some strains showed strong resistance to streptomycin. In the future, we plan to investigate the antibiotic resistance characteristics of strains isolated from the Chungnam region through more bacterial isolation and additional experiments.

## Validation of a Fission Yeast Gene Deletion Library by Whole Genome Sequencing

Miyoung Nam and Kwang-Lae Hoe\*

Department of New Drug Discovery and Development, Chungnam National University

The *S. pombe* gene deletion library is a useful resource for both a functional study and a genome-wide screening of gene-specific barcodes. However, its widespread applications are hampered by non-negligible error rate (6.4~9.5%) in barcode sequences. For the purpose, original 4845 DCs were subject to validate their insertion patterns into chromosome as well as the barcode sequences.

In the study, we performed whole-genome sequencing of the pooled library and individual strains and extracted ~100 megabyte selected read from ~3.8 terabyte sequencing raw data through blast analyses. Using the selected read, 4751 DCs were validated to be integrated exactly at targeted positions on chromosome, although either deleting 1 target or extra multiple ORF. Surprisingly, 89 DCs were found to be correctly constructed based on their construction records but lost by human handling errors.

When the barcode sequences of the 4751 validated DCs were compared with originally designed ones, most of UP/DN barcode sequences were same as design but some UP/DN barcode sequences were different. Consequently, the different barcode sequences were newly allocated as correct ones, and the duplicating barcodes would be fixed into unique ones for systematic research.

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## H043

## Relocation of Paraspeckle Proteins to the Perinucleolar Region during HSV-1 Infection

## Seung Yeon Hong<sup>1</sup> and Eui Tae Kim<sup>1,2\*</sup>

<sup>1</sup>Department of Biomedicine & Drug Development, Jeju National University College of Medicine, <sup>2</sup>Department of Microbiology & Immunology Pathology, Jeju National University College of Medicine

The nucleolus is a membrane-less organelle assembled through phase separation of their molecular components and the most prominent subnuclear structure exclusively known as the place for ribosomal RNA synthesis and ribosome biogenesis. Paraspeckles are large ribonucleoprotein domains composed of RNA binding proteins and long noncoding RNAs, such as NEAT1. Although many studies have been reported on the nuclear paraspeckle, the exact function of each component remains to be elucidated. It has been reported that paraspeckle proteins relocalize to the perinucleolar region. It's likely that in common with the nucleolus, paraspeckles are dynamic nuclear body structures that respond to the changing environment of the cell. Many viruses target the nucleolus as part of their replication strategy. In this study, we observed that herpes simplex virus 1 (HSV-1) infection induces the disruption of paraspeckle structure between NEAT1 and paraspeckle proteins and relocalization of the proteins in the nucleolar region. In addition, we biochemically investigated the accumulation of paraspeckle proteins in the nucleolar fraction as a result of viral infection. Experiments employing siRNA-mediated gene silencing demonstrated that the paraspeckle protein is required to replicate HSV-1. Our data suggest that HSV-1 may exploit paraspeckles to promote viral replication. Further studies are needed on how translocation of paraspeckles to the nucleolus by HSV-1 is involved in viral replication.

## Analysis of Full-genome Sequences in Human Adenovirus

Anyeseu Park<sup>1</sup> and Jeong Yoon Lee<sup>1,2\*</sup>

<sup>1</sup>Department of Bioactive Material Science, Jeonbuk National University, <sup>2</sup>Korea Zoonosis Research Institute, Jeonbuk National University

Adenovirus is one of the most common pathogens, broadly infected to the animal and human. The number of adenoviruses has dramatically increased in a short time, and nowadays more than 110 adenoviruses in A to G species have been reported in the NCBI databank. Numerous new adenovirus mutants have been annually reported, and it is getting more important to study adenoviromics and the mechanism of adenovirus mutations. For this study, 106 fully sequenced human adenovirus genomes were collected from the NCBI and analyzed to identify a general human adenovirus sequence and the usages of nucleotides of full-genome sequences. We converted the 106 full-genome sequences to a FASTA file and analyzed using genome analysis programs, which are the on-line or off-line programs such as ClustalW and CLCBio genome workbench. 1) we identify a common adenovirus sequence, which is abundantly used on various human adenoviruses. 2) we have been analyzing homologous recombination hot-spots which are the critical candidates for homologous recombination to make new mutants. Especially, the hypervariable genes (Penton base, Hexon, Fiber and E3 gene) targeting homologous recombination were finely analyzed and the homologous recombination hot-spots were modified. 3) we have synthesized a new human adenovirus full-genome sequences using codon optimization or deoptimization. This study could support important information to develop a new adenoviral vector and understand human adenovirus pathogenesis.

## H045

## Genome-wide Screening of the Fission Yeast Deletion Library against AZD1208

Dohoon Kim and Kwang-Lae Hoe\*

Graduate School of New Drug Discovery and Development, Chungnam National University

AZD1208 is an anticancer drug used to treat Acute myeloid leukemia. It is a potent and selective pan-Pim kinase inhibitor and ATP competitive drug. Pan-Pim kinase promote cell proliferation and survival downstream of cytokine and growth factor signaling pathway. AZD1208 is an orally available drug that effectively inhibits all isoforms at < 5 nM or <150 nM in enzyme and cell assays. The clinical trial was terminated in phase 1 due to hepatotoxicity and lack of observed responses in the clinical setting. For this reason, the development of AZD1208 was terminated. It kills well at low concentrations in fission yeast. These result strongly suggest that AZD1208 have antifungal effects. For the better understanding antifungal action mechanisms of AZD1208, we systematically identified AZD1208 sensitive genes through the drug-induced haploinsufficiency screening of the fission yeast heterozygous and Haploid gene deletion library. Now we received the result of NGS. It is under analysis. [This research was supported by the National Research Foundation (NRF) grants from the Korea government,

Ministry of Science, and ICT (NRF-2017M3A9B5060880)]

**Development of Rapid Detection Method for Xerophilic Fungi Isolated from Confectionery Products** Dong Wook Jung, Jamin Lee, Yu Mi Sim, and Jin Young Beom<sup>\*</sup> Lotte R&D Center

Xerophiles are defined as microorganisms that grow at low water activity levels, below 0.85. Many of these xerophilic fungi can survive in high-sugar environments. They have been found in various foods, including confectionery foods, dried fruit and may lead to spoilage. This study was designed to develop rapid detection method of xerophilic fungi using 3M<sup>™</sup> Petrifilm<sup>™</sup> Rapid Yeast and Mold Count (RYM) Plates. Some of xerophilic fungi are difficult to detect in RYM. Treat sugar containing solution to make proper environment in RYM can be the method to detect xerophilic fungi within 3 days and allowing to respond to product quality related claims quickly. 6 kinds of xerophilic fungi (*Eurotium* sp., *Aspergillus chevalieri, A. penicilloides, E. rubrum, E. herbariorum, Wallemia sebi*) were used on this study. To select proper pretreatment solution, various concentration of sucrose and glycerol were tested and the most effective concentration on xerophilic fungi in 3 products and compared to currently method(M40Y). No significant difference was observed between pretreatment solution added RYM (3 days) and M40Y (7 days) after incubation at 25°C. Through this method, the test period could be reduced from 7 days to 3 days, and it was concluded that the rapid test method is as good as the current method in detecting xerophilic fungi in confectionery products.

## H047

## Identification of Multiple dsRNA Mycoviruses of *Trichoderma polysporum* NFCF205 and Their Antifungal Activities of the Mycoviruses on *T. polysporum*

Hae-Ryeong Yoon<sup>1</sup>, Jeesun Chun<sup>2</sup>, Ngoc My Tieu Le<sup>1</sup>, Beom-Tae Kim<sup>3</sup>, and Dae-Hyuk Kim<sup>1,2,4\*</sup> <sup>1</sup>Department of Bioactive Material Science, Jeonbuk National University, <sup>2</sup>Institute for Molecular Biology and Genetics, Jeonbuk National University, <sup>3</sup>Department of Bioenvironmental Chemistry, Jeonbuk National University, <sup>4</sup>Department of Molecular Biology, Jeonbuk National University

We reported the 15 agarose gel band patterns of double-stranded RNA (dsRNA) from *Trichoderma* spp.. We revealed that band pattern XIII in *Trichoderma polysporum* NFCF205 was co-infected with two mycoviruses related to the members of families "Fusagraviridae" and *Partitiviridae*. Based on the evaluation of gene organization and sequence similarity, we have named Trichoderma polysporum Fusagravirus 1 (TpFV1) and Trichoderma polysporum partitivirus 1 (TpPV1), respectively. Through each single-spore isolation, we were able to obtain mycovirus-free strain and strains with either virus of families "Fusagraviridae" or *Partitiviridae*. We observed growth rate of four strains mentioned above. And we also checked growth inhibition rate of all four strains through dual culture method, water soluble assay and VOCs profiles exposed to *Rhyzoctonia solani* and *Pleurotus ostreatus*. In this study, we report the presence of novel dsRNA mycoviruses and investigate their possible antifungal activities on *T. polysporum*.

[Supported by the National Research Foundation (NRF-2022R1A2C3005906 and NRF-2017R1A6A1A03015876) from South Korea.]

## **Identification of Target Genes for VX-680 by a Genome-wide Screening of the Fission Yeast Deletion Library** Kyuyoup Do and Kwang-Lae Hoe<sup>\*</sup>

Graduate School of New Drug Discovery and Development, Chungnam National University

VX 680, a potent inhibitor targeting the Aurora kinase family, is used in the inhibition of a diverse range of human tumor types. VX-680 has progressed to phase 2 clinical trials in patients with chronic myeloid leukemia (CML) or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) containing the T315I mutation. We systematically identified VX-680-sensitive genes screening of the fission yeast heterozygous gene deletion library. After screening, we analyzed through the next generation sequencing (NGS) process and obtained the estimated target. Currently, we are conducting a target validation to select genes sensitive to vx-680 using the estimated target.

Understanding the mechanism of VX-680 suggests discovering new therapies for cancer in the future. [This research was supported by the National Research Foundation (NRF) grants from the Korea government, Ministry of Science, and ICT (NRF-2017M3A9B5060880).]

## H049

**Changes in the Composition of the Gut Microbiome in Commercial Swine Herd with Changes in the Age** Sungseok Lee, Chanyoung Park, Donguk Ha, and San Kim<sup>\*</sup> *BRD* 

The gut microbiome plays various roles such as regulation of immunity, aid in the digestion, promoting growth and eventually the maintenance of host homeostasis. Several attempts were made to elucidate the function and importance of the gut microbiome in Human. However, little is known about animal microbiome, especially industrial animals such as swine species. The composition of the gut microbiome changes by several factors such as ages, environment, immunity, health status and genetical traits of the host. In this perspective, the result of human studies sometimes could be confusing. Compared to the human, swine species are genetically identical among individuals and diet program was applied in the same manner. In the current study, fecal samples were collected at the commercial farm to analyze the composition of the gut microbiome. The most abundant bacterial class was *Clostridia* followed by *Bacteroidia*, *Bacilli*, *Negativicutes* and *Gammaproteobacteria* at the 4 weeks of age. But the ratio was dynamic with changes in the age of the host. At the age of 150 days, the most abundant bacterial class was *Bacteroidia* followed by *Clostridia*, *Negativicutes* and *Bacilli* respectively.

In the current study, the dynamic changes of the gut microbiome were assumed to be attributed from the changes in the diet program and host age. Further study was required to determine the importance of two factors in the swine species.

[Supported by grant from Rural Development Administration]

**Characterization of Integral Membrane Protein**, *CpImp1*, of the Chestnut Blight Fungus, *Cryphonectria parasitica* Jaehyeon Lee<sup>1</sup>, Jeesun Chun<sup>2</sup>, Yo-han Ko<sup>2</sup>, Yeji Kwon<sup>1</sup>, Fatima Alejandra Hernandez Alvarado<sup>3</sup>, Seung Moon Park<sup>4</sup>, Nam-jin Chung<sup>5</sup>, and Dae-hyuk Kim<sup>1,2,3\*</sup>

<sup>1</sup>Department of Bioactive Material Sciences, Jeonbuk National University, <sup>2</sup>Institute for Molecular Biology and Genetics, Jeonbuk National University, <sup>3</sup>Department of Molecular Biology, Jeonbuk National University, <sup>4</sup>Department of Bioenvironmental Chemistry, Jeonbuk National University, <sup>5</sup>Department of Crop Science and Biotechnology, Jeonbuk National University

The chestnut blight fungus, *Cryphonectria parasitica*, and its interaction with hypovirus, Cryphonectria Hypovirus 1 (CHV1), is known to be a useful model to study the mechanism of fungus-virus interaction. In our previous study, the sectorization was observed in *CpBckl, CpSlt2* mutants associated with cell wall integrity pathways. In addition, 73 genes were found to be common differentially expressed genes (DEGs) between the sectored progenies and the corresponding parental mutant strains via RNA sequencing analysis. Among common 73 DEGs, 22 DEGs were found to be affected by CHV1 infection.

In this study, a gene encoding integral membrane protein (*CpImp1*) was selected among those genes affected by both CHV1 and sectorization. To analyze the biological function of *CpImp1* of *C.parasitica*, we constructed *CpImp1*-null mutant. The phenotype was similar to the EP155/2, on PDAmb and no differences in responsiveness was observed to various stressors. To confirm the pathogenicity, Bavendamm assay was conducted. The colony size was not different, but the level of brown coloration was higher than EP155/2. And the virulence assay showed that the *CpImp1-null* mutant produced similar size of necrotic areas compared with the wild type EP155/2. Thus, our study strongly suggests that the protein product of *CpImp1* has an important role in secretion of phenoloxidase but its enhanced secretion does not affect the fungal virulence.

[Supported by the National Research Foundation from South Korea.]

## H051

## **Introduction of Protein Library and Analysis Tools for Successful Hit Compound Identification** Heeseok Yoon, Eunmi Hong, and Huiseon Yang<sup>\*</sup> *New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation*

Korea's advanced healthcare industry is one of the nation's future growth engines along with shipbuilding and the automotive industry and is one of the core industries strategically supported by the national government. The Daegu-gyeongbuk medical innovation foundation (K-MEDI Hub) New drug development center provides support to world-class research and R&D in drug discovery and production. The Structural analysis team is an important research department of the New drug development center and supports protein purification, crystallization, structure determination, and biophysical analysis for hit identification. The structural analysis team will launch the protein library and screening service as early as next year to help develop new drugs that are economical and effective. The Target-Based-Screening service include providing protein library and target protein expression and purification, selecting hit compounds that interact with target proteins by Isothermal titration microcalorimetry (ITC), and surface plasmon resonance (SPR) analysis, and measuring kd value. We Introduce Target-Based screening service here and it will be help to related researchers.

# Trnascriptome, Metabolome and Microbiome Integrated Analysis Revealed Viral Host Response Mechanism to Multi Strain PRRSV Infection

Chiwoong Lim, Young-Jun Seo, and Jun-Mo Kim\*

Animal Functional Genomics & Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University

Porcine reproductive and respiratory syndrome virus (PRRSV), is one of the important virus in the pig industry. In addition, the lung microbiome play an important role from the mechanism of this disease. Therefore, we used BALF and blood RNA for identified interaction between the lung microbiome and the host. Thirty-six pigs were sacrificed and 3 pigs were sampled in each treatment group. The normal control group and the medium pathogenic group (JA142) were sampled on 3, 7, 14, and 21 dpi, and the high (NA10) and low pathogenic group (NA8) were sampled on the 3 and 7 dpi. As a result of functional analysis at 14 dpi of strain JA142 and 3 dpi of strain NA10, which has the highest viral concentration in blood, response to virus pathways were significant, and at NA 10 3 dpi, immnue response was the most significant. In addition, Mycoplasma was most dominant at this dpi. and Bordetella increased in JA142 21 dpi and NA10 10 dpi both. As a result of the functional analysis, pathways such as Alzheimer, Vitamin B6 metabolism related to the immune response shown as significant terms. The results showed that common pathways were identified to be consistent with host transcriptome analysis. Therefore, we provide lung microbiome mediated regulations for PRRSV signaling after host infection.

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## H053

## Korean Agricultural Culture Collection (KACC) as Domestic and International Patent Depository & National Patent Orgaism Integrated Depository

Byeong Hak Han, Eun-Mi Park, Nam-Jeong Kim, Seung-Beom Hong, Soon-Wo Kwon, Jun Heo, and Daseul Lee<sup>\*</sup> Korean Agricultural Culture Collection, Agricultural Microbiology Division, NAS, RDA

Korea Agricultural Culture Collection (KACC) is the national institution in Korea that deposits and preserves domestic and international patented microorganisms.

KACC has been entrusted with domestic patent microorganism deposit duty since it was designated as a Patent Microbial Depository Institution by the Korean Intellectual Property Office in 2002. In addition, KACC has received international patent microbes as it was granted status as a International Depository for Patent Microorganisms from the World Intellectual Property Organization (WIPO) under the Budapest Treaty in 2015. As of December 2021, it is managing 2,350 strains deposited for the patent purpose. KACC is also working as the integrated depository to preserve all the duplicates of microbes held in four patent depositories in Korea since the Korean Intellectual Property Office designated KACC as a National Patent Microorganism Integrated Preservation Center. KACC provided a storage facility that could most safely preserve patented microorganisms such as bacteria, molds, seeds, and genes for more than 30 years using liquid nitrogen at minus 196 degrees Celsius. Presently December 2021, 14,907 strains of patented microorganisms managed by all patent microbial depository institutions in Korea are safely and duplicately preserved in KACC integrated depository.

## A Comparative Study on Antimicrobial Activities of Essential Oils from Citrus Cultivars Peel against Skin Pathogens

Jiyoon Yang, Su-Yeon Lee, Soo-Kyong Jang, Hyunjeong Na, Jeongha Yoon, and Mi-Jin Park<sup>\*</sup> National Institute of Forest Science

The genus *Citrus* comprises some of the most important cultivated fruit trees worldwide. These citrus fruits contain various biologically active compounds such as essential oil. In this study, antimicrobial effect of essential oils from eight citrus cultivars peel were investigated against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Yuzu (*Citrus junos*), lemon (*Citrus limon*), pomelo (*Citrus maxima*), citron (*Citrus medica*), grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), orange (*Citrus sinensis*), and lime (*Citrus × latifolia*) used in this study was collected from Jeju island. The essential oils of citrus cultivars peel were obtained by hydro-distillation, and the chemical composition was analyzed by gas chromatography-mass spectrometry. D-limonene (50.88–95.74%) was found as the major compound with many minor components varying according to the different cultivars. All citrus essential oils were screened to measure antimicrobial activity against *Staphylococcal*. Yuzu and lime essential oils exhibited higher antimicrobial activity than other essential oils. In order to evaluate the microbial growth inhibition of citrus essential oils, time-kill test was performed. Among the eight essential oils, lime essential oil most effectively inhibited *Staphylococcal*.

[This work was supported by research grant from National Institute of Forest Science.]

## H055

# Searching New Genes Related to Alkylating Agent Carmustine Using Genome-wide Screening of *Schizosaccharomyces pombe*

Min-Ki Ha and Kwang-Lae Hoe<sup>\*</sup> Graduate School of New Drug Discovery and Development, Chungnam National University

Carmustine (BCNU) is an alkylating agent of the nitrosourea family. Carmustine is mainly used for the treatment of brain tumors and lymphoma. Carmustine inhibits the replication and transcription via alkylating the DNA, thereby slowing or stopping cell growth. Carmustine also inhibits the conversion of glutathione disulfide to glutathione by carbamylation of glutathione reductase.

Genome-wide targeted gene deletion is a systematic method to study gene function by replacing target genes with deletion cassettes. Fission yeast currently has 5064 annotated protein-coding genes, and a genome-wide deletion collection has been constructed with 4845 genes deleted. We identified carmustine-sensitive genes through screening of the fission yeast heterozygous gene deletion library. The systematic screening of carmustine-sensitive genes was performed as previously described (Han *et al.*, 2013, Kim *et al.*, 2010). And carmustine target of screening with *S. pombe* gene deletion library was analyzed with Next generation sequencing. By doing this research, the expected targets have been secured and they are being validated.

[This research was supported by the National Research Foundation (NRF) grants from the Korea government, Ministry of Science, and ICT (NRF-2017M3A9B5060880).]

## Investigation of Active Antimicrobial Constituents in Essential Oil from Citrus × latifolia

Jiyoon Yang, Su-Yeon Lee, Soo-Kyong Jang, Hyunjeong Na, Jeongha Yoon, and Mi-Jin Park<sup>\*</sup> National Institute of Forest Science

In a previous study, we demonstrated the antimicrobial activities of the essential oils extracted from eight citrus cultivars against *Staphylococcal*. Among them, lime essential oil showed superior antimicrobial effect. This study aims to investigate the active antimicrobial constituents of lime oil. Based on the previous results, we designated the six single compounds ( $\alpha$ -pinene,  $\alpha$ -terpinene, m-cymene, terpinolene, terpinen-4-ol, and  $\alpha$ -terpineol) as active antimicrobial compositions of the essential oils.

All citrus essential oils were screened to measure antimicrobial activity against *Staphylococcal*.  $\alpha$ -Terpinene, mcymene, and terpinen-4-ol exhibited higher antimicrobial activity than other single compounds. In order to evaluate the microbial growth inhibition of citrus essential oils, time-kill test was performed. Among the six single compounds, m-cymene and terpinolene most effectively inhibited Staphylococcal growth. In conclusion, m-cymene showed consistently superior antimicrobial activity against *S. aureus* and *S. epidermidis* and have promising inhibitory effects on skin pathogens.

[This work was supported by research grant from National Institute of Forest Science.]

## H057

## Developing the *In Vitro* Platform Techniques for Investigating Bacteria-fungi Interaction in Gut Microbiome Using *Candida albicans* and *Escherichia coli*

Yujin Lee, Ji-Young Lee, Seong-Ryong Yu, Soojin Yu, Dong-Woo Lee, and Yong-Sun Bahn<sup>\*</sup> Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

The human gut microbiome is closely related to human health and disease state. However, there are not many studies on the interaction between fungi and bacteria comprising the gut microbiota and their effects on the host. For this purpose, we aimed to develop the *in vitro* platform techniques for investigating bacteria-fungi interaction using *Candida albicans* and *Escherichia coli* as model organisms, which are common intestinal bacteria and fungus. To co-culture these microbes, we selected 0.5x blood-heart-infusion (BHI) media as a culture media and perform co-culture under anaerobic condition at 37°C, to simulate the intestinal environment. We analyzed the transcriptional profiling changes occurring during co-incubation. To this end, we collected mono-culture cells of both *C. albicans* and *E. coli* as control samples and co-cultured cells were collected at 1, 6, and 24 h after co-culture, and isolated total RNA using Trizol method. Then, RNA-seq was performed, and the result was analyzed by comparative analysis. The DEG analysis indicates that the expression regulation pattern of the 1-h co-culture sample was most dynamic. KEGG analysis revealed that *C. albicans* and *E. coli* had similar terms, but also showed opposite expression patterns, such as arginine biosynthesis. In future studies, we will further optimize experimental methods for multi-omics analysis of bacteria-fungi interaction. [Supported by grants from NRF]

## A Split Face Study on the Effect of an Anti-acne Product Containing Fermentation Products of *Enterococcus faecalis* CBT SL-5 in Skin Microbiome Modification and Acne Improvement

Bo-Yun Choi<sup>1</sup>, Hye Sung Han<sup>2</sup>, Sun Hye Shin<sup>2</sup>, Nayeon Koo<sup>2</sup>, Sanghyun Lim<sup>3</sup>, Dooheon Son<sup>3</sup>, Kui Young Park<sup>2</sup>, and Woo Jun Sul<sup>1\*</sup>

<sup>1</sup>Department of Systems Biotechnology, Chung-Ang University, <sup>2</sup>Department of Dermatology, Chung-Ang University College of Medicine, <sup>3</sup>R&D Center, Cell Biotech, Co., Ltd.

Acne treatments these days focus on *Cutibacterium acnes* and dysbiosis of the skin microbiome. Enterococcus faecalis SL-5, a LAB strain isolated from the human gut, produces the bacteriocins that kill the *Cutibacterium acnes*. Therefore, we aimed to investigate the effect of *E. faecalis* CBT SL-5-extract-containing lotion in skin microbiome of patients with mild-to-moderate acne. Twenty patients were enrolled in this randomized, placebo-controlled, split-face comparative study. We performed DNA extraction with skin surface samples and sebum samples, and after sequencing using the Illumina MiSeq platform, microbiome analysis was performed through QIIME2 pipeline. In the case of skin surface samples, phylogenetic diversity decreased after applying a lotion containing *E. faecalis* extract compared to before application. However, the lotion couldn't influence the skin microbiome of the infundibular population inside the skin pores. And we predicted that some microbes among skin microbes were removed by bacteriocin contained in *E. faecalis* extract, leaving only species at a close distance in the phylogenetic tree.

## H059

A Study on an Oil Extracted from Extremophile Yarrowia lipolytica for Cosmetic Application Eun-jung Lee, Jeoungjin Ryu, Minji Kim, Youn-Hwa Nho, and Seunghyun Kang\* COSMAX BTI

Here we developed the new cosmetic ingredients to prevent skin aging caused by cold stress. Since cold exposure is known to increase oxidative stress, and as the one of the critical factors of skin aging and skin barrier damage, we used the oil of from oleaginous yeast *Yarrowia lipolytica (Y. lipolytica)* living in the extreme cold environments. *Y. lipolytica* oil up-regulated the expression of SOD2 and COL17 genes in cold-stress induced human keratinocytes. Moreover, *Y. lipolytica* oil significantly increased the expression level of SOD2 and COL17A in menthol-induced keratinocyte. The mRNA expression levels of MMP-1 was decreased and consequently increased the procollagen production even under the UVB irradiation. Furthermore, nitric oxide (NO) production in lipopolysaccharide (LPS)-treated cells was diminished under *Y. lipolytica* oil treatment. Radical scavenging activity by DPPH compared to control was highly increased by the treatment of *Y. lipolytica* oil. Therefore, the oil of oleaginous *Y. lipolytica* that alleviate the cold stress-induced skin damage and improve the skin condition would be a potent active cosmetic ingredient against cold-stress condition as well as anti-aging, anti-oxidation, and skin barrier. [Supported by COSMAX BTI R&I Center]

# The Introduction to the Development of Radio-protective or -mitigative Microbiome against Radiation-induced Gastro-intestinal Tissue Injury

Seong-Jun Cho, Eun-hee Hong, and Ji Young Kim<sup>\*</sup> Radiation Effects Research Section, Radiation Health Institute, Korea Hydro & Nuclear Power Co., Ltd.

Since X-ray has been discovered in 1895, the radiation technology has been rapidly developed and utilized as therapeutic/diagnostic tools in various industrial fields. Despite of wide utilization, radiation still poses a significant risk to living things. For instance, short-term high dose radiation can cause acute radiation syndrome (ARS) in hematopoietic and gastrointestinal (GI) systems and sometimes it can be the reason of radiation-induced mortality of living organisms. Therefore, many researchers have been trying to explore the effective radio-protector or radio-mitigator to overcome ARS. Until recently, various radio-protective agents have been developed, but these agents have still shown as the side effects, such as low efficacy and limitation of target tissue types. Thus, there is a need for the development of safer and more effective radio-protector/-mitigator for GI tissue damage. Therefore, our institute has established a research project for exploration of radio-sensitive microbiota/metabolites and the verification for the radio-protective effects of microbiome candidates against radiation-induced GI tissue damage. Recently, we collected various fecal samples and sera from various radiation-exposed animal or human models (radiation-therapy patients). Now, those fecal samples and sera are analyzed by using metagenome technique for microbiome and mass spectrometry for metabolites to explore the radio-protector/-mitigator candidates. [Grant No. A21IP13, KHNP]

## H061

## Inhibition of *Staphylococcus aureus* Biofilm Formation by Daucosterol

Su-Jin Yum<sup>1</sup>, Seung Min Kim<sup>2</sup>, and Hee Gon Jeong<sup>1\*</sup> <sup>1</sup>Department of Food Science and Technology, Chungnam National University, <sup>2</sup>Division of Human Ecology, Korea National Open University

Staphylococcus aureus is a Gram-positive bacteria that cause a wide variety of clinical diseases. In the biofilm form, this pathogen is more resistant to various antimicrobial treatments. However, various treatments tend to increase antibiotic resistance by the inherent selection of bacteria obtaining more aggressive patterns of infection. In this respect, we focused on novel anti-biofilm without promoting antibiotic resistance. Here, high throughput screening identified selective inhibitors of biofilm formation in *S. aureus*. Biofilm inhibition activities were confirmed and measured by crystal violet assay. We identified 10 novel candidates showed a significant anti-biofilm activity (> 80%, p < 0.05). Among them, 6 candidates including Daucosterol, a natural phytosterol-like compound, induce dispersion of pre-formed biofilm without %) bacterial growth-defect. These results suggest that Daucosterol has the potential to disperse *S. aureus* biofilms, thereby making *S. aureus* more susceptible to the action of antimicrobial agents.

## Reconstruction of Genome-scale Metabolic Network Models of Lactobacillus Species

Shakra Ahmad, Dohyeon Kim, Youngshin Kim, and Sung Ho Yoon<sup>\*</sup> Department of Bioscience and Biotechnology, Konkuk University

Changes in the gut microbiome has profound impacts on human health. Specific strain-based method has been introduced and probiotics have significant roles in intestinal health and immune system strength. The most affective probiotics are considered from *Lactobacillus* genera. In this study, we reconstructed genome-scale metabolic networks of *L. fermentum* and *L. rhamnosus GG*. Genomic reannotation was performed to retrieve a repertoire of metabolic reactions and their associated genes. Draft metabolic models were generated based on automatic reconstruction pipelines and homology search. Strain-specific metabolic reactions were identified using RAVEN and CarveMe tools. The models were validated and updated by comparing the growth simulation with results from phenotype microarray tests. The resulting metabolic models of *L. rhamnosus GG* and *L. fermentum* featured a total of 1551 and 935 metabolic reactions, respectively. The developed metabolic models will contribute to understanding metabolic potential of the strain and community modeling.

## H063

## **Machine-learning Approach to Investigate Bacterial Gene Regulations** Hyeonji Kim, Dohyeon Kim, and Sung Ho Yoon<sup>\*</sup> Department of Bioscience and Biotechnology, Konkuk University

Gene regulatory network temporally and spatially modulates intracellular physiology to optimize resource utilization and integrate genetic information to enable organisms to adapt to various environmental changes. Transcription factor (TFs) bind to DNA regulatory sequences and modulates the rate of gene transcription either by activation or repression. Thus, identification of TF(s) of a gene is a major task to interrogate gene regulation. In this study, we applied the ElasticNet model, one of the regression methods of machine-learning approach, to find the quantitative relationship between transcription factors and genes in the model strain of *Escherichia coli* K-12. Massive transcriptome data gotten from cultivations under various environmental conditions were collected from public database and publications. The processed data was trained to construct the predictive model. Model performance was validated using the experimentally determined TF-gene regulatory relationships. This approach can be applied to microorganisms whose TF regulations are not well known.

## Comparison of Plaque Phenotypes and Mutation Patterns during Serial Passages of Mammalian Orthoreovirus Isolate BatMRV/B19-02 on Different Host Cells

Ha Yeon Kim, Min Chan Kim, Hyun A Lim, Da Young Mun, and Hye Kwon Kim<sup>\*</sup> Biological Sciences and Biotechnology, Chungbuk National University

Mammalian orthoreovirus (MRV) is a virus that causes potential zoonotic diseases with a wide host range. Recent studies have shown that some bat MRVs cause severe respiratory disease and encephalitis in mice (Jiang et al., 2020). Bats can contact other animals that share an ecological space, and since MRV is a virus observed in various animals, it is necessary to secure basic information related to the evolution and interspecies transmission of MRV. Therefore, in this study, MRV isolated from domestic bats was serially passaged into various cell lines to compare and analyze changes in the plaque phenotype and genetic mutations in the infected cells.

As a result of analyzing the morphology of the plaque, Vero E6 and MARC 145 which were derived from African green monkey, the predominant plaque phenotype was different each other. In addition, the plaque number was highly increased in L929-passaged MRV, while the plaque size was higher in Vero E6-passaged MRV. When comparing the rate of increase in viral titer as the passage of MRV increases in each cell, MRV adapted the most rapidly to L929, whereas it adapted the most slowly to MARC 145.

In conclusion, we found that the plaque phenotype patterns of MRVs were different among the host cells during MRV passages. Also, NGS is in progress to analyze the mutation patterns of MRVs during serial passages in different host cells, which can be compared with the plaque phenotype patterns.

[This work was supported by NRF (2021M3E5E3083402).]

## H065

**T-cell Epitope-based Peptide Vaccine Candidates against Middle East Respiratory Syndrome Coronavirus** So-Young Lee, Nayoung Kim, Hyun-Joo Kim, Kyung-Chang Kim, and Hansaem Lee<sup>\*</sup> *Division of Emerging Virus and Vector Research, Center for Emerging Virus Research, National Institute of Health, Korea Disease Control and Prevention Agency* 

Middle-East Respiratory Syndrome (MERS) is a deadly viral respiratory disease caused by MERS virus infection. However, no vaccines have been approved until now. In this study, we aimed to identify peptide candidates preventing MERS-CoV infection through *in vitro* efficacy evaluation using human PBMCs from MERS convalescent patients.

hDPP4 transgenic mice were infected with MERS-CoV KNIH-002 and EMC strains, respectively, and mouse PBMCs were isolated from the extracted lungs and brains. By liquid chromatography and mass spectrometry, approximately 50 peptides were identified from the major histocompatibility complex (MHC) of PBMCs. Properties such as antigenicity, immunogenicity, and allergenicity of these peptides were predicted by *in silico* analysis. In ELISPOT experiments using PBMC from MERS patients, several peptides showed immunogenicity with high potential.

We anticipate these antigenic and conserved peptides can be useful as vaccine candidates to combat MERS-CoV variants. Further studies should proceed with the screened peptides *in vivo* animal models.

[This research was supported by the "National Institute of Health" research project (no. 2022-NI-044-00).]

## Cross Reactivities of Neutralizing Antibodies Induced against SARS-CoV-2 Omicron Variant

Nayoung Kim, Younmin Shin, Jun-Won Kim, Kyung-Chang Kim, Joo-Yeon Lee, and Jeong-Sun Yang<sup>\*</sup> Division of Emerging Virus and Vector Research, Center for Emerging Virus Research, National Institute of Health, Korea Disease Control and Prevention Agency

Severe acute respiratory syndrome corona-virus 2(SARS-CoV-2) causes global health concern. Neutralizing antibodies (NAbs) can reflect the individual's immune status against infectious diseases. In this study, we aimed to investigate the profile neutralizing antibody induced against omicron infection. NAbs were measured by plaque reduction neutralization test using SARS-CoV-2 (S, alpha, beta, gamma, delta, BA.1, and BA.2) obtained from the National Culture Collection for Pathogens in South Korea.

A total of 64 serum samples were serially collected approximately one and two weeks after the diagnosis from 34 patients who were infected with omicron variants (BA.1).

The positive rate at one week after the diagnosis increased of NAbs against BA.1(51.5% to 90%) variant and BA.2(15.2% to 73.3%) variant respectively. Neutralizing activities antibodies were maintained or slightly increased (48.5~72.7% to 63.3~76.5%) against other variant (S type and VOCs).

Based on the vaccination status, titers of NAbs against BA.1 and BA.2 variants increased in both non-vaccinated and vaccinated patients. Information on the patterns of NAbs against various SARS-CoV-2 variants could be helpful to prepare the measures against new variant.

[This study was supported by KNIH intramural research fund no. 2022-NI-043-00 and 6634-325-210.]

## H067

## Identification of Compounds with Antiviral Activity against SARS-CoV-2 Using a High-throughput Screening Systems

Younmin Shin, Junhyung Cho, Jeong-Sun Yang, Joo-Yeon Lee, and Kyung-Chang Kim<sup>\*</sup> Division of Emerging Virus and Vector Research, Center for Emerging Virus Research, National Institute of Health, Korea Disease Control and Prevention Agency

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to the ongoing global pandemic. Thus, it is necessary to develop new therapeutics. Drug repurposing is one of the fast approaches to discovering new therapeutics. In this study, to identify novel antiviral compounds against SARS-CoV-2 infection, we used drug libraries such as immunology/inflammation, protease inhibitors, kinase inhibitors, and cytokine inhibitors libraries. Of the 9,008 compounds, 350 compounds protected over 80% of the cell cytopathic effect compared to the infection control group. Among them, 35 compounds showed dose-dependent inhibition of SARS-CoV-2 N protein. Of 35 drugs, IC50 of Fingolimod and Azeliragon were 5.8  $\mu$ M and 2  $\mu$ M, respectively. It is comparable to the IC50 of the remdesivir treatment group. These results suggest that Fingolimod and Azeliragon have antiviral activity against SARS-CoV-2 infection. Furthermore, it needs to confirm the antiviral activity through animal experiments and antiviral mechanism study of the compounds.

[This study was supported by the Korea National Institute of Health (KNIH) intramural research fund no. 2021-NI-026-01]

# Evaluation of Carbohydrate Material Prediction Model According to Fecal Microbiota Based on Machine Learning Approach

Gyungcheon Kim and Hakdong Shin<sup>\*</sup>

Department of Food Science & Biotechnology, and Carbohydrate Bioproduct Research Center, Sejong University

This study aims to predict fecal microbial biomarkers for carbohydrate materials using supervised machine learning techniques. Fecal samples from healthy donors (n=20) were incubated with 4 types of carbohydrate materials (MOS, FOS, Inulin, and Sucrose) in anaerobic conditions, followed by 16S *rRNA*-based sequencing. Gut microbiota analysis was performed with the Qiime2 pipeline. Gut bacteria biomarkers were evaluated based on unsupervised machine learning techniques (Random forest, Extra trees, Gradient Boosting, AdaBoost, Kneighbors, Linear SVC, SVC) using the q2-features classifier plug-in. The model for carbohydrate materials classification using the gut microbiota features was confirmed with the accuracy of RandomForest (81%), ExtraTrees (84%), GradientBoosting (82%), AdaBoost (86%), Kneighbors (32%), LinearSVC (84%), and SVC (51%). 39 features (out of 782 features in total) to be used as biomarkers for carbohydrate materials classification were predicted using the AdaBoost model with the highest prediction accuracy. Further research could utilize suitable machine learning models to predict classification for various food materials using the fecal microbiota as biomarkers.

## H069

## **Beneficial Effect of Shindari According to the Type of Nuruk on Gut Microbiota Based on** *In Vitro* **Model** Suji Oh and Hakdong Shin<sup>\*</sup>

Department of Food Science & Biotechnology, and Carbohydrate Bioproduct Research Center, Sejong University

Shindari is one of Jeju's traditional liquor and manufacturing process is similar to Makgeolli. Shindari is rich in dietary fiber and is known to contain various lactic acid bacteria (LAB). However, studies on the effects of Shindari on the gut microbiota have not been confirmed. In this study, we analyzed the effect of Shindari on the gut microbiota with 4 types of Nuruk (group R, B, W1 and W2) based on *in vitro* fecal incubation model to identify changes in the fecal microbiota. For fermentation, Shindari was incubated for 12 h at 37°C. Fecal samples from healthy adults (n=6) were collected for the study. The groups were divided into the control, control\_rice, Shindari\_R, Shindari\_B, Shindari\_W1 and Shindari\_W2 and 16S *rRNA* gene-based sequencing was carried out for microbiota analysis. Shindari showed significant changes in pH and acidity, Brix according to fermentation and types of Nuruck. As a result of the alpha and beta diversity, we found significant differences in the control group between the Shindari-treated groups. Also, the relative abundances of *Bifidobacterium, Pediococcus, Faecalibacterium* genus commonly increased in Shindari-treated groups. In conclusion, we confirmed that Shindari could induce changes in the gut microbiome, it suggesting that Shindari may have health benefits, including enhancing beneficial LAB.

## **Extracts of Fruit Peel Induced Variation in the Structure of Gut Microbiota According to Extraction Methods** Hyeonjun Gwon and Hakdong Shin<sup>\*</sup>

Dept. of Food Science & Biotechnology, and Carbohydrate Bioproduct Research Center, Sejong University

Fruit peel waste is an environmental issue and accounts for a large portion of food waste. This study is to confirm the effects of fruit peel extract on the bacterial structure of the gut microbiota and determine whether fruit peel extract has value as a prebiotic material through recycling. The fecal samples were obtained from 6 healthy Koreans for *in vitro* fecal microbiome incubation model. The fruit peel was dried in an oven and ground in a blender. The insoluble and soluble extract was carried out with 70% EtOH and 60°C water. The fecal sample containing extract was incubated for 24 h in anaerobic conditions. Microbial analysis used 16s *rRNA* gene-based sequencing with the QIIME2 pipeline. As a result, the insoluble extract groups showed an increased relative abundance of beneficial bacteria such as *Bifidobacterium*. Also, the relative abundance of *Lachnoclostridium* genus was reduced in the soluble extract groups. The difference in beneficial metabolic functions between groups was predicted by the PICRUSt analysis. This study suggests that fruit peel extracts can be a beneficial effect on the gut microbiota.

## H072

## **Anti-CRISPR Prediction Based on Transformer-based Artificial Intelligence Approach** Chan-Seok Jeong *Korea Institute of Science and Technology Information*

Anti-CRISPR is a family of proteins that inhibit the CRISPR-Cas system of prokaryotic immune system. It has recently emerged as a natural off-switch for CRISPR-Cas system, allowing for post-translational regulation in CRISPR-based gene-editing applications. Although experimental strategies for discovering anti-CRISPR have been developed, bioinformatic prediction may give a more cost-effective screening strategy. However, the lack of validated anti-CRISPR data and low sequence similarity present difficulties in developing prediction methods. Here, we suggest an artificial intelligence approach that predicts the anti-CRISPR function of a protein from its amino acid sequence. We construct the prediction model by adding an additional classification layer to the transformer model pre-trained on unlabeled amino acid sequences. The resulting model is fine-tuned with further training on validated anti-CRISPR and putative non-CRISPR prophage data sets. When comparing the AUC scores, the present method outperforms the existing method, showing 2.1 times higher sensitivity at 95% specificity. In particular, analyzing according to the anti-CRISPR classification, the sensitivity to the AcrIIA7 and AcrIIA9 families was improved by 2.8 times and 3 times, respectively. Unlike existing methods, the present method does not require additional calculations or pre-filtering procedures, and can perform only with amino acid sequences, making it ideal for large-scale data analysis.

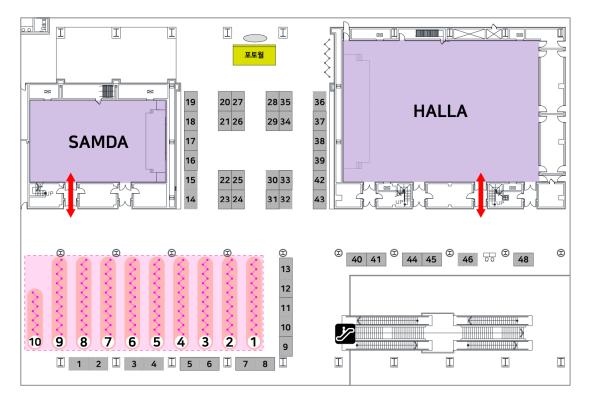
MSK2022 International Meeting of the Microbiological Society of Korea

# Exhibition

## **Exhibition**

- Date: 10.30 (Sun) 11.01 (Tue)
- Place: ICCJEJU, 3F Lobby

## **Exhibition Layout**



## **Exhibitors**

- 1,2. (주)세니젠 / Sanigen Co., Ltd.
- 3,4. (주)마크로젠 / Macrogen, Inc.
- 5,6. CJ바이오사이언스(주) / CJ Bioscience, Inc.
- 7,8. CJ제일제당 / CJ CheilJedang, Inc.
- 9. 레보딕스(주) / REVODIX, Inc.
- 10,11. (주)쎌바이오텍 / Cell Biotech Co., Ltd.
- 12,13. 기산바이오(주) / KisanBio Co., Ltd.
- 14. 주식회사 토모큐브 / Tomocube, Inc.
- 15. (주)인터페이스엔지니어링 / INTERFACE
- 16. 다인바이오(주) / Dyne Bio, Inc.
- 17. (주)녹십자웰빙 / GREEN CROSS WellBeing
- 18. 한국과학기술정보연구원 / Korea Institute of Science and Technology Information
- 19. (주)넥스트진 / NEXTGENE
- 20. 한국표준과학연구원 / Korea Research Institute of Standards and Science
- 21. 국립해양생물자원관 / National Marine Biodiversity Institute of Korea
- 22. (주)디엔에이링크 / DNA Link, Inc.
- 23. 헤밀턴 / Hamilton
- 24. 한국화학연구원 한국화합물은행 / Korea Chemical Bank
- 25. (주)인실리코젠 / Insilicogen, Inc.
- 26. 국립낙동강생물자원관 / Nakdonggang National Institute of Biological Resource
- 27. 주식회사 어큐진 / AccuGene, Inc.
- 28. 크로니텍 / CRONYTEK
- 29. 케이비티(주) / Korea Bio-Tech Co., Ltd.
- 30. 한국벡크만쿨터 라이프사이언스 / Beckmancoulter Lifescience
- 31. 엠피바이오메디칼스 코리아/ MP Biomedicals Korea, LLC.
- 32. 질병관리청 국립보건연구원 국가병원체자원은행 / National Culture Collection for Pathogens
- 33. (주)필코리아테크놀로지 / 1st PhileKorea, Inc.
- 34. 신영코퍼레이션 / ShinYoung Corporation

- 35. 인성크로마텍(주) / InSung Chroma-Tech Co., Ltd.
- 36. 벡톤디킨슨코리아/ Becton Dickinson Korea
- 37. 한국생명공학연구원 영장류자원지원센터 / Primate Resources Center, KRIBB
- 38. 지더블유바이텍(주) / GWVitek Co., Ltd.
- 39. (주)제노믹베이스 / GenomicBase Co., Ltd.
- 40. (주)랩지노믹스 / LabGenomics Co., Ltd.
- 41. 과학기술연합대학원대학교 / University of Science and Technology
- 42. 한국생명공학연구원 / Korea Research Institute of Bioscience and Biotechnology
- 43. 국가생명연구자원정보센터 / Korea Bioinformation Center
- 44. 웰펩(주) / Wellpep Co.

- 45. 경북대학교 차세대시퀀싱 핵심연구지원센터 / KNU NGS Core Facility
- 46. 몰레큘러디바이시스코리아유한회사 / Molecular Devices Korea, LLC.
- 48. 한국해양과학기술원 / Korea Institute of Ocean Science and Technology

#### Sanigen Co., Ltd.

#### (주)세니젠

CEO: Jeong Woong Park Homepage: www.sanigen.kr Address: #411 Pyeongchon Digital Empire, 16, Heungan-daero 427 beon-gil, Dongan-gu, Anyang-si, Gyeonggi-do 14059, Korea

#### Main Technology and Products

NGS Analysis Service NGS panel & Instrument Real-Time PCR Kit & Instrument Sanitary Products & Experimental supplies

#### Macrogen, Inc.

#### (주)마크로젠

CEO: Su Gang Lee /Chang Hoon Kim Homepage: www.macrogen.com Address: 238 Teheran-ro, Gangnam-gu, Seoul 06221, Korea

#### Main Technology and Products

Macrogen, a leading company in precision medicine and biotechnology, was established on June 5, 1997 based on the Genomic Medicine Institute of the Seoul National University College of Medicine. In February 2000, Macrogen became the first ever bio venture in Korea to be listed on the KOSDAQ. Since then, Macrogen has continued to be actively engaged in R&D fields of genetic and genomic analyses. Today, Macrogen has become a global expert in genomic analysis and a leader in Korean biotechnology, working closely with over 18,000 research clients across 153 countries worldwide. In addition to providing services to clients all over the world, Macrogen contributes to the advancement of bioindustries through a wide range of R&D and CSR activities.

CJ Bioscience, Inc.

#### CJ바이오사이언스(주)

CEO: Jongsik Chun Homepage: www.cjbioscience.com Address: 7F, Area B, Grand Central, 14 Sejong-daero, Jung-gu, Seoul 04527, Korea

#### Main Technology and Products

NGS-based microbiome genomic analysis, State-of-the-art NGS bioinformatics total solution service (EzBioCloud, TrueBacID), Microbiomebased new drug discovery platform (Ez-Mx Platform)

#### CJ CheilJedang, Inc.

#### CJ제일제당

CEO: Eun Seok Choi Homepage: www.cj.co.kr Address: CJ Blossom Park, 55, Gwanggyo-ro 42 beon-gil, Yeongtong-gu, Suwon-si, Gyeonggi-do 16495, Korea

#### Main Technology and Products

CJ PHACT is a 100% bio-based polyester produced by fermentation. Using CJ Bio's innovative fermentation technology, PHACT is a 100% bio-based, marine biodegradable plastic. PHACT is an eco-friendly material that provides a solution to address plastic waste and is capable of all modes of biodegradation including marine, soil, home, and industrial composting.

#### **REVODIX**, Inc.

#### 레보딕스(주)

CEO: Jong Hoon Shin Homepage: www.revodix.co.kr Address: #334 ITECO B/D, 150, Jojeong-daero, Hanam-si, Gyeonggi-do 12930, Korea

#### Main Technology and Products

REVODIX Inc. is founded in 1995, and has been a leading company in Laboratory and Nano Fluidics field as a supplier of Basic Lab Instrument, Analytical Instrument, Precision Pump, and Other Consumable.

#### Booth No. 1, 2

Booth No. 3, 4

Booth No. 5, 6

Booth No. 7, 8

#### Cell Biotech Co., Ltd.

#### (주)쎌바이오텍

CEO: Myung Jun Chung Homepage: www.cellbiotech.com Address: 50, Aegibong-ro 409 beon-gil, Gaegok-ri, Wolgot-myeon, Gimpo-si, Gyeonggi-do 10003, Korea

#### Main Technology and Products

- DUOLAC: Brand of premium probiotics products

- Nutra DUOLAC: Multi-functional products of DUOLAC

- LACTOClear: Microbiome skincare products

#### KisanBio Co., Ltd.

기산바이오(주)

CEO: Ji Woon Sun Homepage: www.kisanbio.com Address: Kisan B/D, 11, Yangjaecheon-ro 31-gil, Seocho-Gu, Seoul 06746, Korea

#### Main Technology and Products

Kisanbio is microbial culture medium specialist that supplies high quality microbial medium "MBcell". We have been supplying customized medium, and standard medium that is suitable for domestic and foreign Test method; such as Korean Food Code and Pharmacopoeia, as well as professional academic consultations on the whole product. We will do our best to repay you with the best quality and best service.

#### Tomocube, Inc.

### 주식회사 토모큐브

CEO: Kihyun Hong Homepage: www.tomocube.com Address: 4th Floor, 155, Sinseong-ro, Yuseong-gu, Daejeon 34109, Korea

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#### INTERFACE

#### (주)인터페이스엔지니어링

CEO: Sunki Kim Homepage: www.interface.co.kr Address: 22, Yeoksam-ro 7-gil, Gangnam-gu, Seoul 06244, Korea

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Since its foundation in 1991, Interface Co., Ltd. has been a reliable partner for domestic researchers.

We are striving to expand domestic analysis equipment technology by rapidly introducing and distributing advanced technologies of famous overseas brands.

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Booth No. 12, 13

Booth No. 14

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#### 다인바이오(주)

CEO: Je Hyeon Lee Homepage: www.dynebio.co.kr Address: B-B205, 14, Sagimakgol-ro 45 beon-gil, Jungwon-gu, Seongnam-si, Gyeonggi-do 13209, Korea

#### Main Technology and Products

Dyne Bio Inc. provides reagents and instruments for life science, medical science & biotechnology research more than 20 years as a manufacturer & distributor of domestic and foreign brands such as Roche, KAPA, Merck, etc. including more than 200 DYNE products with the best seller Dyne LoadingSTAR, developed independently.

Through biotechnology, we will do our best to become the great bio corporation in the hopes of making contribution to the health and happiness of human beings.

#### **GREEN CROSS WellBeing**

#### (주)녹십자웰빙

CEO: Sanghyun Kim Homepage: www.greencrosswb.com Address: 33rd fl. Par1 Bldg Tower-II, 108, Yeoui-daero, Yeongdeungpo-gu, Seoul 07335, Korea

#### Main Technology and Products

GC Wellbeing is a global customer-centric company in health, nutrition & aesthetics to improve human life. GC Wellbeing's purpose is to provide personalized nutritional solutions from hospital to home with professional suggestion.

The products provided by GC Wellbeing can be categorized into: (1) The first human placenta extract (Laennec\*) in Korea(ROK), (2) Medical Doctor designed Personalized Nutrition Therapy (Dr. PNT\*), (3) Non-covered pharmaceutical injections and dietary supplements, (4) Probiotics (Providence\*) & phytogenic (Joinsist\*, Greencera\*-F).

GC Wellbeing's products and solutions address increasing aspects for human wellbeing with sustainable health.

#### Korea Institute of Science and Technology Information

#### 한국과학기술정보연구원

CEO: Jaesoo Kim Homepage: www.kisti.re.kr Address: 245 Daehak-ro, Yuseong-gu, Daejeon 34141, Korea

#### Main Technology and Products

Korea Institute of Science and Technology Information (KISTI) supports bio-related big data analysis and artificial intelligence research through the Korea Bio Data Station (K-BDS) analysis system utilization support program (Annual allocable resources: 148,000 node hours). - Resource provision: Free support for analysis system calculation/storage resources and development environment required for bio-related

big data and AI related research

\*For more information: kbds@kisti.re.kr

#### NEXTGENE

#### (주)넥스트진

CEO: Jiyoung Hwang Homepage: www.nextgene.co.kr Address: C-#720 Daedeok Biz Center, 17, Techno 4-ro, Yuseong-gu, Daejeon 34186, Korea

#### Main Technology and Products

Nextgene.

Contribution to the advancement of science and technology in Korea. Utmost dedication to complete customer satisfaction in research field. Booth No. 17

Booth No. 19

#### Korea Research Institute of Standards and Science

#### 한국표준과학연구원

CEO: Hyun-min Park Homepage: www.kriss.re.kr Address: 267 Gajeong-ro, Yuseong-gu, Daejeon 34113, Korea

#### Main Technology and Products

As the national metrology institute (NMI) of Korea founded in 1975, KRISS (Korea Research Institute of Standards and Science) has developed measurement standards technology and played a pivotal role in upgrading Korea's main industries to the global level. As a strong supporter for SMEs, KRISS is striving to enhance the quality of national metrology and industrial competitiveness by providing calibration/testing services. KRISS will continue to strengthen its leadership in the metrology communities as well as partnerships with international metrology institutes in both advanced and developing countries for the sustainable development of humankind in the world.

#### 국립해양생물자원관

CEO: Wan-Hyun Choi Homepage: www.mabik.re.kr Address: 75, jangsan-ro, 101 beon-gil, Janghang-eup, Seocheon-gun, Chungcheongnam-do 33662, Korea

#### Main Technology and Products

Distribution of the resource information and useful materials obtained from basic research to applications of marine biodiversity.

#### DNA Link, Inc.

#### (주)디엔에이링크

CEO: Jongeun Lee Homepage: www.dnalink.com

Address: 2F, Cluster Center, Ewha Womans University, 150 Bukahyeon-ro, Seodaemun-gu, Seoul 03759, Korea

#### Main Technology and Products

We provide a variety of NGS services such as WGS, WES, WTS, and Denovo-seq, and various services such as Array and Genotyping are also available.

#### Hamilton

#### 헤밀턴

CEO: Steve Hamilton Homepage: www.hamiltoncompany.com Address: 4970 Energy Way, Reno, NV 89502, United States

#### Main Technology and Products

As one of 5 business divisions of Hamilton Company, Hamilton Process Analytics has been an innovator in the Process Analytics field for the past 30 years. As a market leader of process sensors, we offer application-oriented process sensor systems including pH, dissolved oxygen, viable cell density, and optical density. Customer collaboration drives our vision for excellence in measurement technology that works seamlessly with the infrastructure of today and the possibilities of tomorrow.

#### **Korea Chemical Bank**

#### 한국화학연구원 한국화합물은행

CEO: Mihye Yi Homepage: www.chembank.org Address: 141 Gajeong-Ro, Yuseong-gu, Daejeon 34114, Korea

#### Main Technology and Products

Establishment of national platform for new drug discovery and chemical biology through collection, management, and provision of biologically relevant chemical libraries. Open platform sharing chemical compound and data for dug discovery

Booth No. 20

Booth No. 21

Booth No. 22

Booth No. 23

#### Insilicogen, Inc.

#### (주)인실리코젠

CEO: Nam-woo Choi Homepage: www.insilicogen.com Address: A-#2901~2904, HEUNGDEOK IT VALLEY, 13, Heungdeok 1-ro, Giheung-gu, Yongin-si, Gyeonggi-do 16954, Korea

#### Main Technology and Products

Insilicogen is a company, specializes in Bioinformatics. Our company provides a platform to share and execute various biological data analysis effectively. A wide range of next-generation-sequencing (NGS)-based bioinformatic services is available, including genomic, transcriptomic, and variant analyses. We communicate research approaches with you to find the best way for your research.

#### Nakdonggang National Institute of Biological Resource

#### 국립낙동강생물자원관

CEO: Ho Yoo Homepage: www.nnibr.re.kr Address: Donam 2-gil 137, Sangju-si, Gyeongsangbuk-do 37242, Korea

#### Main Technology and Products

Security of future value via biodiversity conservation and diversity research Development of raw materials and technologies that support innovation growth in the bioindustry

#### AccuGene, Inc.

#### 주식회사 어큐진

CEO: Soon Jae Kwon Homepage: www.accugenelab.com Address: 73, Gaetbeol-ro, Yeonsu-gu, Incheon 21999, Korea

#### Main Technology and Products

We align our values with the increasing interest in the Health Care market and utilize advanced technologies such as NGS (Next Generation Sequencing) and ddPCR (Digital Droplet PCR) to enable comprehensive genetic tests that help reduce and eliminate factors that shorten the human life span and promote the extension of human life.

#### CRONYTEK

#### 크로니텍

CEO: Jae Wan Lee Homepage: www.cronytek.com Address: A-#811, 66, Beolmal-ro, Dongan-gu, Anyang-si, Gyeonggi-do 14058, Korea

#### Main Technology and Products

As a company that specializes in basic research and bio products necessary for life science research, it has been steadily growing in the life science tools distribution industry with the corporate philosophy of 'trust' since its establishment in 2013.

#### Korea Bio-Tech Co., Ltd.

#### 케이비티(주)

CEO: Hyeong-kil Kim Homepage: www.kbt.co.kr Address: 5, Dongwon-ro 21-gil, Bundang-Gu, Sungnam-Si, Gyeonggi-Do 13547, Korea

#### Main Technology and Products

Cell/Gel Imaging System, Mixer/Shaker, Incubator, Sonicator, Electrophoresis system, Spectrometer, Microscope, Speed Vac, Freeze Dryer, etc.

Booth No. 26

Booth No. 27

Booth No. 28

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CEO: Seung Han Baek Homepage: www.beckman.kr Address: 3rd Fl., Suseo Bldg., 281 Gwangpyeong-ro, Gamnam-gu, Seoul 06349, Korea

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CEO: Simon Choi Homepage: www.mpbio.com Address: B-#809, Garden Five Works, 52, Chungmin-ro, Songpa-gu, Seoul 05839, Korea

#### Main Technology and Products

MP Biomedicals is a worldwide corporation, with headquaters in Santa Ana, Californica, USA and regional offices in Europe, Asia and Australia. The corporation manufactures and sells more than 55,000 products. MP Biomedicals is one of the few corporations in the industry to offer a comprehensive line of life sciences, fine chemicals and diagnostic products.

#### National Culture Collection for Pathogens

#### 질병관리청 국립보건연구원 국가병원체자원은행

CEO: Sung Soon Kim

Homepage: nccp.cdc.go.kr

Address: 220 Osongsaengmyeong2-ro, Osong-eup, heunheung-gu, Cheongju-si, Chungcheongbuk-do 28160, Korea

#### Main Technology and Products

- Collection, receipt of deposits, analysis, and evaluation of pathogen resources
- Management, utilization, and distribution of pathogen resources
- Assistance for operation of specialized pathogen resource bank
- Construction and operation of a cooperation network of domestic institutions and foreign institutions related to pathogen resource
- Establishment and operation of the pathogen resource information system

#### 1st PhileKorea, Inc.

#### (주)필코리아테크놀로지

CEO: Minbong Park Homepage: www.philekorea.kr Address: A-#606B Woorim Lions Valley, 168 GasanDigital1-ro, Geumcheon-gu, Seoul 08507, Korea

#### Main Technology and Products

1st PhileKorea is a company that relays and sells equipment and reagents of several foreign companies such as NEB, nanoString, Azure, DeNovix, and mic.

Cloning-related reagents and equipment are sold, and gene analysis services are in progress.

#### ShinYoung Corporation

#### 신영코퍼레이션

CEO: Yujeong Choi Homepage: www.sycos.co.kr Address: 2F, 22, Yangjae cheon-ro 21-gil, Seocho-gu, Seoul 06748, Korea

#### Main Technology and Products

Shinyoung Corporation was established in April 2000 and we are doing our best for customer satisfaction for 22 years.

We are a domestic exclusive distributor of Starlab (Germany) and PanReac AppliChem (Spain), and we supply the equipment and consumables (TipOne Tips, Tube, PCR Products) and experimental reagents for bio-experiments.

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### InSung Chroma-Tech Co., Ltd.

#### 인성크로마텍(주)

CEO: Geun Sung Yoon Homepage: www.insung.net Address: InSung Bldg. #58-9, Shinmok-ro, Yangcheon-gu, Seoul, Korea

#### Main Technology and Products

Founded in 1982, Insung Chromatech Co., Ltd. supplies best-in-class analytical instrumentations and provides technical services such as installation, maintenance and training. Insung's team is focused on "best technology" and "customer satisfaction". Phenotype Microarray (Biolog Inc), FFF, MALS, DLS (Wyatt Technology)

#### **Becton Dickinson Korea**

#### 벡톤디킨슨코리아

CEO: Jason Hwang Homepage: www.bd.com/ko-kr Address: 16F Arc Place Bldg. 142, Teheran-ro, Gangnam-gu, Seoul, 06236, Korea

#### Main Technology and Products

BD is one of the largest global medical technology companies in the world and is advancing the world of health by improving medical discovery, diagnostics and the delivery of care. The company supports the heroes on the front-lines of healthcare by developing innovative technology, services and solutions that help advance both clinical therapy for patients and clinical process for healthcare providers. BD and its 75,000 employees have a passion and commitment to help enhance the safety and efficiency of clinicians' care delivery process, enable laboratory scientists to accurately detect disease and advance researchers' capabilities to develop the next generation of diagnostics and therapeutics.

#### Primate Resources Center, KRIBB

#### 한국생명공학연구원 영장류자원지원센터

CEO: Ji-Su Kim Homepage: portal.kribb.re.kr/prc Address: 351-33, Neongme-gil, Jeongeup-si, Jeollabuk-do, Republic of Korea

#### Main Technology and Products

Primate Resources Center (PRC) are managed by extensive microbiological monitoring to ensure specific pathogen-free (SPF) NHP resources. PRC efforts to construct collaborative networks and support industries, academia, and institutes focusing on NHP research, including neurodegenerative disease modeling, and pharmaceutical research.

### GWVitek Co., Ltd.

#### 지더블유바이텍(주)

CEO: Jaewon Yang Homepage: www.gwvitek.com Address: #1101 Byucksan-digital valley V, 244, Beotkkot-ro, Geumcheon-gu, Seoul 08513, Korea

#### Main Technology and Products

GW Vitek has been growing with the development of domestic science technology for over 30 years, providing research equipment and science technology services in the field of biotechnology. In addition, as a bio-infrastructure company specializing in providing total genetic analysis solution and contract manufacturing solution for RNA-based vaccines/ therapeutics to create core values for the future of bio.

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#### GenomicBase Co., Ltd.

#### (주)제노믹베이스

CEO: Chan Do Yun Homepage: www.genomicbase.com Address: #F315, 25-23, Dasanjungang-ro 19 beon-gil, Namyangju-si, Gyeonggi-do 12248, Korea

#### Main Technology and Products

GenomicBase Co., Ltd. handles Gene Explore PCR and Real-time PCR (LineGene 9600, QuantaGene 9600, MiniS QuantaGene), which are analysis devices in the life science field and field analysis equipment POCT (Lifeready 1000 and various diagnostic cartridges). We are also dealing with culturing equipment (including lab-scale real-time microorganisms or microalgae)

#### LabGenomics Co., Ltd.

#### (주)랩지노믹스

CEO: Seunghyeon Chin Homepage: www.labgenomics.co.kr Address: 700, Daewangpangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do 13488, Korea

#### Main Technology and Products

LabGenomics offer a broad range of NGS services and bioinformatic services including WGS, WES, RNA-seq, scRNA-seq, and Metagenome. Labgenomics have various NGS Platform such as Novaseq 6000, Miseq Dx, NextSeq 500/550Dx, etc.

Labgenomics provides the best results to a customer by applying the latest analysis technology and Know-how. Please experience various services with LabGenomics.

#### University of Science and Technology

#### 과학기술연합대학원대학교

CEO: Yihwan Kim Homepage: www.ust.ac.kr Address: 217, Gajeong-ro, Yuseong-gu, Daejeon 34113, Korea

#### Main Technology and Products

UST is a graduate school that has given 32 government-funded research institutes an educational function in order to cultivate future talents who will lead Korea's fields of science and technology.

Through Field-oriented education, UST cultivates creative convergence experts who will lead the future by developing students' competencies required for becoming experts in science and technology.

#### Korea Research Institute of Bioscience and Biotechnology

#### 한국생명공학연구원

CEO: Jang Seong Kim Homepage: www.kribb.re.kr Address: 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Korea

#### Main Technology and Products

KRIBB is a professional research institute leading the bioeconomy in Korea. We have been leading the innovation in biotechnology and the development of the bio-industry through open innovation by conducting basic and fundamental research, constructing infrastructure, and developing bio-ecosystem in accordance with national & social R&D needs.

#### **Korea Bioinformation Center**

#### 국가생명연구자원정보센터

CEO: Seon-Young Kim Homepage: www.kobic.re.kr Address: 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Korea

#### Main Technology and Products

Bio-resource Information, Computing Infrastructure and Development, Bioinformatics

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#### Wellpep Co.

#### 웰펩(주)

CEO: Yeongik Kweon Homepage: www.wellpep.co.kr Address: #302 Bispace, 162 Hambakmoe-ro 347 beon-gil, Namdong-gu, Incheon 21629, Korea

#### Main Technology and Products

Wellpep® has been providing reliable custom peptide synthesis services for many scientists worldwide for 4 years. With our proprietary "Wellpep® NET" peptide synthesis platform, Wellpep® is now able to offer high-quality peptides with 100% guaranteed quantity at industryleading speed to help expedite your research.

#### **KNU NGS Core Facility**

#### 경북대학교 차세대시퀀싱 핵심연구지원센터

CEO: Jae-ho Shin Homepage: ngs.knu.ac.kr Address: 1-#225, College of Agriculture and Life Sciences, 80 Daehak-ro, Buk-gu, Deagu 41566, Korea

#### Main Technology and Products

NGS, Bacterial 16S V4 gene amplicon sequencing, Fungal ITS2 amplicon sequencing, Whole metagenome sequencing, Whole genome sequencing

#### Molecular Devices Korea, LLC.

#### 몰레큘러 디바이시스 코리아 유한회사

CEO: Edward Lim Homepage: ko.moleculardevices.com Address: 15F Samsung Bldg. 623 Teheran-ro, Gangnam-gu, Seoul 06173, Korea

#### Main Technology and Products

Molecular Devices is based in Silicon Valley, and provide innovative solutions including microplate reader, high-Content Imaging, and clone screening systems, with AI-based analysis software that accelerate advancing discovery.

#### Korea Institute of Ocean Science and Technology

#### 한국해양과학기술원

CEO: Yoong Seo Kim Homepage: www.kiost.ac.kr Address: 385, Haeyang-ro, Yeongdo-gu, Busan 49111, Korea

#### Main Technology and Products

KIOST aims to guide Korea toward fulfilling its Dream of becoming a maritime power by pursing innovative and creative developments in basic and applied marine science and technology!

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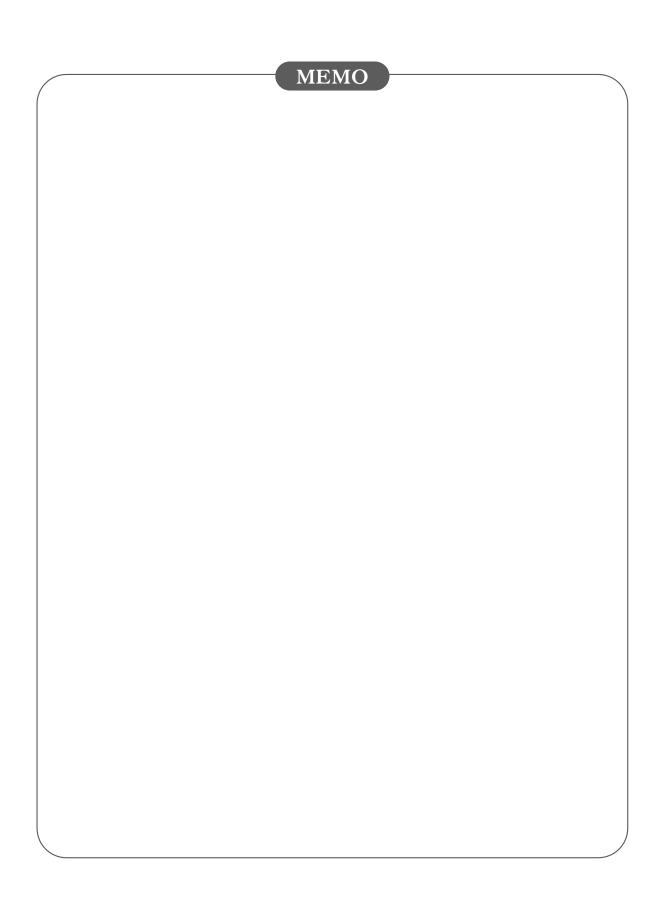
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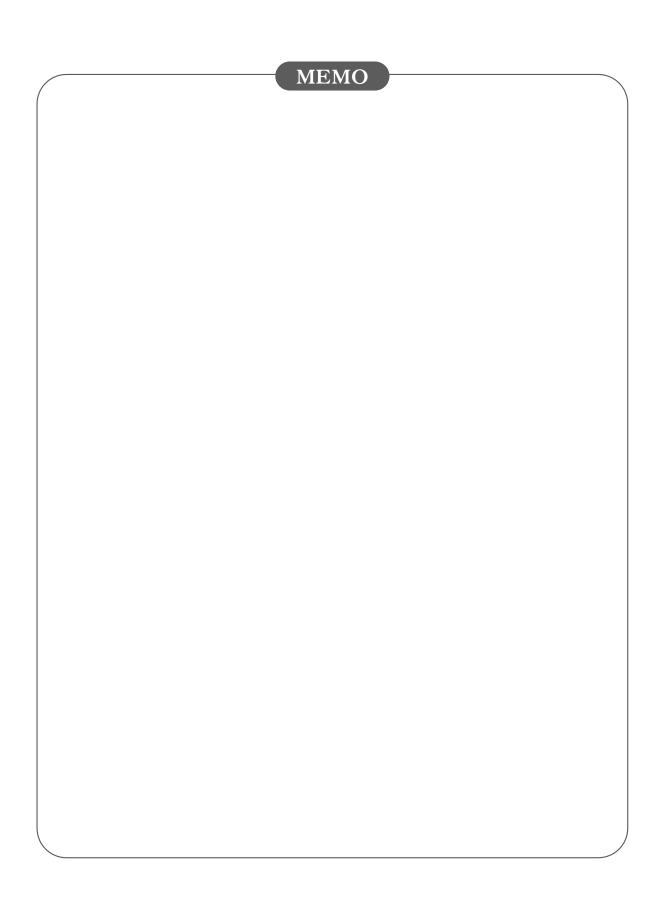
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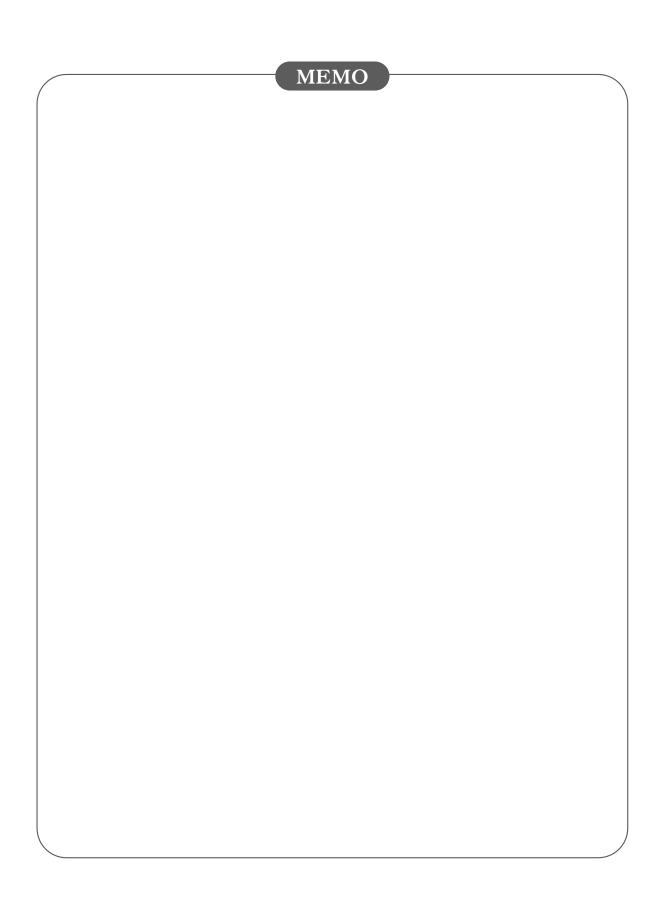
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김민수 김성보 김우성 박진호 신학동 임태규 장수진 전숭종 최학종	S15-2 S15-9 S15-4 S15-1 S15-6 S15-6 S15-6 S15-3 S15-5 S15-8







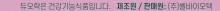


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이러한 플라스틱 오염은 결국 해양 동물들뿐만이 아닌 인류를 포함한 지구의 모든 생명체의 삶에 영향을 끼치고 있습니다. 이제는 우리 모두가 함께 지구를 보호하고, 바다를 보존하며, 인류에게 안전하고 건강한 환경을 제공 해야 합니다.



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# CJ Bioscience의 UBCG를 이용한

### 샷건 메타지놈 분석 결과로

# 마이크로바이옴 연구를 업그레이드하세요.



### 92개 Core Gene (UBCG)을 이용한 독자적인 파이프라인으로 완벽한 종 수준의 Taxonomic Profiling 분석

제공결과 : Taxonomic composition table, Pie chart, Krona chart, Core gene mapping coverage

### KEGG BRITE/NCBI NDARO/VFDB를 이용한 Functional Profiling 및 항생제 내성 / 독성유전자 분석

제공결과 : KEGG-annotated functional gene list, KEGG metabolic pathway, Antibiotic resistant gene and Virulence factor profiling

### 다양한 통계 기법을 적용한 비교 분석으로 바이오마커 탐색

제공결과 : Taxonomic and functional gene composition (stacked bar and heat map), Beta diversity (PCoA, UPGMA clustering, PERMANOVA), Biomarker discovery (LEfSe plot and cladogram)

BIOSCIENCE

Na, SI., Kim, Y.O., Yoon, SH. et al. UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol. 56, 280–285 (2018). https://doi.org/10.1007/s12275-018-8014-6

### NGS서비스 문의하기 E-mail:bs.ngs@cj.net Tel:02-6078-3456

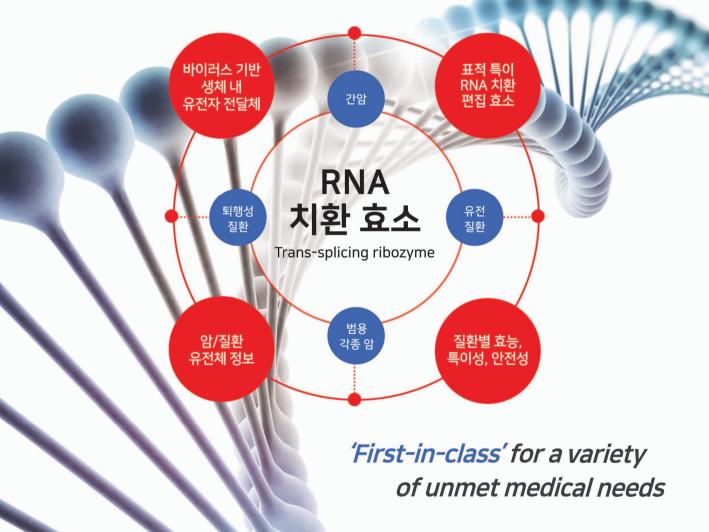
CJ바이오사이언스는 최상의 미생물 데이터베이스로 정확한 생명정보분석 솔루션을 제공합니다.

미생물군집분석[MTP] 샷건 메타지놈 분석[SG] 세균전장유전체분석[WG] 비교유전체분석[CG] 세균전사체분석[RS] 프로바이오틱스 제품검사[GRIIS]



# **Ribozyme and omics**

알지노믹스는 RNA Platform 기술 기반 유전자치료제 개발로 다양한 unmet medical needs를 충족 가능한 핵심 역량을 보유하고 있습니다.



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본사

| 경기도 용인시 수지구 죽전로 152, 단국대학교 소프트웨어ICT관 327호 TEL.031-706-8730 | E\_mail : rznomics@rznomics.com

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# Shotgun Metagenome Sequencing

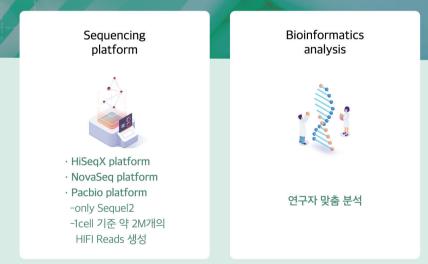
Human에서 식품, 환경까지 다양한 미생물 샘플을 시퀀싱하고 분석한 마크로젠이 고객님의 귀중한 샘플로부터 양질의 데이터 생산 및 분석까지 책임지겠습니다.



### Shotgun Metagenome Sequencing

16S rRNA와 같은 마커 유전자를 포함해 시료에서 추출한 total DNA에 존재하는 모든 미생물의 유전체 정보를 시퀀싱하는 방법으로, 환경/숙주 샘플 내 미생물 종 다양성과 기능 분석이 가능한 연구입니다. 분변, 구강, 질 분비물 등 다양한 human microbiome과 특정 질환의 상관관계를 연구하거나 환경/식품 내 존재하는 항생제 내성균 연구 등 다양한 분야에 응용되고 있습니다.

Shotgun metagenome 연구로 미생물을 종(species)부터 균주(strain) 수준까지 정확히 식별하는 일은 매우 중요합니다. 또한 이들이 가진 기능 유전자부터 대사 경로 분석까지 microbiome을 한층 더 깊이 이해 하기 위해 shotgun metagenome 연구는 이제 필수입니다.



Macrogen META사업부에는 샘플의 전처리 방법 논의부터 시작하여 연구자께서 원하시는 최적의 연구 결과물을 제공해 드릴 수 있는 기술 영업팀, 실험팀, 분석팀이 구축되어 있습니다.



### NGS Analysis Solution provider : Sanigen Co., Ltd.

㈜세니젠은 일루미나의 Microbiology & Food Safety 분야 공식 채널 파트너로서, 일루미나의 장비 및 소모품 뿐만 아니라 일루미나 플랫폼을 활용한 NGS 분석 서비스를 공급하고 있습니다.





Microorganism

Health functional foods

such as probiotics



Human/Animal Microbiome



Food Processing

Plant



Soil & Environment

### 16S rRNA amplicon sequencing 미생물의 대표적인 바이오마커인 165 rRNA gene의 variable region 염기서열 분석을 통해, 해당 미생물

Metagenome Sequencing

### 군집의 종류를 추정하는 마이크로바이옴 분석법입니다. Shotgun metagenome sequencing

특정 환경에서 분리한 모든 유전체 DNA (metagenome)의 염기서열 분석하는 마이크로바이옴 분석법입니다. 방대한 양의 유전 정보를 분석하기 때문에 미생물의 조성 뿐만 아니라 이들의 유전자까지 모두 확인이 가능합니다.

### Bacterial gene expression profiling

일정 조건에서 발현되는 mRNA를 추출 후, 이들의 염기서열만을 분석하는 유전자 발현 분석법입니다. 특정 순간에 활성화된 박테리아의 유전자 정보를 알아내고 이를 정량화 및 구조적, 기능적으로 분석하여, 어느 유전자가 더 많이 혹은 더 적게 발현 되었는지 확인합니다.

Whole Genome Sequencing

Transcriptome Sequencing

### **Bacterial genome sequencing**

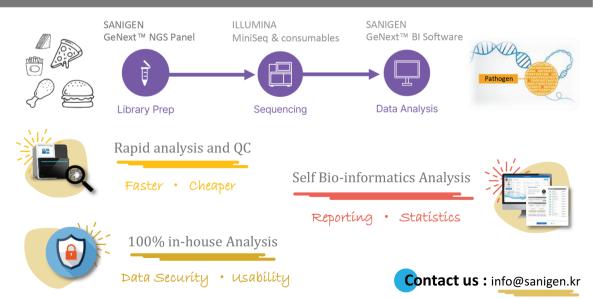
박테리아의 전장 유전체 (whole genome) 염기서열 정보를 밝혀내고, 유전체 상에 존재하는 유전자의 위치와 그 기능을 예측할 수 있습니다. 또한 비교 유전체 분석 등의 추가 분석을 통해, 각 미생물이 가진 생물학적 특징을 매우 상세하게 알아낼 수 있습니다.

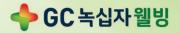
### Hybrid whole genome sequencing

Illumina & ONT 플랫폼을 동시에 사용하여 기존에 상용화된 long read sequencer와 동일한 Quality의 complete genome sequencing을 합리적인 가격으로 수행합니다.

### GeNext<sup>™</sup> Food borne Pathogen NGS Panel & BI Workstation

㈜세니젠은 식품안전센터에 대규모 식품 샘플의 신속한 분석과 QC 보고를 위한 솔루션을 제공하여 고객이 직접 NGS 분석 연구소를 운영할 수 있도록 지원합니다.







# 4년간의 연구 끝에 개발한 호흡기 특허 유산균 프로비던스 숨맑은 유산균





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# 생물자원센터 소개

### ○ <mark>생물자원센터</mark>

#### - KCTC (Korean Collection for Ttpe Cultures)

- · 국내 최대 표준미생물 및 특허미생물 보유기관
- · 생물자원의 개발 및 활용 지원

### – 보유 자원의 종류 및 특성

- ·표준균주, 참조균주, 특허균주 등 국제공인균주
- · 세균, 방선균, 혐기성균, 극한세균, 고세균, 미세조류, 곰팡이, 효모, 동·식물 세포주, 특허균주 등

### ○ <mark>수탁가능 생물자원</mark> 종류

### ○ 연혁

- · '85. 2 과학기술부 유전자은행사업 승인, 국제 미생물균주보존연맹(WFCC) 가입
- · '86.1 세계미생물자원정보센터(WDCM) 가입
- · '90. 6 부다페스트조약에 따른 국제공인 특허미생물 기탁기관 지정(WIPO)
- · '03. 5 연구성과 기탁 및 활용 중심기관 지정
- · '04. 2 ISO 품질경영시스템 인증
- · '08.8 국가 연구성과 전담기관 지정(생명자원)
- · '16.1 전북분원(정읍) 이전







### 〇 <mark>시설 및 장비</mark>



(Lyophilized ampoules)



2. Preservation room (LN<sub>2</sub>Tank)



3. Preservation room (Deep Freezer)

4. Back-up Preservation



### ○ KCTC 홈페이지



 $\bigcirc$ 

 $\bigcirc$ 

### 생명을 향한 연구, 내일을 여는 기술



# K-BIO의 새로운 물결 (WAVE)을 선도하는 글로벌 연구원 !







YouTube

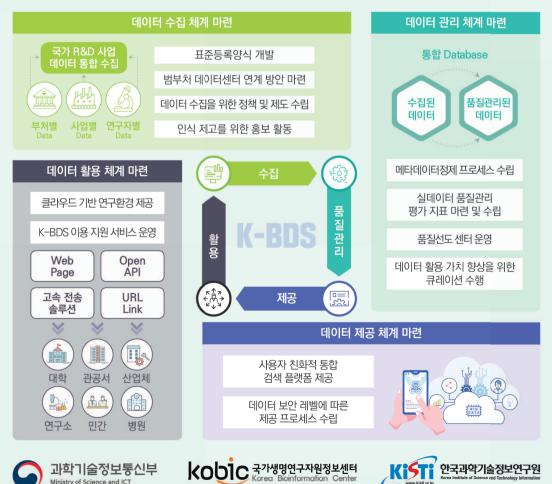
# 데이터 기반 바이오 연구 환경 이렇게 달라집니다! 국가 바이오 데이터 스테이션 Korea BioData Station(K-BDS)





### 국가 바이오 데이터 스테이션(K-BDS) 운영 개념도

Ministry of Science and ICT



# BioPS (주)바이오피에스

### Introduction



- BioPS bio-pharmaceutical safety verification center is a bio-venture specializing in the Microorganism Safety Testing of biopharmaceuticals.
- BioPS service maintain robust quality management systems that comply with ISO standards (ISO 9001:2015, ISO 14001:2015, and ISO 13485:2016).
- All services are provided based on regulatory compliance (MFDS, FDA, EMA, ICH etc.).

### **Our Service**

Cell Line Validation	Viral Clearance Studies	
Cell line characterization	Verification of the virus inactivation/removal in downstream process	
Sterility Testing	<ul> <li>Virus clearance study involves:</li> </ul>	
<ul> <li>Mycoplasma Detection</li> </ul>	Recovery assay (if necessary)	
<ul> <li>Agar and semi-solid broth methods (28 days)</li> <li>Quantification Real-time PCR (&gt;108 species)</li> </ul>	<ul> <li>Determination of process sample cytotoxicity</li> <li>Determination of process sample interference to the virus</li> </ul>	
<ul> <li>Adventitious Agent Testing</li> </ul>	<ul> <li>Virus clearance study in the scaled-down process step</li> </ul>	
In vitro & In vivo Assay	Evaluation of virus inactivation/removal	
Retrovirus Testing	<ul> <li>Virus titer determination (virus quantification)</li> </ul>	
<ul> <li>Adventitious Virus Detection</li> <li>Transmission Electron Microscopy (TEM) services</li> </ul>	<ul> <li>Virus-specific cell-based infectivity assay (TCID<sub>50</sub>)</li> <li>Quantitative PCR (qPCR)</li> </ul>	
Cell Line Authentication	<ul> <li>Viral clearance process establishment service according to the</li> </ul>	
CO1 barcode assay	characteristic of biopharmaceuticals	
<ul> <li>Karyotyping</li> <li>DNA analysis (DNA fingerprinting, genotyping)</li> </ul>	<ul> <li>Selection of the process step capable of inactivating/removing viruses</li> <li>Selection of viruses for viral clearance studies</li> </ul>	
• DIVA anarysis (DIVA imgerprinting, genotyping)	• Selection of viruses for viral clearance studies	
Microbiological test	Regulatory and Technical Support	
<ul> <li>Adventitious Virus Detection</li> </ul>	<ul> <li>Analysis of guidelines for verification of bio-pharmaceutical safety</li> </ul>	
Human viruses     Bovine viruses	Analytical Method Validation	
Porcine viruses	<ul> <li>Provide QC analytical method validation solution</li> </ul>	
Salmon viruses		
Murine and more specific target viruses	Development of QC Kit	
Mycoplasma Detection	<ul> <li>Myco-Sure® Mycoplasma detection Kit</li> </ul>	
Sterility Testing	<ul> <li>MVM-Sure         Minute virus of mice detection Kit     </li> </ul>	
Adeno-Associated Virus (AAV) Testing	<ul> <li>CHO-Sure<sup>™</sup> CHO residual DNA detection Kit</li> </ul>	
Replication Competent Adenovirus (RCA) Testing		

### Why choose BioPS ?

- BioPS have been involved in the implementation of microorganism safety test for the last 15 years.
- BioPS is able to support you with discussions with regulatory agencies in relation to interpretation of data.
- BioPS is building a QA system that can be implemented with high quality and reliability of the test.
- BioPS provide customized services with expert researchers.
- BioPS have various high-titer viruses and cell lines.

BioPS Co., Ltd. 720161 Ho, Jilligwan, Daedeok Valley Campus, Hannam University, Daejeon, 34054, Korea Homepage : www.biops.co.kr E-mail : biops@biops.co.kr Tel : (+82)-42-629-8754 Fax : (+82)- 42-629-8891

#### Ciracle Anti-Wrinkle Drama Peptide Cream

씨라클 안티링클 드라마 펩타이드 크림 / 30ml, 1.0 fl.oz

### Skin Permeable Biological Active Peptides Make Anti-Wrinkle

미소나 찡그린 반복적인 얼굴 표정으로 생기는 미세한 주름과 외부 환경 스트레스에 영향으로 생긴 얼굴, 목 등의 주름개선에 도움을 주는 라이트한 텍스처 처방으로 피부 흡수가 빠른 바르는 Botox Peptide 크림



Ciracle

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### FOR YOUR ANTI-AGING

#### **Acetyl Hexapeptide-8**

Commonly referred to as 'Botox in a jar', it is a peptide compound used to reduce the appearance of fine lines caused by repeated facial expressions, such as crow's feet, glabellar lines or nasolabial lines.

Due to its similar structure to botulinum toxin, acetyl hexapeptide 8 in considered an excellent, non-invasive alternative to Botox.

It **promotes the skin's natural production of type 1 collagen**, the crucial protein which forms large, eosinophilic fibers to rebuild muscles, reducing fine lines and wrinkles. This ingredient blocks signals from the nerves to the muscles, these muscles can no longer retract, causing the wrinkles to relax and soften.

It also **optimizes collagen function**, which, along with the skin-smoothing effects, improves moisture levels in the skin.

In a in vivo test, it reduced wrinkle depth by up to 30% when used for 30 consecutive days (Figure 1).

#### **Palmitoyl Tripeptide-1**

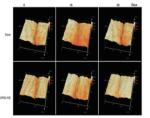
Palmitoyl tripeptide-1, also called pal-GHK and palmitoyl oligopeptide (Sequence: Pal-Gly-His-Lys), is a messenger peptide for collagen renewal. Comparable to retinoic acid with regards to its activity, it does not trigger irritation.

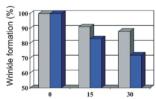
Collagen and glycosaminoglycan synthesis are stimulated, the epidermis is reinforced, and wrinkles are diminished.

It is used in cosmetic **anti-wrinkle skincare** and make-up products. In a study with 15 women, a cream containing palmitoyl tripeptide-1 was applied twice daily for four weeks, leading to **statistically significant reductions in wrinkle length**, depth and skin roughness.

### **Copper Tripeptide-1**

Several copper(III)-peptide complexes occur naturally. In human plasma, the level of GHK-Cu is about 200 ng/ml at age 20. By the age of 60, the level drops to 80 ng/ml. In humans, GHK-Cu is proposed to promote wound healing, attraction of immune cells, antioxidant and anti-inflammatory effects, stimulation of collagen and glycosaminoglycan synthesis in skin fibroblasts and promotion of blood vessels growth. Other studies revealed its ability to modulate expression of a large number of human genes, generally reversing gene expression to a healthier state. Synthetic GHK-Cu is used in cosmetics as a reparative and anti-aging ingredient (Figure 2).





Days of treatment

Figure 1 Acetyl Hexapeptide 8 exhibits *in vivo* activity. Skin topographic imprints of the preorbital region of a healthy healthy volunteer(age 38) treated with and O/W emulsion without(base) or with 10% the hexapeptide. Silicone imprints were taken before the onset of treatment, after 15 days treatment and after 30 days treatment. Imprints were processed by confocal microscopy. Three-dimensional reconstructions were obtained as described in methods.

Ref. Blanes-Mira, C., et al. A synthetic hexapeptide (Argireline) with antiwrinkle activity. Int. J. Cosmet. Sci. 2002;24:303–310



Figure 2 Before (left) and after (right) digital images illustrate typical improvements after 12 weeks of treatment. Note the improvement in skin firmness, peroicular fine lines, crow's feet wrinkles, and nasolabial fine lines (51-year-old subject). The GHK-Cu cream decreased significantly compared with baseline by 12weeks, an indication of skin firming and anti-wrinkle.

Ref. Peter Elsner, et al. Cosmeceuticals and active cosmetics. Drug versus Cosmetics. 2nd Edition. 2005. p.550-561



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# <sup>최고의 실험 파트너</sup> Socorex 프리미엄 피펫



SPECIFICATION SHEET

# QuantStudio Absolute Q Digital PCR System

The Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> Absolute Q<sup>™</sup> Digital PCR System combines the power of absolute quantification via digital PCR with the simplicity of a qPCR workflow. With a single hands-on step that takes 5 minutes to complete, digital PCR is now easier than ever. The QuantStudio Absolute Q system provides a high-quality, consistent, and easy-to-use solution for researchers who want to join the quantification revolution.

#### Features of the QuantStudio Absolute Q system:

- Easy and convenient qPCR-like workflow with only 5 minutes of hands-on time
- Helps reduce errors and manual inputs with all functions integrated into a single instrument
- Increases productivity, with results in as little as 90 minutes
- Helps minimize downtime with Smart Remote Support; troubleshooting by technical support for speedy resolution
- Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> Absolute Q<sup>™</sup> Digital PCR Software enables intuitive setup, monitoring, and analysis
- Security, auditing, and e-signature features to support 21 CFR Part 11 compliance











### "Better Health, Better Life, Better Future"

### 면역증강기술을 통한 글로벌 백<mark>신</mark>·면역치료제 전문 생명공학기업

### 독보적인 면역증강 플랫폼 기술을 보유한 차백신연구소에서 전문역량을 갖춘 최고의 인재를 모십니다.

### 모집분야

### ■ 백신·면역치료제 개발 연구원

- 생물학, 분자생물학, 면역학, 유전공학, 바이오공학 등 전공자

### RA (Regulatory Affairs)

- 인허가 프로젝트 관리, 가이드라인 · 규정 ·법규 검토
- R&D 기획
  - R&D 전략수립, 기술 · 시장 동향 분석

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┃ 우수기업연구소 ┃ 기술평가 우수기업 ┃ 청년친화강소기업 ┃ 기술역량 우수기업 ┃ 고용창출분야장관표창 ┃ 기술혁신형 중소기업

# 더가든오브내추럴솔루션 2022 <mark>채용공고</mark>



\*QR코드를 스캔하시면 채용안내 사이트로 이동합니다.

### K-MEDI hub 신약개발지원센터

### 단백질 라이브러리 & 스크리닝 기술지원 서비스 안내

	약개발지원센 <sup>[</sup> 단백질 라이		1팀
Name	Residues	Expression system	Purity (%)
TNFRSF9	24-180	Insect	≥90
TRAILR1	125-232	E.coli	≥90
IL-2	23-153	Mammal	≥99
IL-8	28-99	E.coli	≥90
PARP1	664-1010	E.coli	≥99
PARP2	235-583	E.coli	-
VEGFA	34-135	E.coli	≥99
HRAS	1-165	E.coli	≥71
MRAS	10-193	E.coli	≥97
TRKB	282-384	E.coli	≥95
HIF2a PASB	239-349	E.coli	≥97
PDCD1	27-147	Mammal	≥94
CTLA4	38-161	Mammal	≥95
FGFR1	464-765	Insect	≥90
HER1	25-642	Mammal	≥98
HER2	23-652	Mammal	≥95
HER4	26-650	Mammal	
Neuropilin-1	273-427	Mammal	-
Neuropilin-2	276-595	Mammal	in.
TNFRSF10B	73-183	Mammal	-
lgG	Fab	E.coli	127

시야개바지의세터 그ㅈ브서티

#### 이 밖에도 다수의 단백질이 확보되어 있으며, 새로운 단백질 확보도 가능 하오니 기술지원 서비스 담당자에게 문의하시기 바랍니다.



- 담당자: 홍은미 책임연구원 turtulee@kmedihub.re.kr 053.790.5252
- 담당자: 양희선 연구원 yhs86@kmedihub.re.kr 053.790.5218





유전자 재조합

단백질-화합물 상호작용 분석

\*본 홍보용 리플릿에 사용된 아이콘은 Flaticon의 Dreamstale, Peerapak Takpho, Ali Syaifullah, Ze Leah 작가에게 저작권이 있습니다.

표적 단백질

발현·정제

<추가 기술지원서비스 가능 항목>

- Kd 측정
   - SPR, DSF 분석을 통해 선별된
  - hit 화합물의 kd 측정
- 단백질-화합물 복합체 구조 분석



# Anaerobic Chamber

### 전세계유일, 세계최고 Coy만의 독자적 기술 Vinyl Type Anaerobic Chamber



✓ Flexible한 재질의 chamber는 사용자의 활동성을 유용하게 하며, 이를 통한 넓은 workspace로 효율적인 실험 가능 ✓ 완벽 밀폐로 더 완벽한 Anaerobic 환경 조성 가능 (0-5 PPM) ✓ 투명한 비닐 재질로 작업환경의 시야를 넓혀 줌





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### MSRS II IVC - RACK



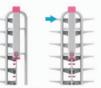
- MSRS II는 실험동물을 외부의 오염원인으로 부터 완전히 차단하여 연구자가 요구하는 Cage환경을 유지하여 정확한 실험결과를 도출 할 수 있도록 제작된 제품입니다.
- Pre/HEPA 필터 및 Carbon 필터로 여과된 공기를 배출하여 Cage 안의 환경을 항상 청정하게 유지시켜줍니다.
- · 실험실 내 환경의 질을 향상시켜 안정하게 실험할 수 있고 오염으로 인한 변이, 실험동물의 스트레스를 최소화 하도록 설계되었습니다.



### Easy Moving



**Air Flow** 



### **Hybrid Rack System**

- 필요시 Single Rack 또는 Double type으로 사용 가능한 Hybrid rack system
- 초보자도 쉽게 모든 부품의 분리 및 조립 가능



### **Cage Guide**

- Cage 장착 후 고정 가능한 돌기를 설치하여 흔들림 없는 안정적인 장치
- Cage guide는 필라스틱으로 제작되어 가벼운 경량 구조
- Cage의 탈착 및 장칙이 손쉬운 구조로 제작됨

LyseNTech

# 박테리오파지전문기업 LyseNTech

박테리오파지(Bacteriophage)와 박테리오파지 유래의 효소인 엔도라이신 (Endolysin)을 이용하여 항생제 내성세균을 제어하는 항생제 개발기술과 박테리오파지를 이용하여 표적 세포에 유전자 전달 플랫폼을 통한 유전자 치료제, 백신, 및 항암제 개발기술을 기반으로 하고 있습니다.



### A New Concept of Antibiotics

그람음성균 타겟 엔도라이신 항생제 (세계 최초 전임상 진입) 박테리오파지를 이용한 항생제 대체제



### **Microbiome modulation**

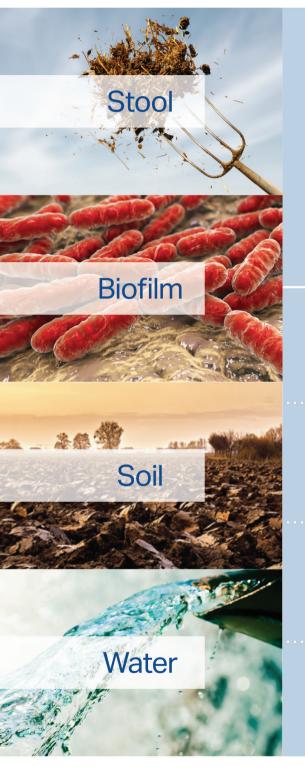
최적의 박테리오파지 칵테일을 도출 타겟균 제거를 통한 마이크로바이옴 조절



### **Gene Delivery**

박테리오파지를 이용한 gene delivery platform개발 유전자치료, 백신의 신규 플랫폼





# 복잡한 microbiome 연구를 위한 최적의 핵산 추출 시스템을 소개합니다!

다양한 종류의 샘플마다 최적의 핵산 추출 솔루션 제공

까다로운 시료마다 최적화된 샘플 균질화 방법

16S gene sequencing과 같은 민감한 downstream application을 가능하게 하는 특허 받은 PCR inhibitor 제거 기술

Spin column, magnetic bead 등 편의성 높은 workflow 선택 가능

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