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Poster

A. Systematics
B. Ecology and Environmental Microbiology
C. Applied Microbiology
D. Immunology and Microbial Pathogenesis
E. Physiology and Biochemistry
F. Genetics
G. Biotechnology
H. Others

A001

Gramella fulva sp. nov., Isolated from a Dry Surface of Tidal Flat

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A novel Gram-stain-negative, aerobic, motile by means of gliding, and short rod-shaped bacterium, designated strain SH35^T, was isolated from a dry surface of tidal flat in Hwasung-si, South Korea. Growth occurred at 10–40°C (optimum 30°C), at pH 6.0–8.0 (optimum pH 7.0), in 1–12% NaCl (optimum 2%), and was inhibited in the absence of NaCl and Ca^{2+} ions. The phylogenetic analysis based on the 16S rRNA gene sequences showed that strain SH35^T belonged to the genus *Gramella*, a member of the family *Flavobacteriaceae*, with the highest sequence similarity to *Gramella flava* JLT2011^T (96.1%), followed by *Gramella oceani* CC-AMSZ-T^T (95.6%), and 93.0–94.9% to other recognized *Gramella* species. The major cellular fatty acids (>5% of the total) of strain SH35^T were iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$, and iso-$C_{17:0}$ 3-OH, and summed feature 9 ($C_{16:0}$ 10-methyl and/or $C_{17:1}$ iso ω9c). The major polar lipids were phosphatidylethanolamine, two unidentified aminolipids, and nine unidentified lipids. The major respiratory quinone and the predominant polyamine were menaquinone-6 (MK-6) and *sym*-homospermidine, respectively. The DNA G+C content was 40.5 mol%. Based on the phylogenetic analysis and physiological and biochemical characterization, strain SH35^T represents a novel species of the genus *Gramella*, for which the name *Gramella fulva* sp. nov. is proposed. The type strain is SH35^T (=KACC 19447^T, =JCM 32369^T).
[Supported by grants from Saehyun tech Co. Ltd., corporation R&D program.]

A002

A Novel Actinobacteria Isolated from Human Feces

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A novel actinobacterial strain, KGMB04489^T, which is a strictly anaerobic, Gram-stain-positive and short-rod-shaped, was isolated human feces. On the basis of 16S rRNA gene sequence similarity, strain KGMB04489^T belongs to the genus *Olsenella* and was most closely related to *Olsenella scatoligenes* SK9K4^T (94.27%), *Olsenella uli* DSM 7084^T (93.51%), *Olsenella umbonata* DSM 22620^T (93.44%), and *Olsenella profuse* D315A-29^T (93.30%). The major cellular fatty acids (>10%) of strain KGMB04489^T was $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:1}$ *cis*9 DMA. Based on phylogenetic, physiological, biochemical, and chemotaxonomic characteristics, strain KGMB04489^T is consider to represent a novel species within the genus *Olsenella*, for which the name *Olsenella faecalis* sp. nov. is proposed. The type strain is KGMB04489^T (=KCTC 15699^T).
[This work was supported by the Bio & Medical Technology Development program of the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MSIT) of the Republic of Korea a grant from the Korea Research Institute of Bioscience & Biotechnology (KRIBB) Research initiative program.]

A003

A Novel *Clostridium* Species, KGMB01110^T, Isolated from Human Faeces

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A Gram-stain-positive, obligately anaerobic, non-motile, and non-spore-forming bacterial strain, designated KGMB01110^T, was isolated from a faecal sample of a healthy Korean man. Phylogenetic analysis based on 16S rRNA gene showed that strain KGMB01110^T belonged to *Clostridium* cluster XIVa and was most closely related to *Clostridium glycyrrhizinilyticum* KCTC 5760^T (95.9% 16S rRNA gene sequence similarity). The DNA G+C content of strain KGMB01110^T based on its genome sequence was 44.04 mol%. The major cellular fatty acids were $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ ω9*c* dimethyl acetal (DMA) and $C_{14:0}$ DMA. The strain KGMB01110^T was negative for catalase, urease, N-Acetyl-β-glucosaminidase, β-glucosidase, α-arabinosidase, and α-glucosidase. The strain KGMB01110^T produced acid from D-glucose, maltose and galactose, and hydrolysed gelatin. Based on the phylogenetic and phenotypic characteristics, strain KGMB01110^T represent a novel species of the genus *Clostridium.* The type strain is KGMB01110^T (=KCTC 15684^T).
[This work was supported by the Bio & Medical Technology Development program of the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MIST) of the Republic of Korea a grant from the Korea Research Institute of Bioscience & Biotechnology (KRIBB) Research Initiative Program.]

A004

Sulfitobacter aestuarii sp. nov., a Marine Bacterium Isolated from a Tidal Flat of the Yellow Sea in Korea

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A novel bacterial strain hydD52^T was isolated from a tidal flat sediment of the Yellow Sea in Korea. The cells were motile, rod-shaped and Gram-stain-negative. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain hydD52^T was a member of the genus *Sulfitobacter* and most closely related to *Sulfitobacter dubius* DSM 16472^T (98.0%), *Sulfitobacter indolifex* HEL-45^T (97.8%) and *Sulfitobacter delicatus* DSM 28223^T (97.6%). The major fatty acids (> 5%) of hydD52^T were $C_{18:1}$ ω7*c*/$C_{18:1}$ ω6*c*, $C_{16:0}$, $C_{18:1}$ ω7*c* 11-methyl and $C_{19:0}$ ω8*c*. The respiratory quinone of strain hydD52^T was ubiquinone-10. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, and an unidentified amino lipid. The G+C content of this strain was 64.0 mol%. The DNA-DNA relatedness of hydD52^T with the type strains of *Sulfitobacter dubius, Sulfitobacter indolifex*, and *Sulfitobacter delicatus* was 18.8, 13.1 and 15.7%, respectively. Based on the morphological, physiological and chemotaxonomic characteristics as well as DNA-DNA hybridization relatedness and 16S rRNA genes analysis, we concluded that strain hydD52^T represents a novel species, for which the name *Sulfitobacter aestuarii* sp. nov. is proposed. The type strain is hydD52^T (=KCTC 32982^T =TISTR 2562^T).

A005

Isolation and Identification of Microorganism from Inset Gut and Their Funtional Properties

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Insects are rich in nutrients such as proteins and have high fertility, which makes them easy to breed. Recently, its value as food has been increasing, and attention has been focused on new microorganism discovery fields such as insect intestinal bacteria. The purpose of this study is to isolate useful microorganisms from insect intestinal microflora and utilize them for the whole industry. In the study, insects using three insect larvae were *Tenebrio molitor*, *Protaetia breviarsis seulensis*, *Allomyrina dichotoma,* and cultivation condition is 37°C, 48 h in NA and MRS medium. The microorganisms isolated from insects were identified as 58 species of *Tenebrio molitor*, 48 kinds of *Protaetia breviarsis seulensis* and 51 species of *Allomyrina dichotoma*. Based on the shape, color, size and enzyme activity of the colonies, a total of 22 microorganisms were identified using 16S rRNA sequence analysis. Three isolates of *Lactobacillus* sp., four kinds of *Bacillus* sp., and one kind of *Saccharomyces* sp. isolated, and microorganisms such as *Chryseobacterium* genus which has not been used in this countries but researching abroad were isolated. Enzyme activities of cellulase and amylase were low in the enzyme activity of microorganism, but enzyme activity of protease and lipase was found to be high.
[This research was supported by a grant (20170328-A-001) from 「Jeonbuk Research&Development」 Program funded by Jeonbuk Province.]

A006

Psychrosphaera aquimarina sp. nov. a Marine Bacterium Isolated from Seawater Collected from Asan Bay, Korea

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Strain SW33^T isolated from the seawater of Asan Bay, Korea was characterized as Gram-negative, aerobic, rod-shaped, and motile, non-spore forming bacterium by a polyphasic taxonomic approach. The 16S rRNA sequences of strain SW33^T revealed a high similarity to *Psychrosphaera saromensis* SA4-48^T (98.7%), *Psychrosphaera haliotis* KDW4^T (97.4%) and *Psychrosphaera aestuarii* PSC101^T (97.3%). The major fatty acids were $C_{16:0}$ (27.9%), summed feature 3 (32.2%), and summed feature 8 (17.2%). The predominant quinone was Q-8, and the polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and an unidentified amino lipid. The G+C content was 38.3 mol%. The DNA-DNA relatedness with the three species of *Psychrosphaera saromensis* KCTC 23240^T, *Psychrosphaera haliotis* KCTC 22500^T and *Psychrosphaera aestuarii* KCTC 32274^T was 22%, 23%, and 18%, respectively. Based on the phenotypic characteristics and taxonomic analyses, we propose that the strain SW33^T represents a novel species within the genus *Psychrosphaera*, for which the name *Psychrosphaera aquimarina* sp. nov. with the type strain SW33^T (KCTC 52743^T =CICC 24249^T) is proposed.
[This work was supported by grants from the KRIBB Research Initiative, Republic of Korea and a fund from Ministry of Environment, Forests and Climate Change (MoEF), New Delhi, India.]

A007

The Intestinal Bacterial Community Analysis of Edible Insects

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Insects are the most abundant animals on Earth, and the microbiota within their guts play important roles by engaging in beneficial and pathological interaction with these hosts. In this study, we compared the intestinal bacterial diversity of edible insects larvae, *Allomyrina dichotoma, Protaetia brevitarsis* and *Tenebrio molitor,* using pyrosequencing of 16S rRNA genes. A total of 24 bacterial phyla and unclassified bacteria were detected. The insect gut microbiota were dominated by *Firmicutes* (47.8% of the total reads), *Proteobacteria* (23.5%), and *Bacteroidetes* (13.2%). The majority of sequence were those of the *Firmicutes* (52.6%) and *Bacteroides* (28.0%) in *Allomyrina dichotoma*. In *Protaetia brevitarsis*, the *Firmicutes* (47.8%), *Proteobacteria* (23.5%) and *Bacteroidetes* (13.2%) were dominant. The *Proteobacteria* (42.6%), *Firmicutes* (37.2%) and *Tenericutes* (15.7%) were dominant in *Tenebrio molitor*. These results indicate that the gut microbiota provides insights into the relationships between insects and their gut bacterial communities.
[This research was supported by a grant(20170328-A-001) from 「Jeonbuk Research&Development」 Program funded by Jeonbuk Province.]

A008

Description of *Kitasatospora acidophila* sp. nov., Isolated from Pine Grove Soil, and Reclassification of *Streptomyces novaecaesareae* to *Kitasatospora novaeceasareae* comb. nov.

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A polyphasic study was carried out to establish the taxonomic positions of an isolate designated MMS16-CNU292^T, from pine grove soil. On the basis of 16S rRNA gene sequence similarity, the strain formed a novel evolutionary lineage within the genus *Kitasatospora* and shared the highest similarity with *K. azatica* KCTC 9699^T (98.75%), *K. kifunensis* IFO 15206^T (98.74%), *K. purpeofusca* NRRL B-1817^T (98.61%), *K. nipponensis* HKI 0315^T (98.42%), and *Streptomyces novaecaesareae* NRRL B-1267^T (98.41%), respectively. The isolate possessed MK-9(H6) and MK-9(H8) as the major quinones, anteiso-C15:1-A, anteiso-C15:0 and iso-C15:0 as the major fatty acids, *meso*-diaminopimelic acid, galactose, glucose and mannose in the cell walls, and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and phosphatidylinositol mannosides as the polar lipids. The DNA G+C content was 70.1 mol%. The phylogenetic, chemotaxonomic and phenotypic properties enabled distinction of MMS16-CNU292^T from related species, and thus the isolate should be recognized as a new species of the genus *Kitasatospora*, for which the name *Kitasatospora acidophila* nov. (type strain= MMS16-CNU292^T) is proposed. It is also proposed that *Streptomyces novaecaesareae* should be reclassified as *Kitasatospora novaecaesareae* comb. nov.
[This work was supported by the National Institute of Biological Resources (NIBR) of the Ministry of Environment (MOE), Republic of Korea.]

A009

Comparison of Microbial Community Profiling on Traditional Fermented Soybean Products (Deonjang, Chungkukjang) Produced in Chungcheong-do, Jeolla-do

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Microbial communities were analyzed by next generation sequencing to evaluate the diversity of microbial population collected 84 samples from Chungcheong-do and Jeolla-do. A total of 17,525,441 reads were utilized for microbial community analysis. The operational taxonomic units (OTUs) of 52-185 Deonjang and 21-52 Chungkukjang were analyzed by CD-HIT. Species diversity was 0.61–4.82 in chuncheong-do samples and 1.29-2.82 in Jeolla-do samples. The dominant bacteria of Deonjang from two area were *Bacillus paralicheniformis* and *Tetragenococcus halophilus. Bacillus* was major dominant bacteria in Chungcheong-do samples (31.91%) and Jeolla-do samples (32.86%). The dominant bacteria of Chungkukjang from two area were *B. paralicheniformis* and *B. hisashii,* and those were major dominant bacteria in Chungcheong-do samples (74.13%) and Jeolla-do samples (74.77%). Based on results in the two regions, the microbial diversity of genus level was not showed significant regional specificity. However, the microbial community of species level showed the specific difference between the sample types. *B. coagulans* was found only in the Chungkukjang. Based on these results, it was confirmed that there was a difference by types of the samples rather than the regional characteristics.
[This work was supported by the Technology Innovation Program (or Industrial Strategic technology development program, R004073) funded by the Ministry of Trade, industry & Energy (MI, Korea)].

A010

Muriicola soli sp. nov., Isolated from Soil

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A Gram-stain-negative, aerobic, orange-pigmented, rod-shaped and non-motile bacterium, designated strain MMS17-SY002^T, was isolated from soil of Seonyu Island, Korea. The isolate grew at 20–37°C (optimum, 30°C), at pH 6.0–9.5 (optimum, pH 7) and in the presence of 0.5–4.0% (w/v) NaCl (optimum, 2.0%). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain MMS17-SY002^T was mostly related to the genus *Muriicola* and shared highest sequence similarity of 96.82% with *M. marianensis* A6B8^T and *M. jejuensis* EM44^T, but formed a distinct phylogenetic line. Chemotaxonomic analyses showed that menaquinone 6 (MK-6) was the predominant isoprenoid quinone, the major fatty acids were iso-$C_{15:0}$ G and iso-$C_{15:0}$, and the main polar lipids were phosphatidylethanolamine, an unidentified phosphoamino lipid, an unidentified phospholipid and two unidentified lipids. The genomic DNA G+C content was 40.8 mol%. MMS17-SY002^T could be distinguished from related species by the combination of trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase and β-glucosidase activities. Based on the phenotypic, phylogenetic and chemotaxonomic characterizations, MMS17-SY002^T (=JCM 32370^T) is considered to form a novel species of the genus *Muriicola*, for which the name *Muriicola soli* sp. nov. is proposed.
[This work was supported by the National Institute of Biological Resources (NIBR) of the Ministry of Environment (MOE), Republic of Korea.]

A011

Isolation and Identification of a Novel Strain of *Bacillus coagulans* from Soil

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Bacillus coagulans is classified as Gram-positive, rod, catalase positive, facultative anaerobe bacteria. They were distributed in fermented soybean food and soil. Some strains of them have abilities to decompose carbohydrate, protein and fat. Also they can produce lactic acid. Furthermore, they are used probiotic in feed to prevent foul smell and antibiotics. This study was aimed isolation and identification about *B. coagulans* from soil as used to probiotic in feed. The soil around the street tree was collected and streaked on TSA plate. After that, only the colony estimated to be *B. coagulans* was collected and DNA extract for PCR. PCR was performed using in-house species-specific primers, and 37.5% (three out of eight) colony is confirmed *B. coagulans* by PCR. In this study *B. coagulans*, which can be used as a probiotic, can be isolated from the soil and found to be a new strain.
[Supported by the National Research Foundation NRF-2016RICIB2016246]

A012

Pan-genome Analyses of *Tetragenococcus halophilus* Reveals Its Metabolic and Fermentative Features

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In this study, we sequenced the type strains of *Tetragenoccocus* (*T. halophilus* subsp. *halophilus* DSM 20339^T, *T. halophilus* subsp. *fladriensis* LMG 26042^T. *T. osmophilus* JCM 31126^T, *T. koreensis* KCTC 3924^T) and retrieved all genomes belonging to the genus *Tetragenococcus* (*T. halophilus*, *T. solitarius*, and *T. muriaticus*) from GenBank. The low quality genomes were excluded using CheckM. Average nucleotide identity, *in silico* DNA-DNA hybridization, and phylogeny tree based on housekeeping gene indicated that all strains of *T. halophilus* (15) have been separated into different phylogenetic lineages. The pan-genome analysis of 15 genomes belonging to *T. halophilus* was found to contain 3494 pan- and 1467 core-genes. The COG analysis using the core-genome revealed that *T. halophilus* contains high fractions for carbohydrate transport and metabolism and transcription categories. The KEGG analysis showed a pentose phosphate metabolism is the main metabolic pathway in the core-genome of *T. halophilus*. The reconstructed metabolic pathways of the *T. halophilus* using their pan-genomes showed that *T. halophilus* have an ability to metabolize diverse carbon sources aerobically or anaerobically and produce various metabolites such as lactate, ethanol, acetate, CO_2. This study provides insights into the genomic, metabolic, and fermentative features of the *T. halphilus*.

A013

Latrinibacter intestinalis gen. nov., sp. nov., Isolated from Human Faeces

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A long rod-shaped, obligate anaerobic strain, SNUG30099, was isolated from a fecal sample of healthy Korean subject. Taxonomic analysis based on 16S rRNA gene sequence distinguished the strain from other species within *Longibaculum* and *Erysipelatoclostridium* genus, which its nearest relatives are *Longibaculum muris* (92.9% similarity), *Erysipelatoclostridium spiroforme* (92.8%), and *Erysipelatoclostridium saccharogumia* (92.33%). Phylogenetic analysis also divided the strain into a unique branch differ from one belong to genus *Erysipelatoclostridium*. In addition, enzymatic activity and cellular fatty acid composition confirmed that the strain is distinct from these relatives. Furthermore, DNA G+C content was 29.2 mol% based on whole genome sequence, which is comparable to that of *L. muris*. Taken together, a novel genus and species, name *Latrinibacter intestinalis* gen. nov., sp. nov. is proposed to clearly classify strain SNUG30099. The type strain is SNUG30099(=KCTC15631^T= JCM32256^T).

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A014

Screening of Non-biogenic-amines-producing *Lactobacillus brevis* SCML432 Having Antibacterial Activity against *Bacillus cereus*

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Biogenic amines (BAs) are low-molecular-weight organic bases with aliphatic, aromatic or heterocyclic structure which can be present in fermented food and beverages. However, tyramine and histamine can evoke unwanted symptoms such as nausea, vomiting, migraine, hypertension and headache. BAs are thus considered a risk for human health and their toxicity has led to the universal concept that they should not be allowed to accumulate in food and beverages. In this study, 407 strains were isolated from Korean traditional Meju manufacturing Sunchang by first screening of non-biogenic-amine-producing microorganisms present in the foods. And then, we investigated the antibacterial activity against *Bacillus cereus* that cause diarrhea, stomachache and so on in food as harmful microorganisms. Finally, we selected SCML432 based on the various properties such as antioxidant activity, extracellular activity and so on. SCML432 was named as *Lactobacillus brevis* SCML432 by 16S rRNA sequencing and biochemical characterization using the API kit.

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A015

Pyrosequencing Analysis of Microbial Community Using Traditional *Ganjang* in Chungcheong-do and Jeolla-do Area

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This study was aimed to evaluate distribution of microbial population in 35 Ganjang samples from chungcheong-do and jeolla-do. Total of 3,078,499,539 sequences were utilized to microbial community analysis. The operational taxonomic units (OTUs) of 46-189 Ganjang assessed by analysis of CD-HIT. The 3,078,499,539 sequences were investigated to three phyla, of which *Firmicutes* was the most predominant phylum, according to 69.82% in chungcheong-do and 64.93% in jeolla-do of all sequence. Major genera were *Bacillus* (16.36%), *Enterococcus* (10.31%), *Halanaerobium* (3.95%), *Staphylococcus* (5.47%), and *Lactobacillus* (0.76%) in chungcheong-do. And genera of jeolla-do Ganjang were showed to *Bacillus* (15.55%), *Enterococcus* (1.62%), *Halanaerobium* (4.93%), *Staphylococcus* (3.21%), and *Lactobacillus* (18.18%). Also, predominant species in ganjang were *Tetragenococcus halophilus* (26.34%), *Enterococcus hirae* (10.31%), *Bacillus paralichiniformis* (9.83%) in chungcheong-do Ganjang. Dominat species of Jeolla-do ganjang were indicated to *Tetragenococcus halophilus* (19.07%), *Bacillus paralicheniformis* (13.85%), *Chromohalobacter beijerinckii* (12.01%). Therefore, chungcheong-do and Jella-do Ganjang samples were not showed a difference of regional microbial community, but showed difference by types of samples.

[This work was supported by the Technology Innovation Program (or Industrial Strategic technology development program, R004073) funded by the Ministry of Trade, industry & Energy (MI, Korea)].

A016

The Distribution of Microbial Community and Pyrosequencing Analysis of *Gochujang* by Chungcheong-do and Jeolla-do Regions

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In this study, we investigated the distribution of microbial clusters in 42 Gochujang samples from Chuncheong-do and Jeolla-do. The operational taxonomic units (OTUs) of 52–185 Gochujang was examined by analysis of CD-HIT. The chao1 (species richness) and shannon (species diversity level) were analyzed for comparison of Chuncheong-do and Jeolla-do Gochujang samples. The species richness was found to be 54–225 in Chuncheong-do and 56-196 in Jeolla-do, and species diversity was 0.61–4.82 in Chuncheong-do and 1.29–2.82 in Jeolla-do. The microbial distribution analysis of genus level based on previously results in the two regions not showed regional specificity. And, analysis of PCA and UPGMA dendrogram also were not showed regional specificity. However, the microbial distribution in species level was showed the specific difference that *Bifidobacterium animalis, Lactobacillus acidophilus, Lactobacillus rennini*, and *Thermoactinomyces vulgaris* were appeared in the case of Jella-do samples. Ratio of dominant species was confirmed the 43.17% *Bacillus paralichniformis*, 16.9% *L. homohiochii*, and 13.25% *B. hisashii* group in Chuncheong-do samples, and the 35.62% *B. paralichniformis*, 14.69% *Aerosakkonema funiforme*, and 7.95% *L. homohiochii* in Jella-do samples. Consequently, we confirmed that Chungcheong-do and Jella-do samples were not dramatically showed a difference of microbial community.

A017

Piscibacter intestinalis gen. nov., sp. nov. Isolated from Freshwater Fish Intestine

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Gram-stain-negative, non-spore-forming, motile by means of flagellum, rod shaped bacterial strains designated S1-19^T and S3-11 were isolated from intestine of freshwater fish (*Hemibarbus labeo* and *Lepomis macrochifus*) collected from Nakdong River. Phylogenetic analysis based on 16S rRNA gene sequences revealed that these isolates S1-19^T and S3-11 were formed an evolutionary independent lineage within the family Neisseriaceae. These strains were most closely related to *Aquitalea mangnusonii* TRO-001DR8^T with 93.9% sequence similarities and their 16S rRNA gene sequences were identical between two strains. They exhibited 16S rRNA gene sequence similarity values between 92.2–93.4% to the type strains of the other recognized species within the family Neisseriaceae. Growth occurred at 4–30°C (optimum, 20–25°C), at pH 6.0–8.0 (optimum, pH 7.0) and with 0–2.0% NaCl (optimum, 0%). They contained Q-8 as the predominant menaquinone and summed feature 3(comprising $C_{16:1}\ \omega$7c and/or $C_{16:1}\ \omega$6c) and $C_{16:0}$ as the major fatty acids. Differential phenotypic properties and phylogenetic distinctiveness suggested that strains S1-19^T and S3-11 represent a novel species of a new genus in the family Neisseriaceae, for which the name *Piscibacter intestinalis* gen. nov., sp. nov. is proposed. The type strain is S1-19^T (=KCTC 62300^T=JCM 32356^T).

A018

Aurantimarina amylolytica gen. nov., sp. nov., Isolated from Seawater

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An aerobic, rod-shaped, Gram-negative marine bacterium, designated HME9304^T, was isolated from seawater of the Yellow sea. Growth was observed at 10–30°C (optimum, 30°C), pH 7–8 (optimum pH 7) and with 2–3% NaCl (optimum 2%). Strain HME9304^T was positive for hydrolysis starch, dextrin and gelatin. The major fatty acid of strain HME9304^T were iso-$C_{15:1}$ G, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and Summed feature 3 ($C_{16:1}\ \omega$7*c* and/or $C_{16:1}\omega$6*c*). The major respiratory quinone was menaquinone-6 (MK-6). The major polar lipids were phosphatidylethanolamine, two unidentified aminolipids, one unidentified phospholipid, one unidentified glycolipid and four unidentified polar lipids. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain HME9304^T was affiliated with the family *Flavobacteriaceae*. Its closest relatives were *Spongiibacterium flavum* A11^T (95.4% similarity), *Spongiibacterium pacificum* SW169^T (94.9%) and *Flagellimonas eckloniae* DOKDO 007^T (94.6%). The DNA G+C content of the genomic DNA was 38.1 mol%. Genotypic, phenotypic, phylogenetic and chemotaxonomic characteristics suggested that strain HME9304^T represents a novel species of a new genus within the family *Flavobacteriaceae*, for which the name *Aurantimarina amylolytica* gen. nov., sp. nov. is proposed. The type strain is HME9304^T (=KCTC 32464^T =KACC 17618^T =CECT 8418^T).

A019

Screening of Acetic Acid Bacteria Strains as Potential Acetic Acid Producer and Characteristic

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In this study, we tried to screen Acetic acid bacteria, which have commercially available benefits, from 40 kinds of traditional fermented vinegar. We used acetic acid bacteria-selected medium for first screening, and isolated 6 strains of acetic acid bacteria-like strains. Finally, 1 isolate was selected as acetic acid bacteria with excellent resistance against the toxicity of ethanol and sulfite. This strain grew even in the presence of 350mg/L of potassium metabissulfite used as a preservative and have resistance against 10% ethanol. It was named as *Acetobacter pasteurianus* SRCM102383 by 16S rRNA sequencing and biochemical characterization. To optimize temperature for increasing acetic acid productivity, *Acetobacter pasteurianus* SRCM102383 was incubated at various temperature conditions for 10 days and Acetic acid was measured by means of a high-performance liquid chromatography (HPLC system) that was equipped with an Aminex HPX-87H Column and RI detector. As a result, optimization of the temperature was 25°C and this strain was able to utilize the ethanol completely and produce acetic acid. Futher study, we will establish high-concentrated acetic acid production conditions through medium optimization.

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A020

Spirosoma aquatica sp. nov., a Novel Species Isolated from Freshwater

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A Gram-stain-negative, rod shaped, non-motile, designated strain HMF3257^T was isolated from freshwater in Republic of Korea. Strain HMF3257^T grew optimally on R2A at 30°C, pH 8.0 and 0–0.5% NaCl. Colonies were round, smooth and yellow. On the basis of 16S rRNA gene sequence similarities, strain HMF3257^T was shown belong to the genus *Spirosoma* and closely related to *Spirosoma aerophilum* 5516J-17^T (96.5%) and *Spirosoma linguale* DSM 74^T (96.1%). The predominant quinone was menaquinone7 (MK-7). The major fatty acids were Summed feature 3 ($C_{16:1}\omega$7*c* and/or $C_{16:1}\omega$6*c*; 41.5%), $C_{16:0}$ (17.2%) and $C_{16:1}\omega$5*c* (14.6%). The DNA G+C content of strain HMF3257^T was 47.2 mol%. Based on biochemical analysis, physiological tests and phylogenetic tree, strain HMF3257^T represents a novel species of the genus *Spirosoma*, for which the name *Spirosoma aquatica* sp. nov. is proposed. The type strain is HMF3257^T (=KCTC 62463^T =NBRC 112670^T).

A021

Aestuariibaculum marinum sp. nov., a Marine Bacterium Isolated from Seawater, South Korea

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A Gram-negative, non-motile, aerobic bacterium, designated strain IP7^T, was isolated from seawater at the sea shore of Incheon Eulwang-ri beach, South Korea. Cells of strain IP7^T are straight or slightly rod-shaped and colonies are round and convex. Strain IP7^T is flexirubin-negative, halotolerant, catalase- and oxidase-positive, and produce a yellow-orange carotenoid pigment(s). Growth was optimal at 30°C, pH 7–9, and 0–2.0% NaCl (w/v). On the basis of 16S rRNA gene sequence similarity, strain IP7^T was affiliated with genus *Aestuariibaculum* in the family *Flavobacteriaceae*, the closest relative being *Aestuariibaculum suncheonense* SC17^T (98.3% sequence similarity). The DNA G+C content of the novel strain was 37.4 mol%. The only quinone was MK-6 menaquinone and iso-branched $C_{15:0}$, iso-branched $C_{15:1}$ G and iso-branched $C_{17:0}$ 3-OH were major fatty acids. The major polar lipids are phosphatidylethanolamine, unidentified aminoglycolipid and two unidentified glycolipids. The DNA-DNA hybridization value of strain IP7^T with *Aestuariibaculum suncheonense* SC17^T was 28.87%. Based on the collective DNA-DNA hybridization, biochemical, phylogenetic and physiological data, we report a novel species of the genus *Aestuariibaculum* for which the name *Aestuariibaculum marinum* sp. nov. is proposed. The type strain is IP7^T (=KCTC 52521^T =JCM 31725^T).

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A022

Edaphorhabdus rosea gen. nov., a New Member of the Family *Cytophagaceae* Isolated from Soil in South Korea

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A novel Gram-negative, designated strain UDD1^T, was isolated from soil sample collected at Udo Island, South Korea. Cells of strain UDD1^T were aerobic, pink-pigmented, oxidase and catalase positive, non-flagellated and motile by means of gliding. Colonies are circular of low-convexity with entire margins, smooth and glistening. Phylogenetic analysis based on its 16S rRNA gene sequence revealed that the strain UDD1^T formed a lineage within the family *Cytophagaceae* of the phylum *Bacteroidetes* and forms a distinct clade with type strains of the closely related genus, *Pontibacter,* with similarities of 91.36–93.62%. Strain UDD1^T. The bacterium lacked flexirubin-type pigments. The predominant isoprenoid quinone detected in strain UDD1^T was menaquinone 7 (MK-7). The cellular fatty acid comprises summed feature 4 (iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B) and iso-$C_{15:0}$ as the major fatty acids. The major polar lipids were phosphatidylethanolamine and an unidentified glycolipid. The DNA G+C content of strain UDD1^T was 49 mol%. On the basis of phenotypic, genotypic, and phylogenetic analyses, the strain UDD1^T represents a novel species of the new genus in the family *Cytophagaceae*, for which the name *Edaphorhabdus rosea* gen. nov. is proposed. The type strain of *Edaphorhabdus rosea* is UDD1^T (= KCTC 62117^T = JCM 32366^T).

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A023

Thalassorhabdus aurantiaca gen. nov., sp. nov., a New Member of the Family *Flavobacteriaceae* Isolated from Seawater, South Korea

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A novel Gram-negative, orange pigmented, strictly aerobic bacterium, designated strain IP9^T, was isolated from seawater at the sea shore of Incheon Eulwang-ri beach, South Korea. Cells of strain IP9^T were straight or slightly rod-shaped and colonies were round, convex. Strain IP9^T was catalase- and oxidase-positive, and non-motile. Growth occurred between 10 and 37°C (optimum at 30°C), pH 6–10 (optimum at pH 7–8), 0–7% (w/v) NaCl (optimum at 0–1%). On the basis of 16S rRNA gene sequence similarity and phylogenetic analysis, strain IP9^T was related with family *Flavobacteriaceae*, the closest relative being *Hwangdonia seohaensis* HD-3^T (95.23% sequence similarity). The DNA G+C content of the novel strain was 39.12 mol%. The major polar lipids were found to be phosphatidylethanolamine, three unidentified aminoglycolipids and two unidentified glycolipids. The major fatty acids (>10%) were iso-branched $C_{15:0}$, iso-branched $C_{17:0}$ 3-OH. The predominant quinone was MK-6 menaquinone. Based on the biochemical, phylogenetic and physiological data, we report that strain IP9^T (=KCTC 52523^T =JCM 31732^T) represents a novel genus of the family *Flavobacteriaceae* for which the name *Thalassorhabdus aurantiaca* gen. nov., sp. nov. is proposed.

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A024

Tellurirhabdus rosea gen. nov., sp. nov., a New Member of Family *Cytophagaceae* Isolated from Soil, South Korea

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Strain U15^T, a pink colored, non-motile, catalase and oxidase positive, rod-shaped, Gram-negative bacterium, was isolated from soil of Udo port, Udo-island, South Korea. Growth of strain U15^T was observed at 10–48°C, pH 6.0–11.0 and 0% (w/v) NaCl. Optimum conditions are 30–40°C in temperature, 7.0–10.0 in pH, and 0% (w/v) NaCl concentration. Phylogenetic analysis based on 16S rRNA sequences showed that strain U15^T forms a distinct clade with type strains of the closely related family, *Cytophagaceae*, with similarities lower than 89%. The DNA G + C content of strain U15^T was determined to be 54.3 mol%. Strain U15^T was found to contain MK-7 as the only menaquinone and iso-branched $C_{15:0}$, $C_{16:0}$, $C_{16:1}$ ω5*c*, iso-branched $C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}$ ω*7c* and/or $C_{16:1}$ ω*6c*) as the major fatty acids (>5%). The major polar lipid is phosphatidylethanolamine, two unidentified aminoglycolipids, unidentified aminophosphoglycolipid, unidentified phosphoglycolipid, and unidentified glycolipid. Based on the phylogenetic, phenotypic characteristics and chemotaxonomic analysis data, the strain U15^T (=KCTC 62116^T =JCM 32361^T) should be classified as representing novel species of novel genus within the family *Cytophagaceae*, for which the name *Tellurirhabdus rosea* gen. nov., sp. nov., is proposed.

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A025

Flavobacterium edaphi sp. nov., a Member of the Genus *Flavobacter* Isolated from Soil

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An aerobic, Gram-stain-negative, bright yellow-pigmented, oxidase and catalase-positive, non-motile, non-spore forming, rod-shaped, designated strain DMN11^T, was isolated from soil of crossroads of Jeju Island in South Korea. Phylogenetic tree analysis based on the 16SrRNA gene sequence revealed that strain DMN11^T formed a lineage within the family *Flavobacteriaceae* of the phylum *Bacteroidetes,* it was most closely related to *F. suzhouense* XIN-1^T and *F. hauense* BX12^T (98.61% and 98.19% similarity, respectively). The major isoprenoid quinone was MK-6. The major fatty acids were summed feature 3 (comprising iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B), iso-$C_{15:0}$ and iso-$C_{15:0}$ 3OH. The polar lipid profile of strain DMN11^T showed the presence of Phosphatidylethanolamine as major lipid with several aminophospholipids, aminoglycolipids, unidentified aminolipids and unidentified lipids. The DNA G + C content was 39.4 mol%. The mean level of DNA-DNA relatedness between strain DMN11^T and *F. suzhouense* XIN-1^T and *F. haunse* BX12^T was 20% and 32%, respectively. Thus the data accumulated in this study support the suggestion that strain DMN11^T is considered to represent a novel species of the genus *Flavobacterieum*, for which the name *Flavobacterium edaphi* sp. nov. is proposed. The type strain is DMN11^T (=KCTC 62114^T =JCM 32372^T).

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A026

Lysobacter flavus sp. nov., a Novel Bacterium Isolated from Soil, South Korea

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A yellow-pigmented, Gram-negative, motile by means of monotrichous flagella, short-rod shaped and strictly aerobic bacterial strain U8^T was isolated from a soil sample from Geommeolle wharf sea-coast, Udo-Island, Jeju, South Korea. Strain U8^T grew on a condition of temperature ranged from 25°C to 42°C (optimum 30°C), pH ranged from 6 to 11 (optimum pH 7–10) and in the 0% (w/v) presence of NaCl. Phylogenetic tree was reconstructed based on 16S rRNA sequence, indicating that strain U8^T is related with other recognized members of the genus *Lysobacter* within the family *Xanthomonadaceae*. Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol were identified as the major polar lipids, and four unidentified phospholipids were also detected as the minor polar lipids. Strain U8^T was found to contain ubiquinone-8 (Q-8) as the isoprenoid quinone and iso-branched $C_{15:0}$, iso-branched $C_{16:0}$ and summed feature 9 (comprising $C_{17:1}$ iso ω9c/$C_{16:0}$ 10-methyl) as the major fatty acids (>15%). The DNA G+C content of the novel strain was 70.1 mol%. On the basis of the chemotaxonomic, phylogenetic and other physiological properties, the strain U8^T (=KCTC 62112^T =JCM 32365^T) should be classified as representing a novel species of the genus *Lysobacter*, for which the name *Lysobacter flavus* sp. nov., is proposed.

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A027

Emticicia persicum sp. nov., a New Species of the Genus *Emticicia* Isolated from Soil in Jeju Island, South Korea

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A taxonomic study was carried out on strain C21^T, isolated from soil sample from a parking lot in Jeju Island, South Korea. Strain C21^T was gram-negative, oxidase- and catalase- positive, non-motile and strictly aerobic. Cells of strain C21^T were rod-shaped and the colonies were pale-yellow pigmented, round and convex. Growth occurred between 4 and 30°C (optimum at 25–30°C), pH 7–10 (optimum at pH 7–8), 0–1% (w/v) NaCl (optimum at 0%). Phylogenetic analysis based on 16S rRNA gene sequence showed that strain C21^T is related with family *Cytophagaceae*, the closest relative being *Emticicia ginsengisoli* Gsoil 085^T (98.52% sequence similarity) and *Emticicia fontis* HD-3^T (98.38% sequence similarity). The DNA G+C content of the novel strain was 35.38 mol%. The major polar lipids were found to be phosphatidylethanolamine, three unidentified aminoglycolipids, two unidentified aminolipids, and two unidentified glycolipids. The major fatty acids (>10%) were and iso-branched $C_{15:0}$, $C_{16:1}$ ω5c and summed feature 3 (comprising $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c). The predominant quinone was MK-7 menaquinone. Based on the biochemical, phylogenetic and physiological data, we report that strain C21^T (=KCTC 62113^T =JCM 32360^T) represents a novel species of the genus *Emticicia* for which the name *Emticicia persicum* sp. nov. is proposed.

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A028

Deinococcus koreensis sp. nov., a Gamma Radiation-resistant Bacterium Isolated from River Water

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A gamma-radiation-resistant, Gram-stain-negative, rod-shaped bacterial strain, designated SJW1-2^T, was isolated from freshwater samples collected from the Seomjin River, South Korea. The 16S rRNA gene sequence analyses showed that strain SJW1-2^T was most closely related to *Deinococcus metalli* 1PNM-19T (94.3% sequence similarity) and formed a robust phylogenetic clade with other species of the genus *Deinococcus*. The optimum growth pH and temperature for the isolate were pH 7.0–7.5 and 25°C, respectively. Strain SJW1-2^T exhibited high resistance to gamma radiation. The predominant respiratory quinone was MK-8. The polar lipid profile consisted of different unidentified glycolipids, two unidentified lipids, two unidentified phospholipids and an unidentified phosphoglycolipid. The predominant fatty acids (>10%) were summed feature 3 ($C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c) (25.2%) and $C_{16:0}$ (21.2%), and the DNA G+C content was 69.5 mol%. On the basis of phenotypic, genotypic and phylogenetic analyses, strain SJW1-2T (=KACC 19332^T =NBRC 112908^T) represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus koreensis* sp. nov. is proposed.

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A029

Isolation and Characterization of *Roseothermus cellulosivorans* gen. nov., sp. nov., and Proposal of *Roseothermaceae* fam. nov. Representing a New Family in the Order *Rhodothermales*

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A novel, aerobic, rod-shaped bacterium, designated strain MEBiC09517^T, was isolated from Buksung-Po, Incheon, Republic of Korea. Cell's width is around 0.62–1 μm and length is around 2.3–3.3 μm. It has low 16S rRNA gene sequence similarity with *Rubrivirga profundi* SAORIC-476, which has highest sequence similarity (89.9%) and other cultivated strains have lower similarity than *Rubrivirga profundi* SAORIC-476. Optimum growth condition of strain MEBiC09517^T is 50–55°C, pH 7, and 2–2.5% salt concentration. Strain MEBiC09517^T is an obligate marine bacterium that requires KCl, $MgCl_2$, and $CaCl_2$ as well as NaCl for growth. A phosphatidylethanolamine, a phospholipid, four glycolipids, and an unidentified lipid are the polar lipid components. The fatty acid of the cell wall mainly consists of carbons with 16 or 18 chain lengths such as $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and summed feature 3 ($C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c). The predominant menaquinone is MK-7. The G + C content in DNA is 68.65 mol%. Based on phylogenetic analysis using 16S rRNA gene sequence and results of physiological test, we propose the strain MEBiC09517^T (KCCM=43267, JCM=32374) for type strain of *Roseothermus cellulosivorans* gen. nov., sp. nov., which is a member of *Roseothermaceae* fam. nov.
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A030

Tamlana carrageenovorans sp. nov. Isolated from Seawater

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A Gram-stain-negative, aerobic, non-motile, rod-shaped, carrageenolytic bacterial strain, designated UJ94^T, was isolated from seawater of Uljin in Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequence determined that the sequence similarity of strain UJ94^T was 98.42%, 96.05%, and 95.36% with *Tamlana agarivorans*, *T. sedimentorum*, and *T. crocina*, respectively, indicating the association of strain UJ94^T with the genus *Tamlana*. Strain UJ94^T grew between 4–37°C, pH 7–8, and in the presence of 2–9% NaCl. It contained MK-6 as the predominant menaquinone and its major fatty acids were $C_{16:1}$ ω7c/$C_{16:1}$ ω6c and iso-$C_{15:0}$. The main polar lipid was phosphatidylethanolamine. The DNA G+C content was 35.2%. *In silico* DNA-DNA hybridization value estimated by genome-to-genome distance ranges 47.6–14.6% with the type species of genus *Tamlana*. Polyphasic approach including phylogenetic, chemotaxis, biochemical and genomic data suggested that strain UJ94^T represent a novel species of the genus *Tamlana* and the name *Tamlana carrageenovorans* sp. nov. is proposed. The type strain is UJ94^T (=KCTC 62451^T).

A031

Limnobaculum parvum gen. nov., sp. nov., Belonging to the Family Enterobacteriaceae

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A Gram-staining-negative, aerobic, non-motile, rod-shaped, pale white bacterium, designated strain HYN0051^T, was isolated from aquatic environment. The isolate grew optimally at pH 8.0, at 30°C, and in the presence of 0% NaCl on R2A, TSA, and NA. The major fatty acids of strain HYN0051^T were $C_{14:0}$, $C_{16:0}$, Summed feature 2 ($C_{12:0}$ aldehyde and/or unknown 10.928), Summed feature 3 ($C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c) and Summed feature 8 ($C_{18:1}$ ω6c and/or $C_{18:1}$ ω7c). The only detected isoprenoid quinone was ubiquinone 8. The phylogenetic analysis based on 16S rRNA gene sequence placed the isolate within the family *Enterobacteriaceae*, a member of the order *Enterobateriales.* Though the highest sequence similarity was observed with *Pragia fontium* (97.1%), strain HYN0051^T formed an independent phylogenetic branch in the family *Enterobacteriaceae*. A number of phenotypic properties also supported the distinctiveness of the new isolate from other previously known bacterial genera. Thus, based on the phylogenetic and phenotypic data, it is fair to say that the isolate is a novel genus of the family *Enterobacteriaceae.* The polyphasic study including whole genome sequencing is still underway.
[This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Korean Ministry of Education (2015R1D1A1A01057527) and by a Korea University Grant.]

A032

A Novel *Gramella* Species Isolated from East Sea of Korea

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A Gram-stain-negative, rod-shaped, aerobic bacterial strain designated LPB0144^T was isolated from the East Sea of Korea. Colonies are yellow-orange, circular, and slightly convex with entire margins on MA. The phylogenetic analysis based on 16S rRNA gene sequence placed the isolate within the genus *Gramella*, a member of the family *Flavobacteriaceae*. Strain LPB0144^T has a circular chromosome of 2.98 Mb with DNA G+C content of 38.2 mol%. The genome includes 2,604 protein-coding genes, 58 RNA genes and three copies of rRNA operons. Strain LPB0144^T contained menaquinone-6 and possessed iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$ 3-OH, and anteiso-$C_{15:0}$ as the major fatty acids within the range of *Gramella* genus. The major polar lipids of strain LPB0144^T were phosphatidylethanolamine and aminophospholipid. Strain LPB0144^T is distinguished from other *Gramella* species by many biochemical and physiological characteristics such as activity of glusosidase and hydrolysis of casein. The polyphasic data obtained in this study support the suggestion of strain LPB0144^T as a novel species of the genus *Gramella*.
[This study was supported by the Survey of Korean Indigenous Species Program through the National Institute of Biological Resources (NIBR) funded by the Korean Ministry of Environment.]

A033

Strain HYN0046^T, a Novel Member of the Family *Moraxellaceae*

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Strain HYN0046^T, a Gram-staining-positive, aerobic, white-colored, catalase-positive, oxidase-negative bacterium, was isolated from limnic environment. The isolate aerobically grew on R2A medium in the presence of 0-1% (w/v) NaCl. In the 16S rRNA gene sequence trees, strain HYN0046^T formed a separate branch in the family *Moraxellaceae*. The highest sequence similarity was observed with *Alkanindiges illinoisensis* (93.7%), and followed by *Fluviicoccus keumensis* (93.5%), *Acinetobacter populi* (93.4%), *Agitococcus lubricus* (93.1%), and *Perlucidibaca aquatic* (92.92%). The low sequence similarity and tree topology demonstrated the taxonomic independence of the strain at genus-level. The isolate possessed $C_{18:1}$ ω9c, $C_{12:0}$ 3-OH, $C_{12:0}$, and $C_{16:0}$ as the major cellular fatty acids. The strain hydrolyzed DNA, casein, and Tween 20, Tween 60, and Tween 80, but not CM-cellulose, alginic acid, starch, chitin, adenine, L-tyrosine, xanthine, or hypo-xanthine. The taxonomic position of the isolate is still under investigation with the strong possibility that it is a novel genus of the family *Moraxellaceae*.
[This study was supported by the Survey of Korean Indigenous Species Program through the National Institute of Biological Resources (NIBR) funded by the Korean Ministry of Environment.]

A034

A Novel *Sphingorhabdus* Species Isolated from Sea Water

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A Gram-staining-negative, oxidase-negative, catalase-positive, yellow-pigmented, aerobic bacterial strain designated LPB0140^T was isolated from sea water. Best growth condition was observed at 35°C and pH 6 on marine agar. The 16S rRNA gene sequence similarity demonstrated that the closest relative of the isolate is Sphingorhabdus wooponensis (96.1%), but the new isolate formed an independent phyletic line within the radiation of the genus Sphingorhabdus. Strain LPB0140^T possessed $C_{14:0}$ 2-OH, $C_{16:1}$ ω7c/$C_{16:1}$ ω6c, $C_{18:1}$ ω7c as the major cellular fatty acids, and diphosphatidylglycerol, phosphatidylglycerol, sphingoglycolipid, phosphatidylethanolamine and phosphatidylcholine as the major polar lipids. Its genome is composed of a circular chromosome of 2.53 Mb with DNA G+C content of 64.8 mol%. The genome includes 2,359 protein-coding genes and 51 RNA genes. Based on phylogenetic, genomic, and phenotypic data presented in this study, strain LPB0140^T should be classified as a novel species in the genus Sphingorhabdus.
[This study was supported by the Survey of Korean Indigenous Species Program through the National Institute of Biological Resources (NIBR) funded by the Korean Ministry of Environment.]

A035

Gemmatimonas aquatica sp.nov., Isolated from River Water

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A Gram-staining-negative, red pigmented, oxidase- and catalase-positive, non-motile, facultatively aerobic and rod-shaped bacterium BK-75^T, was isolated from a Nakdong River of Korea. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BK-75^T belonged to the genus *Gemmatimonas* and was closely related to *Gemmatimonas aurantiaca* and *Gemmatimonas phototrophica* with 96.7 and 96.3% sequence similarities, respectively. Genomic relatedness analyses based on average nucleotide identity and genome-to-genome distance showed that strain BK-75^T could be clearly distinguished from other species of the genus *Gemmatimonas*. The major fatty acids were $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH and iso-$C_{15:0}$. The major respiratory isoprenoid quinone was MK-9 and major polar lipids were phosphatidylethanolamine, phosphatidylcholine and diphosphatidylglycerol. On the basis of phylogenetic analysis and genotypic and phenotypic data obtained in this study, it is concluded that strain BK-75^T re presents the type strain of a novel species of the genus *Gemmatimonas*, for which the name *Gemmatimonas aquatica* sp. nov. is proposed.

A036

Flavobacterium cremeum sp. nov., a Gentamycin-resistant Bacterium Isolated from Han River, South Korea

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A Gram-staining-negative, aerobic, non-motile, rod shaped, and cream-colored bacterium, designated CJ74^T, was isolated from Han River, South Korea. Strain CJ74^T grew optimally at 30°C and pH 6.0 in the absence of NaCl on R2A agar. Flexirubin-type pigments were not detected. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain CJ74^T belonged to the genus *Flavobacterium* and was most closely related to *Flavobacterium subsaxonicum* WB 4.1-42^T and *F. rivuli* WB 3.3-22^T (95.9% similarity). The whole genome sequence of strain CJ74^T was determined and genomic comparison was performed using average nucleotide identity (ANI) analysis. ANI values with *F. subsaxonicum* WB 4.1-42^T and *F. rivuli* WB 3.3-22^T were 73.94% and 73.97%, respectively, indicating that strain CJ74^T represents a novel species in the genus *Flavobacterium.* The predominant polar lipids were phosphatidylethanolamine. The only respiratory quinone was menaquinone 6. The major fatty acids of strain CJ74^T were *iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c). The G+C content of the genomic DNA was 41.2 mol%. Based on polyphasic taxonomy data, strain CJ74^T is identified as a new species, for which the name *Flavobacterium cremeum* sp. nov. is proposed. The type strain is CJ74^T.
[This work was supported by a project of th National Institute of Biological Resources (NIBR) to survey Korean indigenous species.]

A037

Euzebyella algicola sp. nov., a Marine Bacterium of the Family *Flavobacteriaceae* Isolated from Green Algae

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A Gram-negative, yellow-pigmented, non-flagellated, gliding, rod-shaped, and aerobic bacterium, designated MEBiC 12267^T, was isolated from the green algae of the Jeju Island. 16S rRNA gene sequence analysis revealed that the strain MEBiC 12267^T was affiliated to the genus *Euzebyella* of the family *Flavobacteriaceae* and showed the highest similarity to *Euzebyella marina* KCTC 42440^T (98.5%). The DNA-DNA relatedness value of strain MEBiC 12267^T with *E. marina* KCTC 42440^T was 25%. Growth was observed at 10–37°C (optimum, 30–33°C), at pH 6.0–9.5 (optimum, 8.0–8.5) and with 0.5–9.0% (w/v) NaCl (optimum, 2.5–3.5%). The predominant cellular fatty acids were iso-$C_{15:0}$, iso-$C_{15:1}$ G and iso-$C_{17:0}$ 3-OH and the major respiratory quinone was MK-6. Polar lipids included phosphatidylethanolamine, seven unidentified lipids, and two unidentified aminolipids. The DNA G+C content was 40.7 mol%. On the basis of the data from the polyphasic taxonomic study, it was concluded that the strain MEBiC 12267^T represents a novel species within the genus *Euzebyella*, for which the name *Euzebyella algicola* sp. nov. is proposed. The type strain of *E. algicola* is MEBiC 12267^T (=KCCM 43264^T =JCM 32170^T).

[Supported by the MABIK in-house program and the Marine Biotechnology program.]

A038

Polaribacter aureus sp. nov., Isolated from the Gastrointestinal Tract of a Blood Cockle, *Tegillarca granosa*

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A novel isolated bacterium, designated as strain BM10^T was isolated from the gut of a blood cockle *Tegillarca granosa* collected from the tidal flat of Beolgyo-eup in Korea.

Strain BM10^T was Gram-stain-negative, non-motile, strictly aerobic and possessed rod cell shape. Optimum growth occurred at 25°C, in the presence of 1% (w/v) NaCl and pH 7. Comparative 16S rRNA gene sequence analysis showed that strain BM10^T belonged to the genus *Polaribacter* in the family *Flavobacteriaceae*. Strain BM10^T shared sequence similarity (>97%) with *Polaribacter reichenbachii* 6Alg 8^T (98.06% similarity), *Polaribacter dokdonensis* DSW-5^T (97.92% similarity) and *Polaribacter irgensii* 23-P^T (97.08% similarity). The polar lipid profiles of strain BM10^T were composed of phosphatidylethanolamine, two unidentified amino lipids and six unidentified lipids. The major respiratory quinone was identified as menaquinone-6 (MK-6). The major cellular fatty acids were summed feature 3 ($C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c), iso-$C_{15:1}$ G and iso-$C_{15:0}$ 3OH. The G+C content of genomic DNA was 31.4 mol%.

The results of the phylogenetic, phenotypic and genotypic analyses suggest that strain BM10^T represents a novel species in the genus *Polaribacter*, for which the name *Polaribacter aureus* is proposed.

A039

Isolation and Characterization of a Butyrate-producing Bacterium Isolated from the Ruminal Contents of Holstein-friesian Cattle

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Butyrate-producing bacteria are associated with gastrointestinal health in humans and various animal species. This study aimed to isolate and characterize butyrate-producing bacteria in the rumen contents of a Holstein-Friesian cattle. Microbial diluent was prepared by homogenization of 1 ml rumen fluid in 9 ml of M2GSC medium and serially diluted and inoculated in a Hungate roll tube containing anaerobic M2GSC medium. Carbohydrate metabolism, butyrate production, and enzyme activity were analysed using API® 50 CH test kit, HPLC, and DNS method, respectively. PCR was performed for the 16S rRNA gene sequence analysis. A bacterium strain RNAL841125 was isolated and, the optimum growth was observed at 37°C and between pH 5.8 to 6.8. High level of butyrate was produced by *Cl. saccharobutylicum* RNAL841125 (42.39 mmol/L). Enzyme activity showed that the strain was positive for CMCase, FPase, xylanase, and α-amylase activities. The 16S rRNA gene of the strain was sequenced and phylogenetic analyses showed that the strain was closely related to *Clostridium saccharobutylicum* (with a 99.65% sequence similarity). The strain was deposited in the Korean Culture Center of Microorganisms as *Cl. saccharobutylicum* RNAL841125.

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Keywords: Bacteria, Butyrate, Holstein cattle, Rumen

A040

Phenotypic and Genomic Characteristics of Three Freshwater Bacterial Strains Constituting a New Subcluster of Genus *Polynucleobacter*

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The genus *Polynucleobacter* is ubiquitous in freshwater environments. *Polynucleobacter* strains cultured so far have been grouped into four subclusters based on 16S rRNA gene sequence divergence: subclusters PnecA, B, C and D. We report here preliminary analyses on phenotypic and genomic characteristics of *Polynucleobacter* strains IMCC29146, IMCC30063, and IMCC30228 that are not affiliated with any of the four previously-known subclusters. The three strains were isolated from Lake Soyang, an oligotrophic freshwater reservoir, by dilution-to-extinction culturing. Phylogenetic analyses showed that these strains clearly formed a new distinct subcluster within genus *Polynucleobacter*. All three strains were aerobic, facultatively anaerobic, catalase- and oxidase-positive, and non-motile. Growth of strains occurred on R2A and NSY medium at 10–30°C (optimum, 20°C) with 0–0.5% NaCl (optimum, 0%). The predominant fatty acids were $C_{16:0}$ (32.17–37.03%), $C_{17:0}$ cyclo (11.91–26.79%), and feature 3 including $C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c (17.59–30.68%). The draft genome sequences of the strains showed a length of 2.4–2.5 Mb and an average G+C content of 44.5–44.8%. Genes for polyhydroxyalkanoate (PHA) were predicted from the genomes, which was consistent with PHA granules in cultured cells observed using transmission electron microscopy.

[This study was supported by a grant of the Mid-Career Research Program through the NRF.]

A041

Development of Multiplex Real-time PCR Assay for Detecting the Six Most Prevalent Genes of Carbapenemase in Enterobacteriaceae

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The objective of this study was to establish the multiplex real-time PCR (RT PCR) assay for reduction of turnaround time of diagnostics of Carbapenemase-producing (CP) bacteria. We designed two sets of triplex RT PCR for amplifying simultaneously target genes (one was for KPC, NDM, and OXA-48 and the other for IMP, VIM, and GES) using the peptide nucleic acid (PNA)-based probes and optimized into each single reaction. We determined both assay linearity and limit of detection by the 10-fold serial dilutions of DNA extracted from 1−2 colonies by boiling lytic method and regarded a threshold value of Ct ≤ 35 as a positive in each reaction. The designed RT PCR was evaluated the specificity and sensitivity for the previously characterized 296 carbapenem resistant isolates (204 CP and 92 non-CP). In non-CP, there was no detection of six genes. In CP positive, the range of cycle threshold(Ct) value of each gene was from 10 to 35. This assay showed the highest consistency (99.3%) with the results previously examined by conventional PCR. Only two isolates found to carry NDM gene not detected by conventional PCR. These isolates had two different gene types of carbapenemase: one had both KPC, VIM, and NDM, and the other OXA-48 and NDM. In this study, the new developed triplex RT PCR assay provide an easy and fast identification of the presence of six CP genes within only 3 h. In two cases, it was founded that our method is more sensitive than the conventional method in detecting of NDM genes.

A042

Isolation and Genomic Study on Two Novel Bacteriophages Infecting a Freshwater *Comamonadaceae* Bacterium

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Recently, environmental bacteriophages have become a center of attention due to its wide abundance, diversity, and potential as bacterial gene carriers. Although phages of diverse environments have been studied, those of freshwater environment have been understudied. In this study, we report two novel phages, P26059A and P26059B, that were isolated from Lake Soyang in South Korea, and lytically infect bacterial strain IMCC26059, a member of the family *Comamondaceae*, one of the ubiquitous freshwater bacterial groups. The two phages, P26059A and P26059B had different morphologies, each assigned to viral families *Siphoviridae* and *Podoviridae*, respectively. The genomes of the two phages were highly distinct from each other with little sequence similarity. Comparative analysis between phage genome sequences and freshwater viral metagenomes showed that the phage populations represented by the two phages exist in the environment with different distribution patterns. Taken together, although the phages shared a host strain, they showed completely distinctive characteristics from each other in morphological, genomic, and ecological analyses. Considering the abundance of the family *Comamonadaceae* in freshwater habitats and the rarity of phage isolates infecting this family, the two phages and their genomes in this study would be valuable resources for further freshwater virus researches.
[Supported by the Mid-Career Research Program through the NRF funded by the Ministry of Sciences.]

A043

Tropicimonas halophila sp. nov., and *Tropicimonas aestuarii* sp. nov., Isolated from Tidal Flat Sediment

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Two Gram-negative, aerobic, non-motile, rod-shaped bacterial strains, designated IMCC34011^T and IMCC34043^T were isolated from tidal flat of Shin Island in Incheon, Korea. The 16S rRNA gene sequences showed that two strains shared 96.4 % sequence similarity with each other and both of them were most closely related to *Tropicimonas isoalkanivorans* B51^T (95.7%, 96.8%), *Tropcimonas aquimaris* DPG-21^T (95.6%, 96%) and Tropicimonas sediminicola M97^T (95.4%, 96.6%), respectively. Both strains optimally grew at 30°C and pH 6.0–7.0, and contained summed feature 8 ($C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*) as major fatty acid, ubiquinone-10 (Q-10) as the predominant respiratory quinone and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and aminolipid as major polar lipids. However, strains IMCC34011^T and IMCC34043^T were differentiated in several phenotypic properties such as the color of colony, optimum growth in the presence NaCl, proportion of $C_{16:0}$, and numbers of unknown phospholipids. Differential properties of two strains also distinguished them from other *Tropicimonas* species. On the basis of polyphasic taxonomic analyses, it was concluded that strains IMCC34011^T and IMCC34043^T should be classified as representing two novel species in the genus *Tropicimonas*, for which the names *Tropicimonas halophila* sp. nov. (type strain IMCC34011^T =KACC 19597^T) and *Tropicimonas aestuarii* sp. nov. (type strain IMCC34043^T =KACC 19598^T) are proposed.

A044

Phenotypic and Genomic Properties of *Brachybacterium vulturis* sp. nov. and *Brachybacterium avium* sp. nov.

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Strains VM2412^T and VR2415^T were isolated from feces of an Andean condor. They shared 99.3% 16S rRNA gene sequence similarities each other but they were identified as two distinct species based on average nucleotide identity and digital DNA-DNA hybridization values. Among species with validly published names, *Brachybacterium ginsengisoli* DCY80^T shared the highest 16S rRNA gene sequence similarities with them. Major fatty acid was anteiso-$C_{15:0}$. Polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and three unidentified glycolipids. Cell wall contained *meso*-diaminopimelic acid as a diagnostic diamino acid and ribose, glucose and galactose as wall sugars. They had MK-7 as a predominant menaquinone. The genomic G+C contents of the strains VM2412^T and VR2415^T were 70.8 and 70.4 mol%, respectively. Based on phenotypic and genotypic analyses, the strains VM2412^T and VR2415^T are considered to represent two novel species within the genus *Brachybacterium*. The following names are proposed: *Brachybacterium vulturis* sp. nov. for the strain VM2412^T and *Brachybacterium avium* sp. nov. for the strain VR2415^T. The type strains are VM2412^T (=KCTC 39996^T=JCM 32142^T) and VR2415^T (=KCTC 39997^T=JCM 32143^T), respectively.
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A045

Tumebacillus avium sp. nov., Isolated from the Gut of a Cinereous Vulture, *Aegypius monachus*

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A Gram-stain-positive, facultatively aerobic, spore-forming, oxidase-positive, catalase- and DNase-negative, rod-shaped, and motile bacterial strain, AR23208^T, was isolated from the gut of a cinereous vulture (*Aegypius monachus*). Strain AR23208^T grew optimally at 25–30°C, at pH 7, in the absence of NaCl. The phylogenetic analysis revealed the 16S rRNA gene of strain AR23208^T shared 98.2% and 97.1% sequence similarity with those of *Tumebacillus algifaecis* THMBR28^T and *T. lipolyticus* NIO-S10^T, respectively. The predominant fatty acids (>10%) of strain AR23208^T were iso-$C_{15:0}$ (46.5%), summed feature 4 (anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I, 11.7%) and anteiso-$C_{15:0}$ (11.1%) and the primary isoprenoid quinone was menaquinone-7. The polar lipids were phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, six unidentified phospholipids, an unidentified amino phospholipid and ten unidentified lipids. The sugar components of the cell wall peptidoglycan were ribose and arabinose. The amino acids of the cell wall peptidoglycan were L-alanine, aspartic acid, *meso*-diaminopimelic acid, L-glutamic acid, glycine, and L-lysine. The genomic DNA G+C content of strain AR23208^T was 56.0 mol%. In the current study, strain AR23208^T is proposed to be a novel species candidate of the genus *Tumebacillus*, with the strain name *Tumebacillus avium* sp. nov. and strain AR23208^T (=KCTC 33929^T =JCM 32188^T) as the type strain. Supported by grants from NRF and NIBR.

A046

Identification of *Flavobacterium* sp. Strain P2-65^T, A Novel Bacterium Isolated from Water Stream

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A Gram-stained-negative, rod-shaped, aerobic, non-motile bacterial strain, designated P2-65^T, was isolated from water stream. The temperature, NaCl concentration, pH ranges for growth were 10–37°C, 0–3%, 6.5–8.5 with optimum growth observed at 25–30°C, 0–1%, 7–7.5, respectively. Comparison of 16S rRNA gene sequence showed that strain P2-65^T was closely related to *Flavobacterium cauense* (95.39%), *Flavobacterium cheniae* (95.26%). The major fatty acids were iso-$C_{15:0}$, iso $C_{17:0}$ 3-OH, summed features 3 ($C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$), summed features 9 (iso-$C_{17:1}$ ω9c and/or 10-methyl $C_{16:0}$), iso-$C_{15:0}$ 3-OH. The respiratory quinone was identified as MK-6. The main polar lipids detected in strain P2-65^T consisted of phosphatidylethanolamine, two aminophospholipid and one unidentified polar lipid. The G+C content of the genomic DNA of strain P2-65^T was found to be 40.8 mol%. Based on the genotypic, phenotypic and chemotaxonomic characterizations, strain P2-65 is considered to represent a novel species of the genus *Flavobacterium*. The type strain is P2-65T (=KCTC 62055^T=NBRC 112953^T).
[Supported by the Korea Ministry of Environment (MOE) as ''The Environmental Health Action Program'' under Project No. 2016001350005.]

A047

Flavobacterium sp. I3-2 Isolated from River Water

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A yellowish, flexirubin pigment producing strain I3-2^T isolated from river water in Iksan, South Korea was studied to determine its taxonomic position. Cells of the isolate were Gram stained negative, aerobic, non-motile, and showed catalase and oxidase activities. The novel strain was able to grow at 15–30°C (optimum 30°C), pH 6.0–10.0 (optimum pH 7.0) and with 0–1.5% (w/v) NaCl (optimum 1.0%). The major fatty acids were iso-$C_{15:0}$, summed feature 9 (comprising iso-$C_{17:1}$ ω9c and/or $C_{16:0}$ 10-methyl), iso-$C_{17:0}$ 3-OH, summed feature 3 (comprising $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$). Menaquinone-6 (MK6) as the major respiratory quinone. Comparison of the 16S rRNA gene sequence with the sequences of the type strains of the most closely related species showed highest sequence similarity (93.96%) to *Flavobacterium anatolliense* MK3^T and (93.88%) to *Flavobacterium suzhouense* XIN-1^T. Based on phenotypic and phylogenetic distinctiveness, strain I3-2^T is considered as a member of novel species within the genus *Flavobacterium*. The type strain is I3-3^T (=KCTC 62507^T).
[Supported by a fund (2017N-ER5405-00) from Research of Korea Centers for Disease Control and Prevention.]

A048

Epidermidibacterium keratini gen. nov., sp. nov., a Member of the Family *Sporichthyaceae,* Isolated from Keratin Epidermis

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A novel actinobacterial strain, designated EPI-7^T, was isolated on R2A agar from human skin and subjected to a taxonomic study using a polyphasic approach. Strain EPI-7^T showed a Gram-positive reaction, was non-motile, non-spore-forming, and cells had a rod-shape. Colonies were round, convex and pale yellow. Phylogenetic analysis based on 16S rRNA gene sequences showed that the novel isolate formed a cluster with several uncultured bacterial clones and with cultured members of the genera *Modestobacter* and *Sporichthya*. The gene sequence similarities with respect to the type strains of recognized species from the above genera and other phylogenetic neighbours ranged from 92.6 to 93.4%. The G+C content of the genomic DNA was 68.9 mol%. The only isoprenoid quinone was MK-9(H4) and the major fatty acids detected were $C_{17:1}$ ω8c, $C_{16:0}$, and iso-$C_{15:0}$ and summed feature 3. The major polar lipids were found to be phosphatidylethanolamine, phosphatidylinositol, three unidentified phospholipids, phosphatidylglycerol, phosphatidylcholine, two unidentified amino lipids, and three unidentified lipids. The cell wall peptidoglycan contained *m*-DAP, glutamic acid and alanine. Whole-cell sugars present included rhamnose, glucose and galactose. The combination of the genotypic and phenotypic data allowed differentiation of strain EPI-7^T from its closest phylogenetic neighbours and provided evidence that strain EPI-7^T represents a novel genus and species in the family *Sporichthyaceae*.

A049

Plant Growth Promoting Rhizobateria *Pseudomonas koreensis* MU2 Enhance Growth of Lettuce and Chinese Cabbage through Organic Acid and Gibberellin Production

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Plant growth promoting rhizobacteria (PGPR) is an environmentally safe tool employed for the optimum growth and production of plant. Massive application of synthetic pesticides and fertilizer in agriculture have resulted negative effect on nature and human health. To add a brick in the pool of organic agriculture practice, we isolated several strain of PGPR through screening form the diversed agricultural soil from Daegu, South Korea and checked its growth promoting ability on gibberelline deficit rice dwarf mutant *Waito-C*, lettuce and Chinese cabbage. The strain that have higher ability to promote the *Waito-c* growth was selected and identified as *Pseudomonas koreensis* MU2 through 16S rDNA gene sequence analysis. The cultural filtrate analysis revealed that the isolate could produce endogenous phytohormone gibberellic acid (GA1 and GA3). Pot experiment revealed that the inoculation of *Pseudomonas koreensis* MU2 significantly increased shoot length, root length, fresh biomass and dry biomassof chinese cabbage and lettuce. These results suggest that the *Pseudomonas koreensis* MU2 might be the possible candidate of bio-fertilizer as a plant growth promoting rhizobacteria in plant.
[Supported by grants from NRF]

A050

Genomic Insights into the Facultative Methanotrophic Lifestyle of the New *Methylovirgula*-like Isolate from Acidic Forest Soils

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Recently, facultative methanotrophy is demonstrated in several members of the genera *Methylocystis*, *Methylocapsa* and *Methylocella* of the family *Beijerinckiaceae*, that is, capable of growing on the methane as well as some multi-carbon substrates. The knowledge on facultatively methanotrophic in carbon cycle and methane flux remains elusive due to limited availability of pure cultures. In this study, *Methylovirgula*-like strain HY1, a new facultative methanotroph, was isolated from pine forest soils using a sequencing bioreactor and its genome sequence was compared to those of other *Beijerinckiaceae* members. Genome of HY1 contains genes encoding a cytosolic methane monooxygenase (sMMO) while a membrane bound methane monooxygenase (pMMO) was absent. Many genes for methanotrophic growth were present. However, strain HY1 shares highest 16S rRNA gene sequences (98.63%) with *Methylovirgula ligni* BW863^T, a facultative methylotroph (non-methanotroph). Comparative genomic analysis between strain HY1 and BW863 demonstrated that strain HY1 harbors many distinct genes for niche specialization in addition to methanotrophy in terms of sulfur oxidation, micro-aerophilicity and hydrogen utilization. Our finding provides information on ecological niches of facultative methanotrophy in acidic environments.

B001

Artificial Warming Effect on Microbial Community and Humic Substance Degradation in Maritime Antarctic Soil in King George Island

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Although the maritime Antarctic has undergone rapid warming, the effects on indigenous soil-inhabiting microorganisms are not well known. Artificial warming experiments using open-top chamber (OTC) have been performed on the Fildes Peninsula in the maritime Antarctic since 2008. When the soil temperature was measured at a depth of 2–5 cm during the 2013–2015 summer seasons, the mean temperature inside OTC (OTC-In) increased by approximately 0.8°C compared with outside OTC (OTC-Out), while soil chemical and physical characteristics were not changed. Soils from OTC-In and OTC-Out were subjected to analysis for change in microbial community and degradation rate of humic substances (HS, the largest pool of recalcitrant organic carbon). Archaeal and bacterial communities in OTC-In were minimally affected by warming compared with those in OTC-Out, with archaeal methanogenic *Thermoplasmata* slightly increased in abundance. The abundance of heterotrophic fungi *Ascomycota* was significantly altered in OTC-In. Total bacterial and fungal biomass in OTC-In increased by 20% compared to OTC-Out, indicating that this may be due to increased microbial degradation activity for soil organic matter (SOM) including HS, which would result in the release of more low-molecular-weight growth substrates from SOM. Despite the effects of warming on the microbial community over the 8-years-experiments warming did not induce any detectable change in content or structure of polymeric HS.

B002

Endophytic Bacteria from Turmeric Plant as Plant Growth Promoter and Antifungal Agents

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Endophytic bacteria are useful agent in the diverse area of biotechnological applications, and it has already produced commercial formulations as microbial fertilizers and pesticides. In this study, we isolated eight endophytic bacteria from turmeric plant of farmer cultivating area in Jeongeup, Jeonbuk, South Korea. We checked plant growth promoting properties of these endophytes on several ways such as measuring indole-3-acetic acid (IAA) production, chlorophyll contents, root length, and leaf weight. In addition, four plant fungal pathogen, *Ceratocytis fimbriata, Fusarium oxyporum, Colletotrichum gloeosporioides,* and *Fusarium oxysporum f.sp. cucumerinum* were used to screening of antagonistic activity for eight isolates. Isolates were also determined to have the ability to produce enzymes such as protease, cellulose, amylase, and proteinase. We found that isolate D6 is one of the most powerful bacteria that can enhance plant growth, inhibit fungal growth, and produce of enzymatic activities such as protease, cellulose, amylase and proteinase. Based on 16S rRNA sequence analysis, the isolate D6 was identified as *Bacillus velezensis* (formerly referred to as *Bacillus amyloliquefaciens*). Collection of isolates could be promising bio-fertilizer and pesticides for improving plant growth and crop protection. [Supported by grants from Korea Research Institute of Bioscience & Biotechnology]

B004

Comparison of Microbiome and Virome in Chicken Farm under Diverse Environmental Factors

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Microbial/Viral infections are common problem in chicken farming worldwide and it occurred year in and year out in South Korea. However, the studies of metagenomics in chicken farm for prevention of epidemic diseases are quite lack. In this study, we've sequenced and analyzed the microbial or viral composition in chicken farm under diverse environmental factors. Samples from the feces, dust, and soil samples in several chicken farms were collected and sequenced. In virome, every farms showed the different virus families. Most common viruses in chicken farm were Turkey parvovirus 1078, chicken parvovirus ABU-P1. Influenza A virus which is most fatal in poultry industry was not detected in our sequences. Interestingly, host unrelated viruses such as porcine stool-associated circular virus and BeAn 58058 virus were detected. This study will be helpful to understand the composition of virus in chicken farm and establish the relationships between viruses.
[This study was supported by a grant from the Strategic Initiative for Microbiomes in Agriculture and Food, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea as part of the multi-ministerial Genome Technology to Business Translation Program (Grant ID: 914005–04) and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016-R1C1B2016246).]

B005

Microbial Communities Responsible for Glucose and Acetate in Organic-enriched Sediment of the Fin-fish Farm in the South Coast of Korea

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The fish farm sediment is characterized by high organic loading due to uneaten fish food and feces, as a result, various organic carbon (C_{org}) compounds such as glucose, pyruvate, lactate, propionate, ethanol, acetate and fatty acids are highly accumulated. A combination of metabolic rate measurements and molecular microbiological analyses including RNA-stable isotope probing (RNA-SIP) was conducted to identify the active microorganisms responsible for the oxidation of acetate and glucose, a major terminal electron donor in anaerobic respirations, in fish farm sediment. Sediments slurries were amended with glucose (^{13}C and ^{12}C) and acetate (^{13}C and ^{12}C) over 7-days. Anaerobic metabolism was stimulated in the glucose slurries, whereas no increase was observed in acetate slurries. The major bacterium detected in ^{13}C-glucose slurry was related to *Psychromonas* which is known to grow anaerobically by fermenting starch and glucose. They produced acetate, formate, ethanol and lactate, and the gases CO_2, and thus it expect to support other populations relying on the glucose metabolites. In contrast, the major bacterium responsible for the acetate oxidation was affiliated with *Desulfobacter*. Our results indicate that the *Psychromonas* and *Desulfobacter* play an important role in C_{org} oxidation at the initial and final stage of anaerobic food chain, respectively, in fish farm sediments.
[Supported by grants from the National Institute of Fisheries Sciences and BK 21 Plus Program.]

B006

Metagenomic Study of the Upper Airway Microbiome Associated with Childhood Asthma

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Upper respiratory tract is composed of microbiome including viruses, bacteria and fungi, and these have implications for respiratory health and disease. Microbial colonization of the airway plays a role in the pathogenesis of asthma; however, the effect of the upper airway microbiome on childhood asthma is not fully understood. We analyzed the metagenome of airway microbial communities to understand the associated role of upper airway microbiome with childhood asthma. Nasopharyngeal swabs were collected from 147 children with asthma, remission, and control groups. High-throughput sequencing was used to examine the structure dynamics of the airway microbiome according to asthma phenotypes. The amounts of bacteria in upper airway were quantified by real-time PCR. The composition of microbiota differed among healthy control, asthma, and remission groups. Proteobacteria (53.1%) and Firmicutes (25.1%) were the most predominant phyla in childhood airway microbiome. *Haemophilus* and *Moraxella* were predominant in all groups, although the relative abundance of *Haemophilus* were higher in control group (mean, 34%) than in asthma (21%) and remission (23%) group ($P < 0.05$). The proportion of *Corynebacterium* was higher in the asthma group (mean, 11%) than in the remission (8.7%) and control groups ($P < 0.05$). These data suggest that alterations in the composition of the upper airway microbiome could be related with asthma in children.

B007

Synergistic Effect of Antimicrobial Substances from Some Bacteria against Human Skin Pathogens

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The aim of this study is to evaluate antimicrobial activities and synergistic effect by antimicrobial substances produced from isolated bacteria *Paenibacillus elgii* DS381, *Burkholderia gladioli* DS518, *Streptomyces lienomycini* DS620 and *Paenibacillus elgii* DS1515 against several human skin pathogens. Isolated bacteria showed 15.3 to 35.3 mm of inhibition zone diameter against most bacteria and yeast tested, and 1.5 to 27.5 mm of inhibition zone against most target fungi. The purified antimicrobial substances (lipopeptide, antimicrobial peptide, etc.) from isolated strains showed low minimum inhibitory concentrations (0.0000078 to 10 mg/ml) against target organisms. In checkerboard assay, combinations of antimicrobial substances of DS381, DS518, DS620, and DS1515 displayed synergistic effects against each different target organism [0 < fractional inhibitory concentration index (FICI) < 0.75]. Especially, combinations of DS381 + DS1515 and DS620 + DS1515 indicated partial synergy (FICI < 0.75) or total synergy (FICI < 0.5) against most microorganisms. In time-kill assays most combinations exhibited synergistic antimicrobial effect against target bacteria with a reduction of more than 5 log in colony count after 24 h. These results suggest that isolated strains may be utilized as an environment-friendly biocontrol agent against human skin pathogens and combination of antimicrobial substances could be useful for biopreservation in food and cosmetics.

B008

Aflatoxin B_1 Biodegradation and Inhibition against Aflatoxigenic Fungi by *Streptomyces* spp.

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Mycotoxins are secondary toxic metabolites produced by various filamentous fungi including *Aspergillus* spp. Aflatoxin B_1 (AFB_1) is one of the most hazardous mycotoxin and is known for carcinogenic, teratogenic, hepatotoxic and immunosuppressive effect on humans and animals. The worldwide contamination of foods and feeds with AFB_1 is a significant agricultural and medical problem. This study aims to explore AFB_1 degradation and antifungal activity against aflatoxigenic fungi by *Streptomyces sporoverrucosus* JS383 and *S. lavendulae* JS669. JS383 and JS669 degraded AFB_1 (0.1 mg/L) by 93.7 and 96.8%, respectively in nutrient broth for 72 h at 30°C. The antifungal activity of JS383 and JS669 was evaluated by co-culture with three aflatoxigenic *A. flavus* (KACC44986, 45068, and 45146). JS383 and JS669 effectively inhibited mycelial growth of three strains *A. flavus*, also suppressed sporulation of *A. flavus* up to 97.3 and 96.3%, respectively. JS383 and JS669 showed 72.3 and 90.9% of inhibition of spore germination, respectively. Furthermore, JS383 and JS669 decreased AFB_1 produced by *A. flavus* up to 98.8 and 99.6%, respectively. The minimum inhibitory concentrations of ethyl acetate extract from JS383 and JS669 were 8.5 to 24.3 mg/ml against three strains of *A. flavus*. These results imply the prospective applications of JS strains for AFB_1 biodegradation and control of aflatoxigenic fungi in food and feed industry.

B009

Bacterial Degradation of Ochratoxin A and Growth Inhibition of Ochratoxigenic *Aspergillus* spp.

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Ochratoxin A (OTA), one of mycotoxins produced mainly by *Aspergillus* is a common contaminant of stored grains, posing health hazards to humans and animals alike. The aim of this study is to explore ability of isolated bacteria *Bacillus subtilis* AF13 and *Streptomyces shenzhenensis* YR226 to degrade OTA and inhibit ochratoxigenic *Aspergillus*. AF13 and YR226 could degrade 95.15 and 98.84% of OTA (100 μg/L), respectively. When cultures of two strains were separated into washed cells and cell-free supernatant, the supernatant of both strains degraded more than 90% of 100 μg/L OTA, but up to 98% of OTA could be also degraded by washed cells of YR226. Various factors affecting on OTA degradation were examined, such as incubation time, temperature, treatment of metal ion and heat. The antifungal activity against mycelial growth and sporulation of ochratoxigenic *Aspergillus* strains (*A. awamori, A. fresenii* and *A. alutaceus*) was examined by coculture with AF13 and YR226 on agar plate. AF13 and YR226 reduced 77.58 and 78.48% of fungal colony radius, respectively, and both strains inhibited sporulation up to 99%. These results suggest that AF13 and YR226 can be used in a biological method to prevent valuable crops against toxigenic fungi and effectively detoxify mycotoxin contaminated feed and grains and therefore decrease economic damage in agriculture.

B010

Insight the Genome of *Lacinutrix venerupis* Strain DOK2-8 Isolated from Dokdo, Republic of Korea

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The genus *Lacinutrix* is a member of the family Flavobacteriaceae and comprises 10 valid marine species. Although those species were considered nonpathogenic, *L. venerupis* was recently found to be associated with disease outbreak among marine fish, and its genome has not yet been reported. Here in this study, we present the first complete genome of *L. venerupis*. Since 2015, we have screened several indigenous bacterial strains in the Republic of Korea, and the genome of *L. venerupis* DOK2-8 strain that isolated from marine sediment collected from Dokdo, East Sea, was sequenced using PacBio RS II system. Based on the orthologous average nucleotide identity analysis, the DOK2-8 genome showed the highest and lowest similarity with *L. algicola* (80.7%) and *L. himadriensis* (75.5%) among the genus Lacinutrix, respectively. In addition, several potential virulence-related genes, which were homologous to those of other Gram-negative species, were detected in the DOK2-8 strain. These results indicate that *L. venerupis* might have potential virulence to marine organisms, and information regarding its genome will provide important insights into the bacterial diversity in the marine environments.

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B011

An *in vitro* Study to Assess the Impact of Tetracycline on the Human Intestinal Microbiome

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The human intestinal microbiome could be altered by antibiotics in foods derived from animals. To determine if tetracycline could impact the human intestinal microbiome structure and tetracycline resistance genes (TRGs) profile, the effects of 0.15–150 μg/ml of tetracycline, after 24 h and 40 days of exposure, in 3% fecal suspensions, collected from three individuals were assessed using *in vitro* batch cultures. Results were variable, with either no change or minor changes in total bacterial 16S rRNA gene copies after tetracycline exposure, because of the inter-individual variation of human intestinal microbiome. Bacterial community analysis revealed that *Firmicutes* and *Bacteroidetes* were the predominant phyla but the ratio of phylotypes varied among individuals. The evaluation of bacterial community changes at the genus level, from control to tetracycline-treated samples, suggested that tetracycline could lead to slight differences in intestinal microbiome structure. The genus *Bacteroides* was increased 1.68–5.70% and 4.82–8.22% at 0.15 μg/ml or above at both time points for individual A, respectively, and increased 5.13–13.50% and 10.92–22.18% for individual B, respectively. *Clostridium* family XI increased 3.50–25.34% in the presence of tetracycline at 40 days for individual C. *tet*O, Q, W, and X were major TRGs in fecal samples. A variable to slight increase of TRGs copies appeared to be related to tetracycline treatment, inter-individual variability, and exposure duration.

B012

Characterization of Root-associated Microbiome Inhabiting in Different Compartments of Tomato Plants

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The composition and structure of microbial communities are differentiated by plant tissues and compartments, which are specific habitats for microbial colonization. To better understand the complexity of microbiomes associated with tomato roots, we characterized bacterial, archaeal, and fungal communities colonizing in different rhizocompartments (rhizosphere and endosphere) and bulk soil collected from 23 greenhouses in different geographic locations of South Korea using the Illumina MiSeq platform. Microbial communities in endosphere with low species richness and diversity were highly variable and less influenced by edaphic factors. We found robust microorganisms enriched in each compartment and those specifically enriched in rhizosphere and endosphere have potentially beneficial effects on plant growth and health. Microbial communities in rhizosphere represented complex network, while those of endosphere showed smaller and simpler network consisted of several keystone taxa. Microbiomes inhabiting in endosphere and rhizosphere possessed more genes involved in membrane transport than bulk soil, implicating that those actively exchange diverse compounds with plants. These distinct features of microbiome in each compartment of tomato roots provide basic knowledge for better understanding of microbial communities associated with plants.

[This work was supported by Rural Development Administration of South Korea.]

B013

Genomic and Phenotypic Analyses of *Methylobacterium* Species Isolated from a Car Air-conditioning System

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Two *Methylobacterium* species, designated as *Methylobacterium* sp. PR1016A and *Methylobacterium* sp. TX0642, were isolated from the air-conditioning system of a car. Genome analysis indicated that relatively larger genome size of strain PR1016A (approximately 7.7Mb) among *Methylobacterium* species (average; 6.6 Mb) was achieved through horizontal gene transfer, evidenced by high copies of insertion sequences and phage integrases in the genome of strain PR1016A. Comparative genomic analyses of exopolysaccharides operons and high-performance anion-exchange chromatography suggested different EPS production from strain PR1016A and TX0642. Strain PR1016A is low biofilm former, but faster grower and strain TX0642 is high biofilm former, but slow grower. Low attachment of strain PR1016A was overcome by presence of strain TX0642. Biofilm quantification and confocal laser scanning microscopy image analyses showed mixed culture produced more biofilm formation. *Agrobacterium* reporter-based study showed that supernatant of strain PR1016A-grown culture has specific quorum sensing (QS) molecules. More enhanced biofilm of strain TX0642 was observed using the supernatant from strain PR1016A-grown culture. Mixed culture has strong resistance against heat and sodium dodecyl sulfate. Our study demonstrated that divergently-evolved two *Methylobacterium* species isolated from the same niches help each other by exchanging QS signals and dual species biofilm to survive under harsh environments.

B014

Microbial Mechanism for Enhanced Methane Emission by the Introduction of *Phragmites australis* in Temperate Tidal Marsh

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Coastal wetlands sequester *ca.* 44.7 Tg of carbon per year globally. Recently, a large area of tidal marsh has experienced many disturbances including plant invasion. In particular, this may alter the size of stable soil carbon and CH_4 emission in tidal marsh. Previous studies found that plant invasion may increase input of carbon substrate resulting in enhanced CH_4 emission. However, the detailed mechanisms how increases in carbon substrate enhance CH_4 emission is not fully illustrated yet. In this study, we analyzed microbial properties in the chrono-sequence of the introduction of *Phragmites australis* in tidal marsh in Suncheon Bay, and conducted structural equation modeling to determine how the introduction alters CH_4 emission. CH_4 emission reached its maximum within 5 years after the introduction, while soil chemistry and microbial community continuously changed until 10 years. Although DOC and soil moisture had significant correlations with CH_4 emission, increases in DOC and soil moisture due to the introduction was not directly connected with the enhanced CH_4 emission. Instead, DOC and soil moisture affected CH_4 emission through the changes in microbial community structure. Especially, a decrease in relative abundance of sulfate reducing bacteria (SRB) may play a substantial role in the decline of CH_4 emission through reduced competition between SRB and methanogens.
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B015

Dual Species Calcium Carbonate-biofilm Formation by Alkalifying *Lysinibacillus boronitolerans* YS11 and Alkaliphilic *Bacillus* sp. AK13

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$CaCO_3$ precipitating (CCP) bacteria prevail in environment but their interspecies interactions within other species remain unknown. In this study, two bacteria, alkalifying *Lysinibacillus boronitolerans* YS11 and alkaliphilic *Bacillus* sp. AK13, were isolated from a shared habitat, rhizosphere of *Miscanthus sacchariflorus*. Growth and pH measurement of single culture and coculture suggested that alkalization by YS11 facilitates the growth of AK13. YS11 was able to induce $CaCO_3$ precipitation (CCP) through pH increase in the proximity with NH_4^+ production. Alkaliphilic AK13 has optimal growth rate at pH 8 (growth up to pH 11), and no growth at pH 6. Continuous monitoring of unbound Ca^{2+} in the supernatant using ISE showed faster CCP in the coculture than YS11 alone. Spatiotemporal observation of biofilm and $CaCO_3$ using CLSM suggested cooccurrence of two species yields enhanced biofilm formation and broader development of CCP within biofilm matrix. FTIR confirmed the presence of $CaCO_3$ within biofilm. Morphology of coculture-CCP checked by FE-SEM suggested compact particle size of $CaCO_3$ from coculture. Change in the saccharide composition of cocultured EPSs were detected using HPAEC. Our study demonstrates that dual species interaction in the environment shapes CCP characteristics as well as higher biofilm formation.
[Supported by a grant (18SCIP-B103706-04) from Construction Technology Research Program funded by Ministry of Land, Infrastructure and Transport of Korean government.]

B016

Isolation of Benzophenone-3 Degrading Bacterium, *Rhodococcus* sp. S2-17, and Characterization of Its Novel Biodegradation Pathway

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Benzophenoen-3 (BP-3) has been widely used in sunscreens and many other consumer products. Widespread use of BP-3 has led to the release of this compound into aquatic environment. It is necessary to investigate the degradation pathways of BP-3 and its derivatives before their exposures to the ecosystems. Previous study demonstrated a high stability of BP-3 during irradiation period of 72 h under sunlight radiation. Therefore, biodegradation could be the main attenuation mechanism in environment. Aim of this study is isolation of strain with a degradation ability of BP-3 and characterization of the biodegradation pathways of BP-3.
Enrichment with BP-3 as a single carbon source was carried out using various environmental samples and final enrichment culture sample were used for isolation of potentially BP-3-biodegrading bacteria. Additional degradation tests clearly revealed that strain S2-17 had an ability to metabolize BP-3. Three metabolic intermediates, 2,4-dihydroxybenzophenone (BP-1), 2,2'-dihydroxy-4-methoxybenzophenone (DHMB) and 2,3,4-trihydroxybenzophenone (THB), were detected by GC/MS analyses and based on the intermediates a following BP-3 biodegradation pathway by strain S2-17 was proposed: i) BP-3 was sequentially converted into BP-1 and THB by O-demethylation and aromatic hydroxylation, respectively; ii) BP-3 was also converted into DHMB by aromatic hydroxylation. This study will provide insights into the BP-3 biodegradation of *Rhodococcus* sp. S2-17.

B017

Extreme Nitrogen Deposition Event Can Change Methane Oxidation Rate in Moist Acidic Tundra Soil in Arctic Regions

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It has been reported that extreme deposition of nitrogen (N) can occur in Arctic ecosystems where over 90% of the annual N deposition were often found in just a few days. Since Arctic ecosystems are typically N-limited, input of extremely high amount of N could substantially affect ecosystem processes. CH_4 is a potent greenhouse gas that has 25 times greater global warming potential than CO_2 over a 100-year time frame. NH_4^+ is known as an inhibitor of CH_4 oxidation while NO_3-show contradictory with inhibition, no effect, stimulating on it in temperate ecosystems. However, effect of N addition on Arctic ecosystems is still elusive.

We investigated inhibitory effects of extreme N deposition on soil CH_4 oxidation in moist acidic tundra. We conducted lab scale incubation experiment with soil samples. In this experiment, high concentration of NO_3^- (50, 100 μg N/g d.w) showed significant inhibitory effects on CH_4 oxidation. Contrary to these results, NH_4^+ stimulate CH_4 oxidation (10, 50, 100 μg N/g soil d.w). We also conducted field experiment. Potassium nitrate(PN) was added *in-situ* with 3 rates: control (0 mg N/m^2), PN20 (20 mg N/m^2), and PN40 (40 mg N/m^2). CH_4 fluxes were measured using a static chamber method. PN added plots exhibited a lower CH_4 flux than those in control plots, suggesting increased CH_4 oxidation by N additions.

B018

Investigation and Monitoring of Norovirus from Agricultural Water and Fresh Produce in Korea

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Norovirus are the leading causative agent of foodborne disease and the predominant etiological viral agent of acute gastroenteritis worldwide. And fresh produce is known as the primary vehicles of norovirus outbreak. The quality of the water used in irrigation and postharvest washing should be monitored to minimize the potential of cross-contaminating fresh produce. This study was performed to investigate the contamination levels of norovirus in agricultural water and fresh produce. The water samples from 50 facilities and 54 products were collected and tested for norovirus genogroups I and II, respectively. Virus particles were concentrated using centrifugation, viral RNA was subsequently extracted, and transformed into cDNA by reverse transcription.

In this study, one case of noroviruses (GI-2 and GII-4) was detected at the stream water for cultivation. Genotypes of detected noroviruses were reported from previous studies in Korea. The safety assessment system including examination of viruses causing food poisoning is required to obtain the safety of agricultural products.

[Supported by grants from NAQS in 2017]

B019

Fragile Skin Microbiomes in Megacities are Assembled by a Predominantly Niche-based Process

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Given the higher incidence of skin diseases in more urbanized populations and their associations with the skin microbiome, we questioned how the skin microbiome differs depending on the degree of urbanization. Skin microbiomes of 231 healthy subjects in five large cities in China varied mainly depending on the city's environment and socioeconomic status. The differences among microbiomes could be explained by the predominantly niche-based assembly of the microbial communities, which was supported by a dominance test, β-null deviation, and edge-length abundance distribution. Networks among microbes in larger cities were more fragile, which may contribute to the higher incidence of skin diseases in more urbanized environments. These results suggest that microbial ecological theory can provide a framework for understanding crucial health-associated features of the human microbiome.

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B020

The Role of Microorganisms for Making Moonmilk in Baeg-nyong Cave

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The cave is an important environment preserving past climatic information effectively and maintenance of cave environment needs biotic and abiotic mechanisms. Therefore, it is very crucial to identify the relationship between biotic activity and cave environment. We collected secondary calcite as known as moonmilk from Critical Zone Observatory (CZO) in Baeg-nyong (BN) cave and performed the metagenomic analysis. Through the metagenomics, we found different microbial diversity in calcite soil and two different moonmilk samples. Comparison between soil and moonmilk revealed that different microbial communities are distributed even though soil and moonmilk are composed of the same calcite. Bacteria are more important than fungi for making moonmilk, and we also observed different structures of microbial calcite in dry and wet moonmilk respectively via SEM. Metagenomic data showed that a variety of bacterial species affect the moonmilk formation. We selected some OTUs that were highly existed in dry and wet moonmilk compared to soil samples. It showed that dry and wet moonmilk has a large difference in the microbial community depending on their environment. Taken all together, we suggest different bacterial community affects the formation of different types of moonmilk. In a further study, we will identify molecular mechanisms responding to the environment using specific bacteria species.

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B021

Gammaproteobacterial Acidophilic Methanotroph from Sohwangbeyongsan Peat Bog is Capable of Commetabolic Degradation of Chlorinated Solvents

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Methanotrophs have long been studied for applications in bioremediation of soils and groundwater contaminated with chlorinated solvents. Reductive dechlorination using *Dehalococcoides* is the standard protocol in the industry; however, application of reductive dechlorination to acidic soils is not feasible, as vinyl chloride (VC) reduction cannot be carried out in environments with pH < 6.0. Here, we propose cometabolic degradation using acidotolerant methanotrophs for removal of chlorinated ethenes from acidic environments. A moderately acidotolerant methanotroph isolate was isolated from peat samples collected from *Sohwangbyeongsan* bog in Mt. *Odae*. Enrichment of the peat sample with CH_4 in diluted nitrate minimal salt medium adjusted to pH 5.0 and subsequent isolation procedure using the dilution-to-extinction method resulted in successful isolation of a *Gammaproteobactia* methanotroph. The genome of the isolate was sequenced using PacBio sequencing. The chlorinated ethene-degrading capability of the isolated strain was examined by incubating the isolated methanotroph with 20% CH_4 in the headspace and VC at initial dissolved concentration of 50 μM. CH_4 and VC concentrations were monitored until the reactions were complete. The isolated Gammaproteobacterial methanotroph was closely affiliated to *Methylomonas* sp. M5 (95% 16S rRNA gene sequence identity; 97% *pmoA* amino acid sequence identity).
[Supported by grants from NRF (Award 2015M3D3A1A01064881)]

B022

Bovine Intestinal Microbiota

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In the current study, metagenomics and metabolomics approaches were used to evaluate the intestinal microbiota and host metabolism of male, castrated male (CtM), and female Korean brown cattle (n = 30), the Hanwoo, and Holstein Friesian cattle (n = 15). In postpubescent CtM cattle, the animals possessed distinct rectal microbial and serum metabolic profiles, with significantly higher levels of serum branched-chain amino acids (BCAAs) than age-matched male controls. Microbial and metabolic profiling of the different intestinal compartments of adult CtM Hanwoo (n = 10) revealed a strong positive correlation of the serum BCAA levels and the presence of bacteria representing two unclassified genera from the family *Peptostreptococcaceae*, and the genera *Butyrivibrio*, and *Acetitomaculum*, in the small intestine. In addition, the microbial communities of the small intestine and the large intestine of adult male and CtM (n = 20) showed distinct differences. Especially, the increase of *Peptostreptococcaceae* in the small intestine of CtM were remarkable. Fresh striploin meat from CtM carcasses (n = 5 for each) was characterized by higher intramuscular fat accumulation, with significantly higher levels of several amino acids (including BCAAs) and ketone bodies (i.e., β-hydroxybutyrate) than male carcasses. Thus, the data suggest a link between male castration, alterations of the intestinal microbiota, and systemic amino acid metabolism in ruminants.
[Supported by NRF-2016R1E1A1A02921587]

B023

Seasonal Monitoring of Bacterial DMSP in Gwangyang Bay

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The present study estimated biotic and abiotic factors from riverine to coastal waters with seasonality near Gwangyang Bay, Korea. The aim of present study is to know how bacterial habitats are in response to environmental heterogeneity in the coastal system of Gwangyang Bay. In our results, bacterial diversity showed that the habitats from riverine to coastal waters are clustered along spatio-temporal variability, and this pattern was clearly matched with the water mass mixing over the estuary. We found that local bacterial assemblages in response to seasonal variation in Gwangyang Bay, and the sequences of Alteromonadales, Rhodobacterales were especially occurred in Fall and Spring seasons, respectively. In contrast, SAR11 sequences were ubiquitously occurred in all seasons, and the separation between riverine and coastal waters was significantly explained by the distribution of SAR11. The present study estimated the fate of dimethylsulfoniopropionate (DMSP) in Gwangyang Bay by using a dmdA gene detection, and found that the occurrence of dmdA gene from riverine to coastal waters is linked to the distribution of SAR11, suggesting that population dynamics of a bacteria indicator can represent DMSP cycle in coastal zone.
[This research was supported by 'Long-term Marine Ecosystem Research' funded by the Ministry of Oceans and Fisheries, Korea.]

B024

Influence of the Flowrates of the Salinity Gradient Water to Reverse Electro-dialysis (RED) Stack on Performance and Electrochemistry of a Microbial Reverse-electrodialysis Cell (MRC)

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A microbial reverse-electrodialysis cell (MRC), which is the association of a microbial fuel cell (MFC) and a reverse electro-dialysis (RED) stack, can simultaneously treat organic wastewater and generate electricity from salinity gradient. The RED flow rate plays an important role in operating an MRC because it closely relates with energy production rate and economic feasibility. However, influence of RED flow rate on MRC electrochemistry and power production have not been investigated. For this reason, this study purposed to assess optimum RED flow rate. Four flow rates at high and low concentration solutions were tested. By mere increasing the flow rate to MRC, maximum power and optimum current densities increased by 17.7% and 16.2%. EIS results illustrated impedances of anode, cathode and full-cell decreased by 51%, 31%, and 19%, respectively. CV anode test displayed that peak current density increased by 25.7%. To enter into details, the highest optimum current density was 5.36 A/m2 when the flow rate was 7.5 ml/min and the highest maximum power density was 3.71 W/m2 when the flow rate was 10 ml/min. In addition, COD removal and CE were not affected by RED flow rate. In the power generation comparison, there was no significant difference between the RED flow rates of 7.5 ml/min and 10 ml/min. Hence, considering energy production, energy efficiency and energy recovery, the RED flow rate of 7.5 ml/min is a reasonable choice for MRC operation.

B025

Influence of Brush-anode Configurations on Performance and Electrochemistry of a Microbial Fuel Cell (MFC)

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Microbial Fuel Cell (MFC) is a future energy-driven wastewater treatment system. MFC is supplied with energy by using exoelectrogen attached to the anode without a power supplier. For the improved MFC performance, optimization of the system is essential. In particular, the electrochemical properties of the anode and cathode arrangement are optimized by simple and precise analysis. In this study, anode-cathode arrangement (horizontal, vertical) and distances were tested in a single chamber cubic MFC. 1) horizontal closed (H1), 2) horizontal normal (H2), 3) horizontal far (H3), 4) vertical closed (V1), 5) vertical far (V2). The arrangement of electrodes improved the maximum power density and maximum current density. The horizontally-positioned anode configuration (H1) with the closest anode-cathode distance produced the highest power density and current density than vertically-positioned. H1 type EIS showed the lowest anode impedance and full-cell impedance by 60% and 49%, compared to vertically position. 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 MFC electrochemistry.

B026

Cathodic Current Collector Improves Performance in a Microbial Fuel Cell (MFC) by Reducing Cathodic Charge Transfer Resistance

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Microbial fuel cell (MFC) is remarkable environment energy system that converts biomass energy in wastewater into electrical energy. There are many limitations in the practical use of MFC as a wastewater treatment process. Among them, improving cathode performance is one of the significant problem, and introduction of a current collector way to improve cathode performance. In this study, a single-chamber cubic MFC was tested with three current collectors made of stainless steel mesh (SSM) with different contact areas (P 1 cm^2, PC 4.3 cm^2, PM 6.5 cm^2) were tested. Increasing the contacting area enhanced the current generations, power, coulombic efficiency and energy recovery by mainly decreasing cathodic charge transfer impedance. Application of the SSM to the cathode (PM) improved optimum current density, maximum current density, and maximum power density by 3.6%, 6.7%, and 8.8%, respectively. These enhancements in PM are possibly due to a SSM facilitating efficient electron transport and effective oxygen reduction reaction on the surface of the SSM. Application of a current collector decreased charge transfer resistance significantly, but gave negligible effect on ohmic impedance.

B027

Comparison of Performance and Electrochemistry of Anodes with Different Structures and Materials in a Microbial Fuel Cell (MFC)

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Microbial fuel cells (MFC) are sustainable wastewater treatment systems that process wastewater and produce electricity at the same time using exoelectrogen bacteria attached to the anode. And 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, the difference in influence according to anode structure, which is between carbon fiber brush and carbon cloth, was compared and analyzed by Lab scale. Anode were tested in four types: Full Cloth (FC), Full Cloth Electrode Assemblies (FCEA), Full Brush-Horizontal (FB-H) and Full Brush-Vertical (FB-V). Electrochemical analysis has measured polarization curve, CV and EIS. Polarization curve measurements showed the highest maximum power density (1,034 mW/m^2) in FB-H, which is 72% higher than the lowest FC (601 mW/m^2). CV measurement results showed the highest current production (4.5 mA) in FC. EIS measurement results showed the lowest internal resistance value (23Ω) in FB-H. In order to improve MFC performance by modifying anode structures, we suggest the followings: 1) an anode should have large surface area, 2) anodic carbon material and a metal current collector must be tightly connected, 3) locating a brush anode closer to a cathode can be important.

B028

Enhancement of Anodic Performance Enhancement by Modifying Anodic Current Collector in a Microbial Fuel Cell (MFC)

Hyungwon Chai, Junhyuk Kim, and Sokhee Jung*

Department of Environment and Energy Engineering, Chonnam National University

Microbial Fuel Cell (MFC) is environmental energy system that convert the energy contained in organic wastewater into electrical energy by microbial catalysis. MFC generally consists of two electrodes, anode and cathode. Exoelectrogenic bacteria attached to the anode in a biofilm form produce electrons, protons in the process of oxidizing the organic matter. High-performance electrode materials make it possible to achieve high power generation of MFC systems by reducing internal resistance. Carbon-based materials are generally used for the MFC anode electrode, but their conductivity is much lower than metal materials. We investigated the influence of the anode current collector area in MFC performance. In this study, it was hypothesized increasing metal current collector areas improve anodic performance. Carbon-felt anodes with titanium wires (CF-W) and stainless steel mesh (CF-M) were tested. In conclusion, the CF-M had a larger current collect than the CF-W. In the IV polarization test, maximum power density, maximum current density and optimum current density were 33%, 34%, and 30% higher in CF-M (2,311 mW/m^2, 16,815 mA/m^2 and 7,651 mA/m^2) than CF-W (1,737 mW/m^2, 12,566 mA/m^2, and 5,874 mA/m^2), respectively. Anodic polarization resistance and full-cell internal resistance were 81% and 21% lower in CF-M (3Ω and 53Ω) than CF-W (16Ω and 67Ω), respectively. However, we should address the problems of microbial growth and inhibition of adhesion on the carbon felt.

B029

Influence of Different Numbers and Horizontal and Vertical Arrangements of the Brush Anodes on Performance and Electrochemistry of a Microbial Fuel Cell (MFC)

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To maximize wastewater treatment and energy production, it is important to design optimal anode arrangement. In this study, different number and arrangement of the brush anodes were tested in an MFC. As the number of the brush anodes increased in the horizontal arrangement to the cathode, power and current increased and anodic ohmic and charge-transfer impedance decreased. Adding the second brush anode caused great decrease in anodic impedance. In vertical configuration, adding the second brush anode caused great performance enhancement and great decrease in anodic ohmic and charge-transfer impedance, but negligible effects were observed in three and four brushes. In CVs, current production increased as the number of the brush anodes increased in the both arrangement. Different anode-cathode distance might cause heterogeneous anode performance in the vertical arrangement, leading to no increasing performance in three and four brushes.

B030

Increasing Power Production of Microbial Reverse-electrodialysis Cells (MRCs) by Adjusting Intermembrane Distance and Membrane Effective Area for Ion-exchange

Junhyuk Kim, Hyungwon Chai, and Sokhee Jung*

Department of Environment and Energy Engineering, Chonnam National University

Microbial reverse electrodialysis cell (MRC) is an environmental energy system combining microbial fuel cell (MFC) and reverse electrodialysis (RED) stack, to generate electricity from salinity gradient of seawater and freshwater and organic wastewater with simultaneous treatment. To increasing power generation and treatability of wastewater, intermembrane distance (ID) and effective area for ion-exchange (EA) of the RED stack were adjusted and tested in this study. Enlarging EA increased power production. These results show that the MRC performance is improved with increasing flowrate of the saline and the less-saline water into the RED stack and enlarging membrane effective area. However, when inter-membrane distance reduced, the MRC performance is decreased. Considering all aspects of power, current and resistance, it is considered that 1.0 mm-12 cm 2-5 ml/min configuration is the optimal configuration.

B031

The Effects of Methanobactin Secreted by *Methylosinus trichosporium* Strain OB3b on N_2O Reduction in Denitrifiers

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Korea Advanced Institute of Science and Technology

Methanobactin (Mb) is a Cu chelator secreted by methanotrophs for scavenging of Cu from Cu-deficient environments. Cu scavenging may result in deprivation of Cu from the environment and thus, may have a negative impact on the biogeochemical reactions that require Cu for their activities. N_2O reduction mediated by nitrous oxide reductase (N_2OR) is one of such Cu-dependent reactions, which has an environmentally significant function of reducing N_2O emissions. Here, we show that methanobactin secreted from *Methylosinus trichosporium* OB3b inhibited N_2O reduction in denitrifiers.
When *Pseudomonas stutzeri* DCP-Ps1 was cultivated in co-cultures with *M. trichosporium* OB3b or with purified methanobactin from *M. trichosporium* OB3b, stoichiometric N_2O accumulation was observed from NO_3^- reduction, while no significant N_2O production was observed in cocultures with a mutant (*ΔmbnAN*) defective in methanobactin production. In the presence of purified methanobactin, copper uptake by *P. stutzeri* DCP-Ps1 was inhibited with leading to a significant down-regulation of nosZ transcription. Use of soil enrichment inoculum in place of the axenic culture yielded similar results, as permanent accumulation of >80% of NO_3^--N as N_2O-N was observed. Our results suggest that proliferation of methanotrophic activity may have significant implications in soil nitrogen cycling and greenhouse gas emissions in CH_4 and N-rich environments.
[Supported by grants from NRF (2017R1D1A1B03028161,2015M3D3A1-A01064881)]

B032

Resistome of Environmental Antibiotic Resistant Bacteria in Han River

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Environmental resistome has been considered as a major reservoir of antibiotic resistant genes (ARGs) and the resistome of environmental bacteria were shown to be shared with the pathogenic resistome in many studies. We conducted a culture-based survey of environmental antibiotic resistant bacteria (ARB) in Han River to explore the diversity and intensity of resistance phenotypes and characterize factors shaping the environmental resistome. In the year of 2016 and 2017, we collected 2,904 ARB from various locations in Han River using selective media containing 10 different antibiotics. All isolates were identified by 16S rRNA gene sequencing and tested for antibiotic susceptibility by the disk diffusion assay against 18 different antibiotics. *Acinetobacter*, *Pseudomonas*, and *Aeromonas* were the most abundantly isolated genera. Resistance phenotypes of isolated ARB were more correlated with bacterial phylogeny than geography. Interestingly, *Aeromonas* isolates from downstream/urban sites displayed distinctive and stronger resistance profiles compared to the isolates from upstream/pristine sites. Our study suggests that the phylogenetic background is a major determinant of environmental resistome, while anthropogenic influence could selectively act on specific members of ARBs.
[This work was supported by a grant from the Korea Ministry of Environment as “the Environmental Health Action Program (2016001350004)”.]

B033

Antibiotic Resistome and Mobilome in the Urban Sewer System

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Anthropogenic activities have been considered to have significant impacts on environmental resistome and hence the urban sewer system might be involved in the discharge of antibiotic resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) from human to environment. In the present study, Illumina high-throughput sequencing was employed to investigate the microbiome, resistome, and mobilome in the wastewater samples from various parts of a municipal sewer system: two untreated hospital wastewaters, six intercepting sewer contents, and two sludge samples in wastewater treatment plant (WWTP). Metagenomic analysis revealed that resistance against beta-lactam, aminoglycoside and polymyxin was prevalent throughout the sewer system. Among ARGs, *sul1*, *qacH*, and *pmrE* showed the highest abundance. Notably, novel sequence types distantly related to blaKPC-1 and blaKPC-2 were detected in some sewer samples. Hospital wastewaters contained the highest relative abundance of ARGs. WWTP sludge samples contained the lowest relative abundance of ARGs. The abundance of plasmids and integrons was found to be associated with ARGs. Intercepting sewers shared many ARGs each other, while ARGs from WTTP samples were distinct from other samples. Our results suggest that sewer systems convey highly diverse resistome including clinically significant ARGs.

[This research was supported by a fund from Korea Centers for Disease Control and Prevention]

B034

Comparative Genomics of Sulfonamide Resistance in Sulfonamide-degrading Actinobacteria

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Bacterial resistance to sulfonamides mainly occurs because of mutations in *folP* gene encoding dihydropteroate synthase (DHPS) involved in nucleotide biosynthesis, or through acquisition of alternative DHPS genes (*sul* genes). Recently, novel flavin-dependent sulfonamide monooxygenases (SulX) from several sulfonamide-degrading actinobacteria have been characterized to be responsible for resistance to sulfonamides as well as the initial cleavage of the drugs. Comparative genomic analysis revealed that *sulX* gene clusters were highly conserved in a genomic island shared among sulfonamide-degrading actinobacteria, all of which also contained *sul1*-carrying class 1 integrons in another genomic island. Codon usage and GC content analyses suggested that the acquisition of *sulX* gene cluster may have been acquired at later stages of evolution. Our results implied that the sulfonamide metabolism may have evolved in sulfonamide-resistant bacteria which had already acquired the class 1 integron under the sulfonamide selection pressures. Furthermore, the presence of mobile genetic elements associated with the *sulX* gene cluster indicates potential mobilization of the resistance. This is the first study to report that *sulX* is prevalent in sulfonamide-degrading actinobacteria and its genetic signatures indicate horizontal gene transfer of the novel resistance determinant.

[This work was supported by a grant from the Korea Ministry of Environment as the Environmental Health Action Program.]

B035

Regional Comparison of Bacterial Communities Associated with Marine Sponges, *Halichondria oshoro*, *Halicona (Reniera) permollis* by the PCR-DGGE

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The regional difference of bacterial diversity and community structures associated with the marine sponge *Halichondria oshoro* and *Halicona (Reniera) permollis* collected from South sea Goseong and Jeju Island in 2017, were compared by the PCR-DGGE based on the incultivation method. As a result of this study using four *Halichondria oshoro* and two *Halicona (Reniera) permollis*, although there are regional differences, all the bacterial community structure of Halichondria oshoro was composed of 1 phylum, 2 classes : *Proteobacteria* (*Alpha-*, *Gamma-*). Also, the bacterial community structure of *Halicona (Reniera) permollis* collected from different each other site was same and was composed of 2 phylum, 4 classes : *Proteobacteria* (*Alpha-*, *Gamma-*, *Beta-*), *Cyanobacteria*.

The dominant bacterial group of *Halichondria oshoro* belonged to *Alphaproteobacteria* and *Gammaproteobacteria* and The dominant bacterial group *Halicona (Reniera) permollis* belonged to *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Cyanobacteria*. This result revealed that bacterial community profiles of the sponges were host species-specific. There are not regional differences of bacterial community structure of between same sponges.

B036

Maritalea myrionectae HL2708#5 that Produces Cosmetic Compositions

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Herbal extracts as cosmetic component are gaining footlights, and we tried to use marine lava seawater microbes were chosen by esculinase test. Esculinase-positive *Maritalea myrionectae* HL27080#3 showed potential candidate for bio-conversion of herbal extracts for cosmetic industry. Inhibition of melanogenesis and anti-atopic dermartitis could be estimated for herbal extracts from *Clerodendrum trichotomum* Thunberg and *Sambucus williamsii* var. *coreana* (Nakai) Nakai once they were fermented with HL27080#3. Here we report the complete genome sequence of *Maritalea myrionectae* HL27080#3 which has a size of 3,703,346 bp including one chromosome and two plasmids (G+C content of 52.4 %). Protein-encoding sequences (CDS) was 3,696 in numbers and noncoding genes 40 tRNA and 6 rRNA genes. The genome contains a β -glucosidase gene (*bglX*) for esculin hydrolysis, and two homologs of aromatic L-amino acid decarboxylase for tyrosine and dopamine metabolisms.

B037

Microbial Community Responses of Herbivorous Rotifer, *Brachionus calyciflorus*, to P-limited Food

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Global change involves modification to biogeochemical cycles of essential elements such as phosphorus (P). Rotifers, one of smallest metazoan, are raised in the aquaculture industry as a food of fish. We investigate microbial community responses associated with rotifers, *Brachionus calyciflorus*, to P-limited food. Two clones of the rotifer *B. calyciflorus* were obtained from the resting egg banks of one Dutch lakes (D12 and D61). The design of the batch culture experiment consisted of 20 units, i.e. 2 clones (D12 and D61) × 2 food quality treatments (high P and low P) × 5 food replicates. Here, we assessed bacterial composition of zooplankton (rotifer), phytoplankton and bacterioplankton using miseq method based on 16S rRNA gene. A result showed that bacterial communities were significantly different from between phytoplankton, bacterioplankton and zooplankton. These bacterial community were significantly different between P-deficient and P-replete algal food. Genus *Chryseobacterium* was dominated in rotifers associated microbiome. Among rotifers associated microbiome, genus *Pseudomonas* was dominated in only high P food. Rotifers associated microbiome of strain 12 with low P-food was significantly similar with planktonic bacterial community in food chemostat. This study demonstrates that zooplankton might adapt to shifting elemental balances through changes of their microbial communities.

B038

Ecological Genomics of Newly Cultivated Lineages of Family *Halieaceae* (OM60/NOR5 Clade)

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Cells of OM60/NOR5 clade have been detected in high abundance in the surface of coastal ocean, while only a small number of cultured strains from the clade have been investigated. Despite the limited number of cultures and genomes available, comparative genomics and physiological experimentation have shown that members of the OM60/NOR5 clade possess diverse metabolic potential, including bacteriochlorophyll-based anoxygenic phototrophy, chemoheterotrophy, and rhodopsin-based phototrophy. Recently, the novel family *Halieaceae* affiliated within novel order *Cellvibrionales* was proposed to accommodate newly isolated marine gammaproteobacteria belonging to the OM60/NOR5 clade. Here, we report on 6 high-quality genomes of *Halieaceae* strains that were obtained from two different seas (Yellow Sea and East Sea, South Korea) by dilution-to-extinction culturing. Average nucleotide identity and average amino acid identity analysis suggests a clear designation of six strains into six related, but distinct genera within the *Halieaceae* family. The global occurrence of the studied genotypes was also assessed using the Tara Oceans metagenomes. These data set allows for a comparative genomic analysis of representatives from different genera on a species/sub-species level and, moreover, provides insights into diversification and potential niche separation within co-existing *Halieaceae* populations.
[Supported by grants from the Marine Biotechnology Program funded by the MOF, Korea]

B039

Role of Jeotgal, a Korean Traditional Fermented Fish Sauce, in Microbial Dynamics and Metabolite Profiles during Kimchi Fermentation

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In this study, we investigated the effects of jeotgal (fermented fish sauce) on kimchi fermentation using with or without saeu-jeot and myelchi-jeot. Bacterial community analysis showed that *Leuconostoc*, *Weissella*, *Lactobacillus*, and *Tetragenococcus* were the dominant genera; however, their succession depended on the presence of jeotgal. *Leuconostoc gasicomitatum* was the dominant species in kimchi without jeotgal, whereas *Weissella koreensis* and *Lactobacillus sakei* were the dominant species in kimchi with myeolchi-jeot and saeu-jeot, respectively. Metabolite analysis using ^{1}H-NMR showed that the amounts of amino acids and gamma-aminobutyric acid (GABA) were higher in kimchi with jeotgal. Increases in acetate, lactate, and mannitol depended on fructose consumption and were more rapid in kimchi with jeotgal. Moreover, the consumption of various amino acids affected the increase in kimchi LAB. Thus, the role of jeotgal in kimchi fermentation was related to enhancement of taste, the amino acid source, and the increase in levels of functional metabolites.

B040

Variations of Skin Microbiota by the Geographical Locations, Outdoor Activities, and Sunblock Usages during Trips to Antarctica

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Skin microbiota can be reshaped by various potential factors such as extreme climate exposure, cosmetics usage, individual's activities, and residential surroundings. However, there are few precisely defined potential factors which may also be related to the variations or resilience of skin microbiota. Here, two healthy subjects had traveled to Antarctica in which has its unique and extreme environmental features with low temperature below zero Celsius, strong winds, high levels of ultraviolet radiation, and desiccated climate. Such unique environments in Antarctica can lead alterations of skin microbiota. In order to understand the variation of skin microbiota when exposed to extreme environmental changes, time series skin samples were collected during trips and stored until study. Nonculture-based method (16S rRNA gene analysis) was used to explore microbial communities with Quantitative Insights Into Microbial Ecology (QIIME) pipeline. Geographical locations, cosmetic product (sunblock), and indoor/outdoor activity information were applied to analyze skin microbial communities. These results suggest that (i) skin microbiota are distinct across geographical locations, sunblock usage, and indoor/outdoor activities and (ii) completely different skin microbiota are reshaped after subjects returned to their hometown.

B041

Establishment of a New Strategy against *Microcystis* Bloom Using Newly Isolated Lytic and Toxin-degrading Bacteria

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Unwanted, rapid increases in the algal populations of water systems cause harmful algal bloom, which has recently become a major environmental problem. *Microcystis aeruginosa* is the most prevalent bloom species and is responsible for the majority of blooms in freshwater environments. In this study, we attempted to develop an eco-friendly method to suppress *M. aeruginosa* bloom based on a biological control using bacteria newly isolated from the soil. In a screen for bacteria with strong lethal activity toward *Microcystis*, we isolated *Bacillus* sp. T4 and characterized its algicidal activity. *M. aeruginosa* cells were killed via indirect attack by compound(s) secreted by T4 bacteria. ELISA revealed a dramatic increase in extracellular microcystins in *M. aeruginosa* cultures upon treatment with T4. Therefore, we screened for bacteria that could degrade these toxins, and three new isolates (R12, S42, and S65) were identified. Simultaneous application of both T4 as a lytic agent and R12 or S42 as toxin-degrading bacteria could eliminate both *Microcystis* cells and its problematic toxin. Our eco-friendly approach, based on the application of newly isolated bacteria, provides a novel method to control harmful algal blooms.

B042

Comparative Studies on Methods, Membrane Filtration and Most Probable Number by the Colilert-18 System for Detection of Total Coliforms, Fecal Coliforms and *E. coli* in Freshwater

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The aim of this study is to check the routine usefulness of the commercial most probable number (MPN) method called as Colilert-18 system for detecting the fecal indicators in aquatic ecosystem. For the experiment a total of 21 water samples, 19 samples from the middle part of Nak-Dong River, partly used as raw drinking water and 2 samples from its tributary where the effluent of a domestic wastewater treatment plant inflows, were analyzed in duplicate using both the most probable number (MPN) by the Colilert-18 system and membrane filtration (MF) method approved already as the Korean standard methods. The Colilert method was found to be more sensitive and practical than MF method for detection of fecal indicators, total coliforms (TC), fecal coliforms (FC) and *E. coli* obtained from freshwater regardless of degree of its fecal contamination. Based on the results, the easy going Colilert-18 method might be more rapid and feasible method for assessing the actual fecal contamination than traditional methods such as MF.

B043

Culture-dependent and -independent Analyses of Bacterial Assemblages within Microalgae Cultures

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Microalgae are important primary producers in aquatic ecosystems and also biological resources with many industrial uses. Growth of many microalgae are known to be dependent on co-occurring bacteria, suggesting the importance of interaction between microalgae and heterotrophic bacteria. Here, we analyzed bacterial assemblages within cultures of four microalgae (*Tetraselmis* sp. KCTC 12236BP and KCTC 12432BP, *Dunaliella tertiolecta* LB999, and *Synechocystis* sp. PCC6803), using culture-dependent and -independent methods. Amplicon sequencing showed that bacterial communities of the microalgal cultures were dominated by several genera, such as *Oricola* (19% of amplicon sequences; *Tetraselmis* sp. KCTC 12236), *Algoriphagus* (56%; *Tetraselmis* sp. KCTC12432), *Winogradskyella* (55%; Dunaliella), and *Mesorhizobium* (64%; Synechocystis). Several bacterial strains isolated by dilution-to-extinction culturing and dilution plating were found to represent bacterial assemblages of the microalgal cultures. For example, 16S rRNA gene sequence of a *Winogradskyella* strain isolated from Dunaliella culture showed >99.5% identity with 46% of the amplicon sequences from the same culture. A *Mesorhizobium* strain from Synechocystis culture also had 16S rRNA gene sequence nearly identical to 56% of amplicons. Further studies on these microalgae and bacterial isolates would contribute to our understanding on microalgae-bacteria interaction.
[Supported by grant from the Marine Biotechnology Program funded by the MOF]

B044

Environmental Characteristics of Host Species Act as Primary Determinant for Gut Microbiome of Fish

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Fish harbors about 28,000 species, which is greater species diversity than any other group of vertebrates. However, during last decades, there have been plenty of investigations only on mammal's gut microbiota. Here, we comprehensively characterized fish gut bacterial compositions of 230 individuals belonging to 14 orders, 42 families, 80 genera and 82 species, using 454 pyrosequencing of 16S rRNA genes. In total, 1,014,240 sequence reads were obtained, identifying 3,273 operational taxonomic units (OTUs) at a threshold of 97 % sequence identity (in average 90.6±5.5 OTUs per sample). Intriguingly, the fish gut microbiota was dominated by Proteobacteria (51.68%) and Firmicutes (13.54%). Overall fish gut bacterial communities are different from the other vertebrates such as reptiles (ANOSIM; $R = 0.628$, $p < 0.001$), birds (ANOSIM; $R = 0.581$, $p < 0.001$) and mammals (ANOSIM; $R = 0.800$, $p < 0.001$). Specifically, significant differences were found in gut microbiota of freshwater (FW) and seawater (SW) fishes (ANOSIM; $R = 0.287$, $p < 0.001$). In this regard, this fish-class-spanning investigation of the gut microbiota provides insights into the relationships between fish and their gut bacterial communities.
[This work was supported by a grant from the Mid-Career Researcher Program (NRF-2016R1E1A1A02921587) through the National Research Foundation of Korea (NRF) Republic of Korea.]

B045

Impact of Hydrothermal Vent Plumes on Microbial Community of Mid Indian Ocean Water Column Environment

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Depth distribution of microbial diversity in water columns of Indian Ocean around hydrothermal vent area was investigated. Seawater samples were obtained from 3 different area of mid-Indian Ocean by 500 m depth interval to near bottom layer and concentrated on membrane filter for further analysis. Amplicon sequencing was conducted after extraction of DNA from filter paper, amplification of 16S rRNA gene, and attaching tag sequences. Predominant bacterial genera in the surface water were revealed as members of a typical oligotrophic ocean such as *Prochlorococcus, Pelagibacter ubique,* and *Alteromonas macleodii*. Proportion of *Alphaproteobacteria* was substituted by *Gammaproteobacteria* with increase of water depth. However, proportion of hydrothermal environments specific microorganisms was very low even in the water column displaying plume signal, almost no hyperthermophilic microorganisms such as Thermococcus was detected but plenty of sulfur oxidizing *Epsilonproteobacteria* such as the genera *Sulfurovum* and *Sulfurimonas* was detected in the water column displaying plume signal. The result implied that reduced sulfur species were supplied into water column from hydrothermal vent and support growth of sulfur oxidizing bacteria in the mid Indian Ocean Ridge area.
[Supported grants from KIOST & MOF]

B046

A Pesticide-degrading Bacterium Can Mitigate Adverse Effects of Pesticide against Earthworms and Soil Microbial Community

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In the present study we investigated effects of difenoconazole, a triazole fungicide, on earthworms and soil microbial community, and whether a difenoconazole-degrading bacterium *Sphingomonas* sp. strain C8-2 can mitigate the adverse effects. After 5 weeks of incubation in soils contaminated with difenoconazole (4 mg/kg soil), the number and body weights of adult earthworms showed no difference with those in uncontaminated soil but the number of juveniles newly hatched were reduced to 29% of that in uncontaminated soil after 4 more weeks. When strain C8-2 was inoculated, the concentration of difenoconazole decreased from 4 to 1.8 mg/kg soil and the number of juveniles were recovered to that in uncontaminated soil. Contamination with difenoconazole (20 mg/kg soil) did not change the copy numbers of bacterial and archaeal 16S rRNAs in soils for 27 days of incubation but reduced fungal ITS to 4% of that in uncontaminated soil while strain C8-2 decreased the amount of difenoconazole and increased fungal ITS beyond that in uncontaminated soil. Difenoconazole did not change bacterial community structure but reduced the relative portion of a fungal family *Chaetomiaceae* drastically, which however was recovered with inoculation of strain C8-2. This study showed that a fungicide difenoconazole can inhibit reproduction of earthworms and growth of soil fungi, and the presence of a bacterial strain degrading difenoconazole can mitigate the adverse effects.

B047

Gut Bacterial Community of Class *Cephalopoda* Shaped by Host Phylogeny, Diet and Environment

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In contrast to vertebrates, little is known about the factors involved in gut microbiome in invertebrates, especially non-insect invertebrates. Class *Cephalopoda* in the phylum *Mollusca* are only group that have closed circulation system and well-differentiated digestive system to process their carnivorous diet. In this study, we characterize gut microbiota of 6 species of wild cephalopods (cuttle fish, beka squid, nearshore squid, Japanese flying squid, common octopus and whiparm octopus, 5 individuals respectively) by Illumina MiSeq sequencing of 16S rRNA gene amplicons. Each cephalopodal species have unique but shared gut microbiota, by reflecting their host phylogeny. *Photobacterium* and *Mycoplasma* were widely distributed in all cephalopodal guts as core taxa. These genera are differently distributed in olygotype level and their distribution pattern is also associated with host phylogeny. Furthermore, we suggest that host phylogeny, diet and environment are major gut microbiota shaping factors in mollusks.
[This work was supported by a grant from the Mid-Career Researcher Program (NRF-2016R1E1A1A02921587) through the National Research Foundation of Korea (NRF).]

B048

Wastewater from Livestock Industries and Fisheries is the Main Source of Proliferation and Accumulation of Antibiotic Resistance Genes in the Freshwater Environment

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Antibiotics used in livestock are released into the environment and affect the accumulation of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB). In particular, wastewater from livestock industries and fisheries is one of the major contributors to antibiotic contamination in the environment. This study identified the changes in bacterial community and ARGs upstream water, effluent from wastewater treatment and downstream water of the 4 rivers in Korea. The dominant genus in all samples was *Flavobacterium, Limnohabitans, Fluviicola, and Sediminibacterium*. A total of 193 subtypes within 15 ARG types were detected, and the most common types were Sulfonamide, Aminoglycoside, and Tetracycline. Total relative abundance of ARGs increased in the effluent and decreased in the downstream, but relatively higher than upstream. *Arcobacter, Novosphingobium,* and *Acinetobacter* were increased in the effluent, and the pattern of abundance change was similar to that of ARGs. These results indicate that the bacterial community from the wastewater can affect the diffusion and accumulation of ARGs in the downstream environment. Therefore, this study suggests that the intensive use of antibiotics would lead a continuous influx of ARB and ARGs from wastewater into the environment, which would have a significant impact on aquatic environmental contamination and further on human health.
[Supported by grants from Ministry of Environment]

B049

Characterization of Lignocellulose-degrading Potentials in Soil Near the Antarctic King Sejong Station Using Single Molecules Real-time Metagenomic Sequencing

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Lignocellulose, is composed of complex carbohydrates and aromatic polysaccharides, and it is one of the major substance for producing renewable biofuels. Degradation of lignocellulose can be associated with global warming by producing greenhouse gases like methane and carbon dioxide. In concern with on-going global warming, we investigate the degradation of lignocellulose in the extreme environment of Antarctic soil and low temperature specific microbes and catabolic enzymes. We applied metagenomic approaches to taxonomic and functional analysis for the microbes using SMRT sequencing. From a soil near the King Sejong Station in Antarctica, we revealed the dominant members of the phylum Actinobacteria and through a functional gene analysis with CAZy-based annotation identified 381 genes including 117 glycoside hydrolases (GHs) families and 39 auxiliary activities (AAs) families. The CAZymes detected in King Sejong soil (GH5 and GH13) may have low temperature-specific activities and be the candidate of cold-adapted enzymes. The results showed that the genetic capacity of lignocellulose-degrading bacteria was found in extreme Antarctic environments and as global warming progresses, production of carbon dioxide can be facilitated by the activation of lignocellulose-degrading microbes.

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B050

Kraft Lignin Degradation by *Bacillus* sp. Strain K13

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Bacillus sp. strain K13 is a mesophilic spore-forming member of the phylum *Firmicutes*, isolated by enrichment on Kraft lignin (1%) from a compost, South Korea. The strain K13 is capable of growing on lignin and lignin-derived monoaryls as a sole carbon source. To elaborate our findings, we assessed the ligninolytic capability of the strain by studying degradation of lignin-derived monoaryls including coniferyl aldehyde, ferulic acid, vanillin, vanillic acid, sinapic acid, syringic acid, gallic acid, cinnamic acid, *p*-coumaric acid and 4-hydroxybenzoic acid. In addition, decolorization of ligninolytic indicator dyes like, Aure B, methylene blue, malachite green, Congo red and bromophenol blue in strain K13 was also studied. In our results we found that, *Bacillus* sp. strain K13 can degrade nearly all lignin-derived monoaryls especially higher molecular weight compounds and exhibited multiple dye-decolorizing capacities with particular reference for the recalcitrant phenothiazine dye class (Azure B and methylene Blue). The study provides an important basis for lignin degradation by bacteria.

[Supported by Advanced Production Technology Development Program (Project No. 316001-03), Ministry of Agriculture, Food and Rural Affairs in Republic of Korea.]

B051

Biosynthesis of Polyhydroxybutyrate in *Comamonas testosterone* Strain P19 from Aromatic Compounds

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The bacterium *Comamonas testosterone* strain P19 can utilize *p*-coumaric acid, vanillic acid, ferulic acid, protocatechuic acid, gentisic acid, 4-methoxy-benzoic acid and 4 hydroxybenzoic acid as a sole carbon and energy source. Further experiments were carried out to investigate the conversion of aromatic compounds described above into useful bioplastic. The ability of the strain P19 to produce PHB (polyhydroxybutyrate) from those compounds was screened by Sudan black B staining and confirmed by crotonic acid assay method. Results revealed that strain P19 was able to produce PHB along with cell growth using the 2 mM substrates. *p*-Coumaric acid and 4-methoxybenzoic acid showed higher accumulation of PHB in *Comamonas testosterone* strain P19.

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B052

Unique Seasonal Dynamics of Planktonic Archaeal and Bacterial Assemblages in Bays of West Coast of Korea

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The west coast of Korean Peninsula in the Yellow Sea features unique characteristics due to strong tides and nutrient-enriched freshwater outflows from China and Korea. The coupling of archaeal and bacterial assemblages associated with phytoplankton blooms and environmental parameters at two bay areas were investigated. Seasonal variations of archaeal and bacterial assemblages were demonstrated to be greater than spatial variations based on analysis of 16S rRNA gene sequences. The dynamic interactions among archaeal and bacterial populations resulted in unique patterns between abundance and diversity of them. The proportions of sequences of five key taxa, *Thaumarchaeota*, *Euryarchaeota*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria,* comprise ca. 95% of all sequence reads. *Thaumarchaeota* was coincident with high nitrite accumulation in October, indicating comparative dominance of ammonia oxidation to nitrite oxidation. Co-dominance of MGIIb and *Ca.* Nitrosopumilus suggests that assimilation of organic nitrogen by MGII could be coupled with nitrification by ammonia-oxidizing archaea. Distinct spatio-temporal dynamics of archaeal and bacterial assemblages observed in this study may provide insight into Marine microorganisms playing key roles in coastal oceans biogeochemistry greatly affected by tides and inflow of nutrient-rich freshwaters.

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B053

Microbial Communities Associated with Rhizospheres of Pepper and Perennial Ginseng are Distinct from Bulk Soils

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In soils, numerous microorganisms are involved in biogeochemical cycles of elements. Plants can influence on activities and compositions of microbial communities especially around roots via plant-microbe interactions. We compared microbial communities between rhizosphere and bulk soils of pepper and ginseng. Whole DNA was extracted from pepper rhizosphere after 2 and 3 months of cultivation and from rhizospheres of 2- to 6-years old ginseng for analyze microbial composition base on 16S rRNA gene by next-generation sequencing. As controls, bulk soils around the plants were used. Microbial communities in rhizospheres were distinct from bulk soils. Interestingly, rhizosphere microbial communities of ginseng were distinct each year depending on age of growth. Further, the microbial communities of 2-, 4-, 6-years old and 3-, 5-years old roots were separately clustered. Our results demonstrate that microbial communities of plant rhizosphere are distinct from bulk soils and can be changed responding to growth phase and physiology of plants.

B054

Cloning and Characterization of an Extracellular β-1,3-Glucan-degrading Enzyme from *Cellulosimicrobium* sp. DL-13

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The gene (1,650-bp) encoding a new GH64 β-1,3-glucan-degrading glycoside hydrolase (GlnL) from *Cellulosimicrobium* sp. DL-13 was cloned and molecularly analyzed. The acidic protein with a deduced molecular mass of approximately 58.0 kDa was a bi-modular biocatalyst consisted of an N-terminal catalytic GH64 domain and a C-terminal RICN domain, respectively. The GH64 domain was 97% identical to that of *Cellulosimicrobium aquatile* glucan 1,3-β-glucosidase. In this study, to investigate the function of GH64 domain, we purified and characterized rGlnL ⊿RICIN (M.W.: 40.1 kDa) that was overexpressed in *Escherichia coli* BL21. The highest catalytic activity of the enzyme was observed at 45°C and pH 5.5, respectively. rGlnL ⊿RICIN was fairly stable at a temperature below 45°C even after pre-incubation of 1 h, while its thermostability sharply decreased at temperatures exceeding 45°C. Similarly, the enzyme maintained over 90% of its original activity for β-1,3-glucan after pre-incubation of 1 h at a broad pH range of 3.5-10.5. rGlnL ⊿RICIN could efficiently decompose curdlan, pachman, and laminarin but did not exhibit any degradation activity for Avicel, carboxymethyl cellulose, beechwood xylan, and locust bean gum. The biocatalytic activity (540.2 U/mg) of rGlnL ⊿ RICIN for pachyman was approximately 1.7-fold higher than its biocatalytic activity (319.2 U/mg) for laminarin. The present results suggest that GlnL is a new GH64 endo-type β-1,3-glucanase with a unique molecular structure.

B055

Cloning and Characterization of a Cold-adapted Endo-β-1,4-xylanase from *Rugamonas* sp. DR-9

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The gene (1,122-bp) encoding a new GH10 β-1,4-xylan-decomposing glycoside hydrolase (XylZ) from *Rugamonas* sp. DR-9 was cloned, recombinantly expressed, and biochemically characterized. XylZ was a non-modular acidic protein consisting of a single catalytic GH10 domain with a deduced molecular mass of approximately 41.3 kDa. The GH10 domain in XylZ was 86% identical to that of uncultured bacterium BLR13 endo-β-1,4-xylanase. The recombinant enzyme (rXylZ) was most active for beechwood xylan at 40°C and pH 5.5, respectively. Moreover, the enzyme exhibited approximately 12% of its maximum degradation activity for the xylosidic substrate even at 3°C. At 40°C, the half-life of rXylZ was 25 min and its thermostability sharply decreased at temperatures exceeding 40°C. rXylZ was relatively stable at a broad pH range of 3.5–10.5 because it retained more than 80% of its original activity for beechwood xylan even after pre-incubation of 1 h at the respective pH values. The susceptibilities of xylosidic polysaccharides by the enzyme were as follows: beechwood xylan > birchwood xylan > oat spelts xylan > wheat arabinoxylan. The catalytic activities of rXylZ for beechwood xylan and *p*-nitrophenylcellobioside were 274.7 U/mg and 365.1 U/mg, respectively. The enzyme showed relatively strong binding affinity to ivory nut mannan but its binding capacity to lignin was negligible. The results suggest that XylZ is a new cold-adapted GH10 endo-β-1,4-xylanase with distinct catalytic properties.

C001

Studies on Intestinal Colonization and Improved Enterokinesia by Three Layer Coating of *Lactobacillus plantarum* K-1

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Lactobacillus plantarum K-1 (LPK1) was isolated from a Korean fermented food, Kimchi. *L. plantarum* has been well known as an effective probiotics. To achieve beneficial effects continuously, it needs to be colonized in the intestine. Therefore, *L. plantarum* needs coating layer(s) resistant to acidic pH for passing the stomach, because the *L. plantarum* is very weak in acidic pH. In the present study, to investigate the intestinal colonization of LPK1 after three-layer coating with gelatin, casein, and kelp. we measured the stability of delivery of LPK1 from gastrointestinal tract and enterokinesia effects by comparing the intestinal colonization between uncoated LPK1 and three-layer coated LPK1. BALB/C mice were grouped by three experimental conditions; DW, LPK1(LP), and coated LPK1(CLP) groups. All mice were treated with DW, LP or CLP (1×10^8 CFU) once a day for 10 days. After LPK1 administration for 10 days, mice were orally administered with charcoal and white feedstuff, and the enterokinesia was examined at 6 h, 12 h, and 24 h after administration. Stools and the intestine were examined on genomic DNA and the number of lactic acid bacteria using Gram staining. LPK1 gene was detected in ileum and colon of CLP mice for longer than LP mice. The enterokinesia was more improved in CLP treated mice than DW and LP groups. The results of the present study may be helpful to demonstrate the usefulness of three-layer coated LPK1 in the intestinal colonization and improved enterokinesia.

C002

Manufacturing and Utilization of Organic Compost Using Spent Mushroom Substrates of *Pleurotus eryngii*

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By analyzing seed germination indexes of cucumber and radish, lettuce, chinese cabbage, this study aims to evaluate decay degree of compost, which is used by spent mushroom substrates King Oyster mushrooms. In the experimental results, compost using spent mushroom substrates was shown to complete the stabilization process between 4–6 weeks, it was evaluated by the appropriate organic compost maturity for the plantation on the basis of that point. The germination rate and germination index were measured after sowing cucumber, radish, lettuce and Chinese cabbage in order to verify the germination ability of compost. The germination index of cucumber and radish was higher than that of lettuce. In order to investigate SMS production rate and growth characteristics of lettuce, 100% of normal soil and SMS 10%, SMS 20%, SMS 30%, SMS 50%, SMS 70%, SMS 100% and lettuce seedlings were transplanted. First, analysis of chemical composition of soil material was carried out. The higher the amount of SMS added, the higher the total amount of nitrogen. However, when we compared the growth of SMS in lettuce with that of normal soil, weight was similar to SMS 10% 43.9 cm and general soil 46.4 g. Chlorophyll content was also similar to SMS 10% 32.8 and general soil SMS 10% 32.8. Similar results were obtained, but SMS 10% 14.6.

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C003

The TOR Pathway Governs Cell Viability and Pathogenicity of Fungal Meningitis Pathogen *Cryptococcus neoformans*

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Tor1 is a serine/threonine protein kinase that is widely conserved across eukaryotic species. Tor pathway was known as it has been implicated in regulating cellular responses to nutrients, including proliferation, translation, transcription, autophagy, and ribosome biogenesis. Here we identified two homologues of *S. cerevisiae TOR*, CNAG_06642 (*TOR1*) and CNAG_05220 (*TLK1, TOR*-like kinase 1), in *Cryptococcus neoformans*. Both *TOR1* and *TLK1* has rapamycin binding domain but *TLK1* has truncated form of that. To study the TOR1 signaling pathway, we attempted to construct the *tor1* Δ and *tlk1* Δ mutants. But we could construct only *tlk1* Δ mutant. So we confirmed the essentiality of *TOR1* by using a promoter of copper transporter (CTR4) and diploid strain. As a result, *TOR1* is an essential gene for viability in *C. neoformans*. Although we could not construct the *tor1* Δ mutant, we constructed *TOR1* overexpression mutant using a promoter of histone H3 in *C. neoformans*. We found that Tor1 overexpression mutant was resistant to rapamycin but *tlk1* Δ did not affect to resistance of rapamycin. And we also identified that Tor1 is involved in response to diverse stresses including genotoxic stress, oxidative stress, thermo-stress, antifungal drug treatment, and production of melanin.

C004

Aflatoxin B1 Degradation Activity by *Bacillus subtilis* Strain 168 Isolated from *Meju*

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Aflatoxin, known as a group 1 carcinogen by IARC (International Agency for Research on Cancer), is a secondary metabolite produced by *Aspergillus flavus* and *Aspergillus parasticus*. We screened a total of 164 bacilli isolated from *Meju*, a Korean traditional fermented soybean starter, for the reducing ability of Aflatoxin B1 (AFB1). Each of *Bacillus* isolate was cultured in Soytone-NaCl broth at 37°C for 72 h, and the cell free supernatant was used for the AFB1 degradation. The AFB1 reduction ability was analyzed using Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC). *Bacillus* isolate KUFNMS Y63, which showed the highest AFB1 reduction ability, was identified as *Bacillus subtilis* strain 168 by 16S rRNA gene analysis. Crude enzyme prepared from the cell free supernatant of *B. subtilis* strain 168 by ammonium sulfate precipitation (concentration 70%) completely degraded AFB1 (100 ng/ml) at 37°C for 72 h. Aflatoxin B2, G1, and G2 were also degraded by *B. subtilis* strain 168.

C005

Bacterial Diversity Analysis of Korean Commercial Probiotic Products for Quality Evaluation Using Next-Generation Sequencing

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Probiotics are live microorganisms that provide positive effects on the health for humans and animals. In this study, the descriptions of bacterial communities in probiotics were appeared by metagenomic approach using next generation sequencing (NGS) technology on the Ion system. The bacterial gDNA was extracted from forty-two kinds of probiotics and the 16S rRNA genes were amplified using six specific primers targeted on the region of V2, V3, V4, V6-V7, V8, and V9, respectively. Amplicons were sequenced in-house using the Ion Torrent Personal Genome Machine (PGM) and S5 sequencer and analyzed with the Ion Reporter™ software version 5.2. Reads were classified into bacterial 16S rRNA using both the curated MicroSEQ™ ID 16S rRNA reference and the curated Greengenes databases. 86.3 to 100% of valid reads were mapped into the database. The dominant bacteria were *Firmicutes* and *Actinobacteria* at the phylum level. Most bacteria were classified into *Lactobacillus, Streptococcus, Bifidobacterium, Enterococcus* and *Lactococcus* at the genus level which showed similar patterns related to the lot-to-lot differences of bacterial population and all of the bacteria species specified on product labels were detected using the Principal Coordinate Analysis (PCoA). Our study demonstrates that NGS is available tool for examining the bacteria population based on taxonomy analysis and controlling the food quality related to bacteria.

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C006

Characteristic Analysis of *Bacillus* sp. Having Potential Probiotics Activity and Medium Optimization for Improving Biomass by the Response Surface Methodology

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Bacillus has used in various industry such as fermented food, starter, feed, and pharmaceutical industry. And then, they were known as non-toxigenic and non-pathogenic strains including GRAS microbe. We isolated *Bacillus* strains from traditional fermented foods, and finally selected SRCM 100731 by investigation of non-toxin, resistance of acidic and bile condition, antibacterial activity, and cell hydrophobicity. SRCM 100731 was identified as *Bacillus amyloliquefaciens* by 16S rRNA sequencing. Based on preliminary experiments, we optimized culture medium compositions for improving dried cell weight of SRCM 100731 using response surface methodology (RSM). Plackett-Burman experimental design was used for screening of medium constituent, and molasses, potassium chloride, sodium chloride were predicted as important factors for improving cell growth. Through the central composite design, we obtained optimum values as follows: molasses 7.0%, potassium chloride 0.5%, sodium chloride 1.1%, respectively. By model verification, we confirmed that the dried cell weight was increased a 7-fold compared to basal medium from 1.8273±0.0214 g/L to 12.8008± 0.0658 g/L.

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C007

Effect of *Ecklonia cava* Extract on the Growth *Lactobacillus plantarum* SWLP258

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Ecklonia cava is a brown alga (Laminariaceae) that is abundant in the subtidal regions of Jeju Island in Korea. Recently, it has been reported that *Ecklonia* species exhibits radical scavenging activity, anti-plasmin inhibiting activity, antimutagenic activity, bactericidal activity, HIV-1 reverse transcriptase and protease inhibiting activity and tyrosinase inhibitory activity. Lactic ac id bacteria are beneficial microorganisms for the gut health in human as well as animals when consumed. *Lactobacillus* strains are frequently found in the human intestinal tract. Isolation *of Lactobacillus plantarum* SWLP258 from fermented kimchi was carried out in order to study *Lactobacillus plantarum* SWLP258 as probiotics. When 0.1% *Ecklonia cava* extract was added in cultivation, the cell growth of *Lactobacillus plantarum* SWLP258 increased more than 3 times and increased amino acid concentration. Furthermore, the antimicrobial activity of the culture medium of *Lactobacillus plantarum* SWLP258 with *Ecklonia cava* extract was 60% and Antioxidant Activity was 82%.

[Supported by grants from Ministry of Science and ICT]

C008

Isolation of a BTEX-degrading Bacterium, *Rhodococcus* sp. 24, from Nakdonggang and Characterization of Biodegradation Conditions

Hyun Mi Jin, Hye Kyeong Kang, Byung-Gon Ryu, Seung Young Lee, Ji Young Jung, Jin-Woo Jeong , and Kyung Min Choi*

Nakdonggang National Institute of Biological Resources

An enrichment culture was established using freshwater containing BTEX (Benzene, Toluene, Ethylbenzene, o-,m-,p-Xylene) compounds to isolate a BTEX degrading bacterium from contaminated freshwater.

The enriched microbial communities were characterized by culture based ARDRA analysis, which indicated that a *Rhodococcus* species was dominant during the enrichment. Strain 24, able to degrade all BTEX compounds, was isolated and characterized. In addition to its ability to degrade a broad range of single aromatic substrates including BTEX, strain 24 was also able toutilize high amounts of phenol of either up to 1,000 ppm with cell vigorous growth.

Isolate was able to grow in pure culture and in defined mixed culture with other aromatics degrader on phenol compound as a sole source of carbon and energy. NH_4Cl, NaH_2PO_4, cell mass and contaminant concentrations were used as independent variables to optimize the degradation of aromatics by strain 24 in a Nakdong-river and a statistically significant $p < 0.0001$) quadratic polynomial mathematical model was suggested.

C009

Isolation of a Mono-aromatic Ring Compounds Degrading Bacterium, *Microbacterium* sp. 28, from a Freshwater and Optimization of Biodegradation Conditions

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An enrichment culture was established using freshwater containing phenol compound to isolate a phenol degrading bacterium from contaminated freshwater. The enriched microbial communities were characterized by culture based ARDRA analysis, which indicated that a Microbacterium species was dominant during the enrichment. Strain 28, able to degrade phenol compounds, was isolated and characterized. In addition to its also able to utilize high amount of BTEX of either up to 1,000 ppm with cell vigorous growth.
Isolate was able to grown in pure culture and in defined mixed culture with other aromatics degrader on BTEX compounds as a sole source of carbon and energy, NH_4Cl, NaH_2PO_4, cell mass and contaminant concentrations were used as independent variables to optimize the degradation of aromatics by strain 28 in Nakdong-river and a statistically significant ($p < 0.0001$) quadratic polynomial mathematical model was suggested.

C010

Inhibitors of Antigen-induced Degranulation in RBL-2H3 Cells from the *Streptomyces* sp. MJM13788

Chun Whan Choi, Yun-Hyeok Choi, Ji Eun Lee, Jin Kyu Kim, Wonsik Jeong, Changon Seo, Jin Gwan Kwon, Jin A Kim, and Seong Su Hong*

Gyeonggido Business & Science Accelerator (GBSA)

The ethyl acetate(EtOAc)-soluble extract of the liquid-grown culture of *Streptomyces* sp. MJM13788 was found to suppress antigen mediated degranulation of rat basophilic leukemia (RBL-2H3) cells. Three known compounds (**1**–**3**) were isolated from the EtOAc-soluble layer using bioassay-guided fractionation. Their chemical structures were determined based on the spectroscopic data interpretation, particularly MS, 1D and 2D NMR data including HSQC and HMBC. Isolated compounds were identified as phenylacetic acid (**1**), cyclo(L-Pro-D-Leu) (**2**), and cyclo(L-Pro-D-Phe) (**3**). The inhibitory effects of isolated constituents on the release of β-hexosaminidase from RBL-2H3 cells were examined, and compounds **1** and **3** were found to show the inhibitory activity with IC_{50} values ranging between 780.8 and 485.3 μM, respectively.

C011

Studies on Chemical Constituents from *Streptomyces* sp. MJM8088

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Bio-Center, Gyeonggido Business and Science Accelerator

As a result of the Nagoya protocol, it is expected that the securing and utilization of biomass resources will become more difficult. So, it is necessary to invest biotechnology using domestic microbial resources while reducing dependence on overseas biological. Especially actinomycetes are recognized as the most important microorganism in the industry. Because they have a lot of secondary metabolites and the diversity of chemical structures. MJM8088, which we studied, is also a kind of *Actinomycetes, Streptomyces* spp.
MJM8088 is partitioned with various solvents, and we selected ethyl acetate (EtOAc) fraction. Compounds **1**-**8** waere isolated and purified by various column chromatography and HPLC analysis. The structures were identified by analysis of NMR and MS data, along with comparison with those in the literatures. Details of the isolation and structures determination will be presented.

C012

Molecular Characterization of *Weissella cibaria* JC2-3 as a Dextran Producer Isolated from Korean Traditional Kimchi

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A strain of *Weissella cibaria* was isolated from Chonggak kimchi that produced dextran and named as JC2-3. It was characterized in aspects of genetic relationships by random amplified polymorphic DNA PCR (RAPD-PCR) and 16S rRNA sequencing. With primer LAB52, isolates such as *Leuconostoc mesenteroides* JA2-3 and JB1-2 showed exactly same patterns of RAPD-PCR but *W. cibaria* JC2-3 showed a different pattern of RAPD-PCR. With primer LAB65, Both *L. mesenteroides* JA2-3 and *L. mesenteroides* JB1-2 revealed exactly same RAPD patterns however *L. mesenteroides* subsp. *mesenteroides* KCTC 3722 showed more typical RAPD DNA bands. RAPD DNA bands of *W. cibaria* JC2-3 were not the same as those of tested *L. mesenteroides.* This tendency was confirmed by other RAPD primers applied. Thus it is understood that RAPD-PCR is a valuable tool that can distinguish different species of lactic acid bacteria. Also phylogenetic analysis with 16S rRNA sequencing confirmed the results of RAPD-PCR. *L. mesenteroides* JA2-3 and *L. mesenteroides* JB1-2 were grouped into a same cluster and *W. cibaria* JC2-3 was outgroup that was a branch of *W. cibaria*. From results of dextran measurement, *W. cibaria* JC2-3 revealed its maximum dextran production in 20% sucrose (w/v) containing MRS media (3,540 $Pa.S^n$) rather than 30% sucrose (1,900 $Pa.S^n$). However other tested stains of *L. mesenteroides* such as JA2-3, JB1-2 and KCTC 3722 showed no production of dextran in 0–30% sucrose (w/v) containing MRS media.

C013

Inhibitory Activity of *Cladonia furcata* and *Nipponoparmelia laevior* Lichens against Influenza A Virus (H1N1)

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Herein, we investigated the antiviral effect against influenza A (H_1N_1) virus of the extract of 32 untapped species of Korean lichens using the MDCK cell line. Based on the *in vitro* assays, we found that methanolic extract of *Cladonia furcata* and *Nipponoparmelia laevior* showed extremely high activity against influenza A virus (IAV) through various mechanisms, which enhance cells viability and reduce virus replication after influenza virus infections. Lichens treatment showed extremely high activity against IAV, which is significantly higher cell viability (more than 90%); however, relatively low apoptosis and cell death compared to positive IAV-infected cells. Furthermore, the lichens treatment showed a significant reduction (by 9.7 to 11.0 log-fold) of influenza A viral replication with a concentration of 100 μg/ml. The expression of 7 selected genes associated with viral infection was observed during IAV infection; all biomarkers, including annexin proteins (ANXA1 and ANXA2), heat shock chaperones (HSPA4, HSPA5, and HSPA8), the serine-threonine protein kinase (AKT1) and hypoxia-inducible factor 1 alpha (HIF1A) were highly inhibited by lichen treatments. In conclusion, our results show that methanolic extracts of natural lichens highly inhibited IAV replication *in vitro* assays. This knowledge has, in turn, allowed the researchers to further explore the specific molecules from natural lichens for pharmaceutical biomaterials of a new anti-influenza therapy.

C014

Isolation of Biogenic Amine Non-producing *Lactobacillus brevis* SBB07 Having Antioxidant Activity and Medium Optimization to Improve Biomass

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Microbial Institute for Fermentation Industry (MIFI)

Antioxidant are substances that may protect cells from the damage caused by unstable molecules known as free radicals. In this study, 42 strains were isolated from various berries including extract and vinegar, and investigated the biochemical characterization of isolates. SBB07 was selected by results of antioxidant activity and ability of biogenic amine non-producing and identified as *L. brevis* SBB07 by 16S rRNA sequencing. Nextly, we investigated cell growth for application of industrial field, and optimized the culture medium constituents using response surface methodology. Plackett-Burman experimental design was used for screening of medium constituent, and protease peptone, CSP, and yeast extract were selected as important factors. We carried out central composite design for find out optimal concentration on selected constituents. Optimal concentrations of protease peptone, CSP, and yeast extract were predicted to be 2.0%, 2.5%, and 2.0%, respectively. By the model verification, we confirmed about 1.3-fold improvement of the dried cell weight from 2.40 ± 0.01 g/L to 2.97 ± 0.05 g/L when compared to basal medium. Finally, we carried out experiments for establishing culture conditions, and confirmed maximal cell growth 6.25 ± 0.02 g/L at 37°C and initial pH 8.0.

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C015

Change of Microbial Community in Skate (*Raja pulchra*) during Fermentation

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Cham-hong-eo, *Raja pulchra* is a mottled skate which is belonging to the cartilaginous fish. This species has been known as economically valuable fish in South Korea and Japan. Detailed study outlining the functionalities and antihypertensive activity of fermented skates have been mainly performed, but little is known about the microbial community in alkaline fermented skates. In this study, we used the wing parts of the skates (4 females and 2 males) to compare the changes of microbiota before, during and after fermentation. During the fermentation of the skates, pH, total number of bacteria and marine bacteria were increased. These results indicate differences in microbial community during fermentation period. These data will be helpful to used as information for further microbial community analysis.

[Supported by a grant from Marine Biotechnology Program (PJT200620) funded by Ministry of Oceans and BK 21 Plus Program from South Korea.]

C016

Changes in Bacterial Community by Media Component and pH

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Medium is a one of the most important factors in microbiological study. However, there are not fully study for species specific media. In this study, total 6 media (MRS, AB, AC, MRS pH5, AB pH5, AC pH 5) were used for observation to changes of bacterial community in pigs' gut. AB medium was designed with species-specific gene by using genome comparison. Each medium showed different bacterial growth patterns and different bacterial groups had been enriched in each medium. These results provide an efficient method in bacterial isolation and identification.

[Supported by the Strategic Initiative for Microbiomes in Agriculture and Food (Grant ID:914005-04) and BK 21 Plus Program from South Korea.]

C017

Unraveling the Role of Pseudouridylation in a Human Fungal Pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic fungal pathogen that causes cryptococcosis in both immunocompromised and immunocompetent individuals. Due to its clinical importance, revealing the factors that can affect its life cycle is critical. Among the various factors, pseudouridylation of RNA is the most abundant type of post-transcriptional modification. Pseudouridylases isomerize uridine into pseudouridine, therefore can affect the stability of RNA structure. In *Saccharomyces cerevisiae*, 8 proteins exist as stand-alone pseudouridylases, and each protein has specific pseudouridylation sites and roles. To discover the features of pseudouridylases, we aim to identify 6 putative pseudouridylases in *C. neoformans*. We sorted out the enzymes based on the database from FungiDB and NCBI. We used BLAST search with protein sequences to find out any corresponding orthologs in multiple organisms, such as *S. cerevisiae*, *Candida albicans*, *Aspergillus fumigatus* and *Neurospora crassa*. To characterize the function of pseudouridylases, we constructed 10 mutant strains representing 5 putative pseudouridylases and we examined their phenotypic traits under various conditions so far. By using pseudouridylation RNA sequencing, we will identify pseudouridylated RNA transcripts and characterize their role in pathogenicity of *C. neoformans.*

C018

Unravelling of the Melanin Regulatory Signaling Networks of *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic human fungal pathogen. Among its virulence factors, melanin is important to protection against host cellular environment. Melanin production is known to be regulated by several signaling pathways. However, the comprehensive overview of signaling networks regulating melanin production still remains elusive. To analyze melanin-regulating signaling pathways, we employed our phenome database of 155 transcription factor and 129 kinase mutant libraries of *C. neoformans.* First, we select 75 TF and kinase mutants, which exhibit altered melanin production level in niger seed media, confirmed by our phenotypic analysis. Next, we investigate transcriptional levels of each gene at glucose starvation condition, which induces expression of the major melanin production gene *LAC1*. Furthermore, we check melanin production of the selected mutants in different melanin inducing media, such as L-DOPA and epinephrine containing media. Based on the above experiment, we focused on the 13 genes whose deletion showed significant changes in transcriptional levels as well as a clear melanin production defect in all three types of media. These include 9 kinases (*VPS15, GSK3, KIC1*, *CBK1, MEC1, MET3, MPS1, PRO1* and *PKH202)*, 4 TF (*BZP4, USV101, HOB1* and *MBS1)* which are all expected to be positive regulators of melanin production. Our study will provide insights into the regulatory mechanism of melanin-regulating signaling pathways in *C. neoformans*.

C019

Systematic Functional Profiling of Phosphatases in the Human Fungal Pathogen *Cryptococcus neoformans*

Jae-Hyung Jin[1], Dong-Gi Lee[1], Kyung-Tae Lee[1], Yee-Seul So[1], Kwang-Woo Jung[1], Eunji Jeong[1], Yeonseon Lee[1], Dongpil Lee[1], Seung-Heon Lee[1], Jin-Young Kim[1], Eun-Ha Jang[1], Minjae Lee[1], Jaeyoung Choi[2], Anna F. Averette[3], Joseph Heitman[3], Yong-Hwan Lee[4], and Yong-Sun Bahn[1]*

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Cryptococcus neoformans causes fatal cryptococcal meningoencephalitis. Despite its clinical importance, the signaling networks governing its virulence remains elusive and therapeutic options for treatment of systemic cryptococcosis are limited. Here, to understand signaling networks regulating the virulence of *C. neoformans*, we aim to identify and functionally characterize the 139 putative phosphatases, which are major signaling components in the basidiomycete fungal pathogens. We selected putative phosphatases based on annotation in the *C. neoformans* var. *grubii* genome database provided by the National center for Biotechnology Information (NCBI) and performed a BLAST search with their protein sequences to identify any corresponding orthologs in *S. cerevisiae*, *A. nidulans*, *C. albicans* and *F. graminearum*. We classified putative phosphatases into 16 groups based on InterPro phosphatase domain annotation. Thus far, we have successfully constructed 227 signature-tagged gene-deletion strains representing 114 putative phosphatases through homologous recombination methods. We are in the middle of examining their phenotypic traits under 30 different *in vitro* conditions, including growth, differentiation, stress response, antifungal resistance and virulence-factor production. Along with our previous functional genetic studies for *C. neoformans* transcription factors and kinases, this study will provide a comprehensive insight into the fungal signaling networks.

C020

Exopolysaccharide Production and Biofilm Formation in Soil-dwelling *Acinetobacter oleivorans* DR1 under Oxidative Stress

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Many bacteria secrete exopolysaccharides (EPS) that contribute to their nutrient trapping, surface attachment, and protection against abiotic or biotic stresses. Like other *Acinetobacter* species the genome of soil-dwelling *Acinetobacter oleivorans* DR1 contains three distinct EPS operons. Our transcriptomes and quantitative RT-PCR analysis showed that the levels of their expression vary under different EPS-producing conditions. Morphologically distinct biofilms by those abiotic stresses were observed using confocal laser scanning microscopy, indicating involvement of different components in each biofilm formation. The results of RNA sequencing and Northern blot analyses showed that the expression of PNAG1 and K-locus operon genes were highly upregulated under H_2O_2 treatment although genes in the K-locus has constitutively high level of expression. Interestingly, the *Δ oxyR* and *Δ pgaC1* mutants displayed increased production of EPS and higher biofilm formation, but not *Δ pgaC2* and *Δ wzc-wza* mutants which are defective in EPS production from PNAG1 region and the K-locus. Due to the possible replacing role of the K-locus genes products in the PNAG1-knockout background, the *Δ pgaC1* mutant was capable of producing more EPS and biofilm formation. Electrophoretic mobility shift assays using purified OxyR revealed that the H_2O_2-sensing OxyR might be involved in the regulation of those three EPS operons through different degrees of promoter's bindings.

C021

Inhibitory Effect of Bacteriocin Produced by *Lactobacillus brevis* DF01 on the Biofilm Formation of *Escherichia coli* and *Salmonella* Typhimurium

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Biofilm is a bacterial community that attachs to surface of biotic and abiotic materials. Thus, it has become problematic in a wide range of food industries as well as human health. Bacteriocins from lactic acid bacteria are known as antimicrobial peptides and they are usually used to food preservation, but it has not been clearly demonstrated the inhibitory effect of biofilm. In the present study, we investigated that bacteriocin derived from *Lactobacillus brevis* DF01 showed anti-biofilm effect of foodborne pathogens, *Escherichia coli* and *Salmonella* Typhimurium. Bacteriocin of *L. brevis* DF01 significantly inhibited the biofilm formation, while the bacteriocin of *L. brevis* DF01 was less potent for the inhibition of pre-formed biofilms of *E. coli* and *S.* Typhimurium. Furthermore, the inhibitory effect of bacteriocin of *L. brevis* DF01 was confirming by confocal laser scanning microscopy and sanning electron microscopy. In addition, biofilms of *E. coli* and *S.* Typhimurium on the surface of stainless steel were significantly reduced in the presence of bacteriocin of *L. brevis* DF01. These results suggested that bacteriocin of *L. brevis* DF01 can be a natural antimicrobial agent against foodborne pathogens for the use in food processing environment.

C022

Antifungal Activity of Cell-free Supernatants from *Pediococcus acidilactici* HW01 against *Candia albicans*

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Candida albicans is an opportunistic fungal pathogen that is responsible for candidiasis of oral, gastrointestinal and vaginal tracts and it may cause infections. In this study, we investigated whether cell-free supernatants derived from *P. acidilactici* HW01 (HW01 CFS) showed antimicrobial and antibiofilm activities against *C. albicans*. HW01 CFS inhibited the growth of *C. albicans*, whereas bacteriocin, which is a well-known antimicrobial peptide of lactic acid bacteria, failed to inhibit the growth of *C. albicans*. The antimicrobial activity of HW01 CFS was lost at pH 6 to 12, whereas treatment with enzymes including proteolytic enzymes did not affect the antimicrobial activities of HW01 CFS. Moreover, heat treatment of HW01 CFS was not likely to affect the inhibition of *C. albicans* growth. Regarding the antibiofilm activity, HW01 CFS strongly inhibited the biofilm formation of *C. albicans*. Pre-treatment and simultaneous treatment with HW01 CFS showed a significant inhibition of *C. albicans* biofilm. Although post-treatment with HW01 CFS was not able to disrupt the established biofilm of *C. albicans* at early time points, reduced biofilm of *C. albicans* was observed at late time points (12 to 24 h) after post-treatment with HW01 CFS. Taken together, these results suggest that the CFS from *P. acidilactici* HW01 can be used as an alternative antifungal agent to control *C. albicans* infection.

C023

Investigation of Drug-induced Gastrointestinal Side Effects by Comparative Analysis of Gut Bacteriome and Serum Metabolome

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Our previous study using a rat model system indicated that the active ingredients contained in the medications for hypertension, respectively, appeared to induce various bowel problems including constipation and inflammation. It is noteworthy that the probiotic blend tested was found to alleviate intestinal complications caused by medicine. In order to gain more extensive insight on the effects, the present work focused on changes in the microbiota and metabolic level. Specifically, the gut bacteriome was analyzed using fecal DNA to find changes in the gut microbiota and lipid metabolites in the rats were analyzed using serum to look for changes in their metabolism. In gut bacteriome analysis, *Parabacteroides goldsteinii* increased and *Romboutsia ilealis* decreased in amlodipine (AMD)-administered group. They are restored by co-administration of probiotic blend (PB). The AMD-administered group showed that different metabolome patterns with other groups. Some metabolites like sterol derivatives were increased in AMD-administered group, compared to the control group and the AMD+PB-administered group. Several metabolites like ceramides also exhibited significantly decreased in the AMD-administered group compared to the control group and the AMD+PB-administered group. The present work fortifies the hypothesis that dysbiosis is a cause or a contributor to gastrointestinal disorders, although the underlying mechanism is not fully known.

C024

Functions Enhancement through Fermentation of Lactic Acid Bacteria with Korean Traditional Medicines

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Adult (lifestyle) diseases, usually they are caused by abnormal life pattern (especially modern life) and very complicated to treat due to its many different reasons of causes. Targeting based (cell based) treatment works well in a short time, but in a long-term plan it is not enough. Korean traditional medicines and lactic acid bacteria (LAB), they are one of solutions to solve these complex problems. The mechanisms and functions are being clearly explained recently, and they are being generally accepted to use as functional food and medicinal practices.

The aim of this study, is to improve effects of Korean traditional medicine through bio-conversion of its active components by fermentation with LABs. 300 strains of LABs were single isolated from Korean traditional food (Kimchi), and among them, three were new strains, 14 strains had bio-conversion ability on *Illicium verum, Gardenia jasminoides* and some others also. The bio-converted compounds shows activity against adult diseases. This study may provide new insights into bio-conversion of compounds from traditional medicines for potentially treatment of adult disease.

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C025

Unveiling of Complex Signaling Networks Associated with the Developmental Process of *Cryptococcus neoformans*

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The fungal pathogen *Cryptococcus neoformans* causes cryptococcosis by the inhalation of infectious spores generated by unisexual or bisexual reproduction. To understand complex signaling networks modulating the developmental process, a complete understanding of genome-scale transcription factors (TFs) and kinases is needed. Previously we reported that 37 TFs and 42 kinase mutants constructed in *C. neoformans* MATα H99 strain background exhibited altered mating efficiency. To further elucidate the mating regulatory mechanism, we constructed knockout mutants of the mating-regulating TFs and kinases in YL99 strain—MATα isogenic strain of H99 strain—to monitor unilateral and bilateral mating, and to perform an analysis of their function in the developmental process. We constructed 22 gene-deletion strains representing eleven TFs and are currently constructing gene-deletion strains for the remaining mating-regulating TFs and kinases. For confirmed mutant strains, we are examining mating phenotypes during bilateral mating: mating pheromone production, cell fusion efficiency, filamentous growth, formation of basidia and basidiospores. Furthermore, we are examining transcript profiles of mating-regulating TFs and kinases at different developmental stages of sexual reproduction. Ultimately, this study will focus on mapping and discovering the functions of the mating-regulating TFs and kinases, and elucidating complex signaling networks in the developmental process of *C. neoformans*.

C026

Attaching Abilities of Lactic Acid-producing Bacteria are Strain-dependent

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The attaching ability of lactic acid–producing bacteria (LAB) to host intestinal mucus is an important factor for exerting beneficial effects. Still, it is controversial whether the attaching ability is species-dependent, strain-dependent, or host-dependent. In this study, a total of 121 isolates in 15 LAB species were isolated from swine, food, and humans. *L. plantarum*, *W. cibaria*, and *W. confusa* were isolated from all three origins and other LAB were isolated only from one or two origins. The most frequently isolated species was *L. plantarum*; *L. acidophilus* was not detected. Attaching abilities of each isolate were tested with three different intestinal epithelial cell lines: IPI-2I from boars, Caco-2 from Caucasian people, and SNU-C4 from Mongolian people. Ten LAB species (*E. faecalis*, *E. faecium*, *L. rhamnosus*, *Leu. mesenteroides*, *L. fermentum*, *L. sakei*, *L. lactis*, *L. brevis*, *L. amylovorus*, and *L. reuteri*) attached to all three cell lines, but numbers of attached colony-forming units varied. Isolates in five LAB species—*L. paracasei*, *L. plantarum*, *L. salivarius*, *W. cibaria*, and *W. confuse*—had variable attachment abilities. Results showed that the attaching ability of LAB to a specific intestinal epithelial cell lines is strain-dependent and not species-dependent. Each LAB needs to be tested for its attaching ability before being used as a probiotic, because adhesion and persistence in the gastrointestinal tract are necessary for exerting beneficial effects to the host.

C027

Yeast Community Associated with Wild Tiger Lily (*Lilium lancifolium* Thunb.) and Characterization of Biosurfactant-producing Yeast

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Gyeongbuk Institute for Marine Bioindustry

Identification of yeast species that have potential applications in biotechnology is greatly interesting. In this study, we investigated the yeast biota associated with wild tiger lily (*Lilium lancifolium* Thunb.). We compared the efficacy of different yeast media for the isolation of yeasts associated with wild tiger lily, and the media included antibiotics and fungistatic agents for the suppression of fungi. We isolated yeast species from flowers, leaves, and stems of the wild tiger lily because these niches have not yet been used for determining the yeast biodiversity. Yeast isolates were identified by phylogenetic analysis based on internal transcribed spacer region sequencing. Yeasts produce biosurfactants (BSs), which are important amphiphilic compounds that are used in the agricultural industry as well as cosmetic and pharmaceutical industries because of their low toxicity, biodegradability, and both commercial and academic interests. Using these isolated yeast strains, we developed rapid and simple screening methods for BS-producing yeast (BSPY) to design processes for the characterization of high-value yeast BSs and production of eco-friendly BSs. We screened *Aureobasidium pullulans* L3-GPY from flower of wild tiger lily, and developed fermentation processes for BS production. Analysis of the chemical structure of these compounds by mass spectrometry and nuclear magnetic resonance revealed several potential novel BSs, including glycolipids.

C028

Optimization of β-Glucosidase Activity and Characteristics of *Weissella koreensis* Isolated from Kimchi

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This study establishes the optimal fermentation conditions of β-glucosidase (E.C. 3.4.1.21) activities of *Weissella koreensis* isolated from Kimchi. The β-glucosidase activities of this strain were measured at various pH and temperatures in MRS containing glucose as a carbon source. The highest β -glucosidase activity was achieved at 35°C and pH 6.5. Under these optimal culture conditions: the highest β-glucosidase activity was 1.80 ± 0.26 unit/mg protein at 6 hours, a specific growth rate of 0.57 ± 0.01 h^{-1} was observed at 24 hours, and 1.15 ± 0.01 g/L of lactic acid was produced at 24 hours.

[This research was financially supported by the Ministry of Trade, Industry, and Energy (MOTIE), Korea, under the "Regional Specialized Industry Development Program (R0005969)" supervised by the Korea Institute for Advancement of Technology (KIAT).]

C029

Exploring the Potential for the Application of Korean Fermented Food-isolated Bacteria to Enhance Drought Stress Tolerance in Onions

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Microbes play a vital role in food fermentation where drying and salting constitute the majority of the process. Primary motive behind the present study is that bacteria present in fermented food have strong resistance to water stress, enabling them to cope with water deficit. In this regard, this study aimed to examine the potential for applying Korean fermented food-isolated bacteria in enhancing drought stress tolerance in onions. Onions (*Allium cepa* L.) were chosen as they are one of the most consumed fresh vegetables and susceptible to drought stress, resulting in significant yield reduction. Bacterial strains isolated from Korean meju, soy sauce, and soybean paste and stored in individual wells of 96-well plates at -80°C were transferred to another 96-well plates containing different concentrations of polyethylene glycol (PEG), ranging from 0% to 40%. After incubation at 28°C for 72 hours, resistance to PEG-induced water stress of bacterial strains was determined based on their growth through optical density measurements. Selected bacterial strains with water stress tolerance were additionally characterized for plant growth promoting trains, such as nitrogen fixation, production of IAA and siderophores, ACC deaminase activity, and seed germination test after treated with the bacterial strains. The detailed results from the study will be presented at the conference.

[This work has been supported by grants from Rural Development Administration.]

C030

Plant Growth-promoting Rhizobacteria, *Variovorax boronicumulans* PMC12, Confers Resistance to Abiotic and Biotic Stresses in Tomato Plant

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Microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), play important roles in plant growth and health enhancement and render them to resistant to not only biotic stress but also abiotic stress, such as drought and salinity. This study aimed to investigate the capability of PGPR to mitigate biotic and abiotic stress effects in tomato plants. We isolated a novel bacterial strain, *Variovorax boronicumulans* PMC12 from sterile nursery soil. *In vitro* assay exhibited that PMC12 produced ammonia, IAA, siderophore, and ACC deaminase, which are well known traits of PGPR. Under 1,000 kPa complex salt condition, the fresh weight of tomato plants treated with PMC12 increased by 20.5% compared to non-treated plants. The growth of tomato plants treated with PMC12 did not not increase significantly at high temperature condition, but showed a 49.4% increase in fresh weight compared to non-treated tomato plants at low temperatures condition. The survival rate of tomato plants treated with PMC12 was significantly higher than non-treated plants by 47% under drought stress condition induced by no irrigation. PMC12 also enhanced the resistance to bacterial wilt disease caused by *Ralstonia solanacearum*. Taken together, these results indicated that PMC12 could be used as a promising biocontrol and biostimulant agent to reduce susceptibility of plants to both abiotic and biotic stresses.

[This work has been supported by grants from Rural Development Administration.]

C031

Discovering the Polysaccharide Capsule Regulatory Signal Pathways in the *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic pathogen that causes fungal meningitis, which is responsible for more than 600,000 deaths worldwide annually. The polysaccharide capsule of *C. neoformans* is a key virulence factor which interferes with the phagocytosis by host innate immune cells. The cAMP/PKA and HOG pathways are the central signal transduction systems to control the capsule formation. In our previous studies, we qualitatively and quantitatively measured the ability of each TF or kinase mutant to produce capsule under Dulbecco's Modified Eagle's (DME) solid medium at 37°C and found that 50 TFs and 51 kinases appear to be negatively or positively involved in capsule production. This result suggests that capsule production requires direct or indirect involvement of complex signaling pathways. Nevertheless, it still remains elusive how these complex signaling components and pathways are coordinated.

To obtain the holistic view of core capsule signaling pathways, for 50 TF and 51 kinase mutants exhibiting altered levels of capsule production, we examined their capability to produce capsule under other capsule inducing conditions, such as Littman's medium and fetal bovine serum (FBS) medium. Here we found that 11 signaling components were found to be critical for capsule production in all capsule induction media we tested. These include 7 kinases and 4 TFs. This study will allow us to reveal the capsule production related mechanisms in *C. neoformans*.

C032

Improving Amber Suppression Activity of an Orthogonal Pair of *Saccharomyces cerevisiae* Tyrosyl-tRNA Synthetase and a Variant of *E. coli* Initiator tRNA, $tRNA_2^{fMet}$ for the Efficient Incorporation of Unnatural Amino Acids

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The orthogonal pair of *Sc* Tyrosyl-tRNA synthetase (*Sc* YRS) and its cognate tRNA of which anticodon has been changed to recognize the amber stop codon is an effective tool for incorporating unnatural amino acids into protein in *E. coli*. To evolve the amber suppression activity of the orthogonal pair, we generated a library of mutant *Sc* YRS by randomizing two amino acid residues at 320 and 321 which involve recognition of the 3rd base of anticodon in a variant of *E. coli* initiator tRNA, $tRNA_2^{fMet}$. Two positive clones are selected from the library with chloramphenicol resistance by amber suppression. All the clones showed growth resistance at higher concentration of chloramphenicol and their LD_{50} values were 1.7–2.3 fold higher than the wild type YRS. *In vivo* assay of amber suppression activity measured in DH10B(Tn:*lacZam*) reveals that mutant YRS-3 clone containing mutations of P320A and D321A showed 6.5-fold higher activity than the wild type. In addition, *in vitro* amino acylation assay with purified YRS-3 also showed approximately 3-fold higher activity than wild type YRS. Moreover, introduction of the same mutations to AzPheRS-6 (an engineered ARS from *Sc* YRS to incorporate *p*-L-azidophenylalanine) enhanced AzPhe incorporation to GFP target proteins by 3.2-fold higher than the original one. These results demonstrate that optimization of anticodon recognition of suppressor tRNA by engineered ARS improves the efficiency of unnatural amino acid incorporation.
[Support by HUFS]

C033

Prediction of Secondary Metabolites from Genome Sequences of *Streptomyces* sp. MEBiC10311 Isolated from Seawater

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Genome of a Streptomyces strain isolated from seawater was sequenced with PacBio RSII system SMRT sequencing technology and its genes related to the production of secondary metabolites were investigated via anti-SMASH web-based tool. Strain MEBiC10311 showed no difference with *S. rutgersensis, S. gougerotii*, and *S. diabolicus* in 16S rRNA gene sequence, however, ANI values between strain MEBiC10311 and genomes of previously sequenced strains were lower than 91%. Genome of strain MEBiC10311 was featured as 7.28 Mbps size, 73.3 mol% of DNA G+C ratio, 5,882 CDSs, and includes 14 gene clusters related to the production of secondary metabolites. The 14 clusters includes four NRPSs, one type 1 PKS, one siderophore, and 7 PKS-NRPS hybrids. Among the 14 predicted products, 11 were novel compared to previously known compounds. This result confirmed that bacterial secondary metabolites displayed strain by strain diference and revealed that small differences in the genome made novel differences in the production of natural products.
[Supported grants from KIOST & MBRB].

C034

Chemico-physical Method for the Transformation of Various Bacterial Species

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A chemico-physical transformation method that combines rubidium chloride (RbCl) based chemical method and sepiolite-based physical method has been reported to widely apply with bacterial species. The sepiolite concentration utilized in this method has been optimized with Escherichia coli (DHα). The transformants also can be obtained from *Bacillus subtilis, Bacillus megaterium, Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Bacillus* sp. *Enterococcus faecalis*, *Enterococcus malodoratus* and *Enterococcus mundtii* by chemico-physical transformation method. Furthermore, to accelerate the advance of bacteria biotechnology we optimize our method by evaluating chemical compounds (rubidium chloride, lithium acetate, and cesium chloride) for increasing membrane permeability and physical materials (sepiolite, gold(III) chloride, multi-walled carbon nanotube, and chitosan) for piercing membranes with *Lactococcus lactis* subsp. *Lactis*, *Enterococcus faecalis*, and *Bacillus subtilis*. The industrially attractive bacterial species *Ralstonia eutropha* and *Methylomonas* sp. DH-1 can also be obtained transformants by optimal chemico-physical transformation method with reliable efficiency. The best transformation efficiencies were achieved as follows; 2.84×10^4 CFU/μg in *Lactococcus lactis* subsp. *Lactis* [0.1 M CsCl and gold(III) chloride], 3.60×10^4 CFU/μg in *Enterococcus faecalis* (1 M Li-acetate and MWCNT), 2.41×10^4 CFU/μg in *Bacillus subtilis* (0.01 M RbCl and sepiolite), 3.49×10^4 CFU/μg [0.1 M RbCl and gold(III) chloride] in *Ralstonia eutropha* and 8.78×10^4 CFU/μg (1 M RbCl and chitosan) in *Methylomonas* sp. DH-1. The efficiencies are 100-fold higher than those without optimization. Consequently, our chemico-physical transformation with chemicals-materials optimization allows efficient DNA entry into various bacterial cells with high efficiency.
[This research was supported by C1 Gas Refinery Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2016M3D3A1A01913244).]

C035

Reduction of *Listeria monocytogenes* on Polyethylene Surfaces by Heat and UV Light

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This study determined the efficacy of heat treatments and UV light exposure on the reduction of *Listeria monocytogenes* viable cell loads on polyethylene surfaces. The efficiency of heat treatment was tested with two types of dry and wet at various thermal conditions (55, 60, 65, 70, 75, and 80°C) and times (1, 3, 5, and 10 min). The polyethylene film inoculated with *L. monocytogenes* were treated with UV light (254 nm) for 5, 10, 15, 20, 25, and 30 min. *L. monocytogenes* populations on polyethylene surface were reduced 1.0 to 5.2 by dry heat treatment, while the density of *L. monocytogenes* was not declined with wet heat treatment below 65°C. In the wet heat treatment, the reduction of *L. monocytogenes* populations was observed only above 70°C. *L. monocytogenes* counts on polyethylene surface were significantly reduced after 5 min of exposure to UV light compared with the initial density. These results indicate that dry heat treatment and UV light are potential candidates for elimination of *L. monocytogenes* on polyethylene surfaces.

C036

Site-specific Incorporation of *p*-L-Azidophenylalanine Using a New Orthogonal Pair of *Saccharomyces cerevisiae* Tyrosyl-tRNA Synthetase (*Sc* TyrRS) and a Variant of *E. coli* Initiator tRNA, $tRNA_2^{fMet}$

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Orthogonal pair of *Sc* TyrRS and a variant of *E. coli* initiator $tRNA_2^{fMet}$ (*fMam* $tRNA_{CUA}$) was a useful tool for site-specific incorporation of unnatural amino acid (UAA) into protein in *E. coli*. Aiming to incorporate p-L-azidophenylalanine (AzPhe) into protein by using this orthogonal pair, a rational design was applied to construct three different mutants of *Sc* TyrRS by site-directed mutagenesis in the putative active site which was deduced by analogy with *Methanocalococcus jannaschii* TyrRS. *In vivo* incorporation of AzPhe by these mutant TyrRSs (designated as AzPheRS-1, -2, and -3, respectively) was analyzed by several ways. AzPheRS mutants conferred 2−3 fold higher chloramphenicol resistance generated by CAT amber suppression in the presence of AzPhe compared to the absence of UAA. B-galactosidase activity produced by AzPheRS-mediated amber suppression in DH10B (*Tn:lacZam*) strain was 1.6-2.4 fold higher in the presence of AzPhe. Consistently, fluorescence measurement and SDS-PAGE also showed approximately 1.7−2.1 fold higher production of $sfGFP_{150TAG}$ by AzPheRS-mediated amber suppression in the presence of AzPhe. LC-MS analysis of sfGFP protein purified from AzPheRS mutant showed shift of spectra to the expected position, which further confirmed incorporation of AzPhe at the 150 residue of sfGFP. This is the first report demonstrating AzPhe incorporation by using orthogonal pair of *Sc* TyrRS and *fMam* $tRNA_{CUA}$.

[Supported by GRRC grant]

D002

Construction and Evaluation of Chikungunya Virus Pseudotyped Virus for Neutralization Assays

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Chikungunya virus (CHIKV) with a single stranded positive-sense RNA genome, belongs to *Alphavirus* genus of *Togaviridae* family. Its infection mainly causes abrupt high fever, rashes, headache, and especially severe joint pain that can last for several months or years. CHIKV, a mosquito-borne arbovirus, is considered to be an emerging/re-emerging pathogen that becomes one of the most important global health concerns due to rapid increase in epidemics. There is no currently available vaccine or antiviral against CHIKV. Due to the limitation of handling CHIKV at Biosafety Level 3 facilities, we set to generate CHIKV glycoproteins pseudotyped virus (CHIKV*pseudo*) using lentiviral vector systems. In this study, we firstly identified the structure protein sequence of a CHIKV strain isolated in Korea (KNIH/2009/77). Based on this information, we constructed a few types of lentiviral vector-based pseudotype viruses expressing the structural proteins of CHIKV. The production yields of CHIKV*pseudo* significantly differed, depending on the vector backbones. We then examined potential application of CHIKV*pseudo* for neutralization assays. IC_{50} values of neutralizing assays with CHIKV*pseudo* were similar to those of plaque reduction neutralization assays using CHIKV, suggesting a useful and safe method to test the neutralizing activity of anti-sera against CHIKV by using CHIKV*pseudo*.

[This research was supported by a grant (16172MFDS272) from Ministry of Food and Drug Safety in 2016-2018.]

D003

Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) Isolates from Pigs and Farm Workers in Korea

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Recently, LA-MRSA has frequently been reported worldwide, especially in pig farms in Europe. The emergence of LA-MRSA in food-producing animals has raised public health concerns on the possible transmission of LA-MRSA among livestock animals and subsequently to humans. In this study, a total of 1011 swab samples (760, 137, and 114 samples from pigs, farm workers, and farm environment, respectively) were collected from 7 provinces in Korea and 34 MRSA strains (3.4%) were isolated from pigs (26 strains), farm workers (6 strains), and farm environment (2 strains). The major genotype of LA-MRSA from pigs was ST398-SCC*mec* type V-*agr* type I (35.3%) followed by ST541, ST9, and ST1. Two MRSA strains isolated from farm environment were also ST398 and six MRSA strains from farm workers were ST2084 (5 strains) and ST541 (1 strain). All the MRSA strains were resistant to multiple antibiotics (at least 4 of 10 antibiotics tested) and were highly resistant to ampicillin (97%), cefoxitin (97%), tetracycline (72.7%). Our findings indicate that although the prevalence of MRSA among pig herds in Korea is lower than other European countries, the ST398 lineage of LA-MRSA has become prevalent among pigs in Korea.

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D004

Flagellin-specific IgG2c and IgA Antibody Response Requires Type I Interferon Signaling Activated by Toll-like Receptor 5

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Flagellin is a main protein component of flagella and activates Toll-like receptor 5 (TLR5) to trigger the innate immune responses. In addition, flagellin is recognized as an antigen by adaptive immune system. Toll-like receptor 5 (TLR5) recognizes flagellin in the cell surface and induces secretion of many pro-inflammatory cytokines. However, it was not known if flagellin-mediated TLR5 stimulation also promotes type I interferon (IFN) production. In this study, we found that flagellin induced secretion of IFN-β and several type I IFN-stimulated genes (ISGs) in both human and mouse cell lines. Production of IFN-β, but not IL-6 and TNF required the internalization and pH-dependent endosomal signaling of TLR5. We also found that flagellin induced IFN-β expression in primary monocytes and neutrophils by using IFN-β reporter mice. Serum IFN-β level was rapidly increased by injection of flagellin in wild type and TRIF-deficient mice, but not in TLR5- or MyD88-deficient mice. Interestingly, type I interferon receptor (IFNAR)-deficient mice showed an increased level of serum IFN-β compared to wild type mice, indicating that IFN-β is rapidly consumed by IFNAR binding. Type I IFN is known to enhance humoral immune responses and promote immunoglobulin class switching. Among isotypes of flagellin-specific antibodies, serum IgG2c and mucosal IgA depended on IFNAR signaling. Our study suggests that IFN-β produced by TLR5 controls the humoral immunity against flagellin.

D006

Effect of Live VHSV Immersion Vaccine in Juvenile Olive Flounder, *Paralichthys olivaceus*

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VHSV is the causative agent of VHS, one of the most serious diseases in cultured marine fishes worldwide. Although several kinds of VHSV vaccines have been developed, none of VHSV vaccine is not currently available. Our result was revealed that the optimal condition was $10^{5.5}$ $TCID_{50}$/ml for live VHSV immunization in olive flounder. The presence of live VHSV in organs from vaccine treated fish was analyzed by qRT-PCR, it was not under-detection limit in all the tested organs. During the immunized period, we estimate the immune related-gene and specific VHSV antibody by using qRT-PCR and ELISA method. The significant difference of immune genes was not observed in this study. Also VHSV antibody was detected in fish blood. The cumulative mortality of vaccinated fish was < 40%, whereas the cumulative mortality in positive control was 100%. This study was observed that the effect of live VHSV immersion vaccine treatment could provide a protection in olive flounder against VHSV infection. Future study is needed to confirm the immune gene expression and VHSV-specific antibody in VHSV-infected fish after vaccine treatment.

[This research was a part of the project titled 'Fish Vaccine Research Center', funded by the Ministry of Ocean and Fisheries.]

D007

Distribution of Nervous Necrosis Virus (NNV) in Dead Sevenband Grouper, *Hyporthodus septemfasciatus* by Intramuscular Injection (IM) or Immersion Challenge

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Viral nervous necrosis (VNN) is one of the most serious diseases affecting over 120 species of cultured marine fishes worldwide. NNV, the causative agent of VNN, its genome consists of two positive senses single stranded RNA1 and RNA2. Several studies have detected viral genome using qRT-PCR, but there is no information about RNA1 segment status and also did not describe whether NNV was infective or not. In this study, we compare NNV genomic segments RNA1 and RNA2 through qRT-PCR and viral infectivity in eight organs of dead fish by NNV challenge. IM injection with NNV at $10^{0.5}$, $10^{1.5}$, $10^{2.5}$, and $10^{3.5}$ $TCID_{50}$/100 µl/fish resulted in cumulative mortality of 0%, 20%, 40%, and 100%, respectively. Immersion challenge with NNV at $10^{1.5}$, $10^{3.5}$, and $10^{5.5}$ $TCID_{50}$/ml resulted in cumulative mortality of 0%, 40% and 60%, respectively. NNV infectivity and its gene from dead fish were detected in all tested non-nervous tissues but their levels were much lower than those in nervous tissues. The ratio of the RNA1 segment and infective particles in dead fish was higher than RNA1/$TCID_{50}$, which indicates that RNA1 was overproduced than RNA2 segment. The gill, spleen and kidney of viral infectivity and its gene was detected higher than the other non-nervous tissues, suggest that these tissues can be relate to its defensive role and early antigen processing. [This research was a part of the project titled 'Fish Vaccine Research Center', funded by the Ministry of Ocean and Fisheries.]

D008

Anti-virulence Effect by Propeptides of Extracellular Proteases on *Pseudomonas aeruginosa* Infection

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Pseudomonas aeruginosa infections in the respiratory tracts such as ventilator-associated pneumonia and cystic fibrosis (CF) cause huge problem. *P. aeruginosa* secretes virulence factors that may subvert host innate immunity, disrupts tight junctions, and causes proteolytic damages on host tissues. We found that some major extracellular proteases of *P. aeruginosa* (Protease IV, LasA, and LasB) are inhibited by their propeptides. Since they play a key role in the *P. aeruginosa* infections as virulence factors, in this study, we have attempted to lower the virulence of *P. aeruginosa* through the propeptide treatment. For this, we used multiple infection models, *Caenorhabditis elegans*, *Tenebrio molitor* and mouse. In *P. aeruginosa* infection, the survival rate of *C. elegans* and *T. molitor* are increased when *P. aeruginosa* was treated with the propeptides. Mouse models of acute and chronic lung infection have been used in studying *P. aeruginosa* for assessing *in vivo* behavior and for the evaluation of novel therapies. *P. aeruginosa* enmeshed in agar beads can be used in the mouse to reproduce the lung pathology of cystic fibrosis patients with advanced chronic pulmonary disease. We conducted several experiments on mouse to find out that treated with propeptides of *P. aeruginosa* virulence factors are effective or not to cure *P. aeruginosa* infection.

D009

Inhibitory Effect of Culture Supernatant Fractions Derived from Marine *Penicillium* sp. on the Biofilm Formation of *Streptococcus mutans*

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Streptococcus mutans is a primary causative agent which is associated with dental caries in humans. One of the virulence properties of *S. mutans* is an ability to form biofilm known as dental plaque on the surface of teeth, resulting in the dental caries by decalcifying enamel. In this study, the culture supernatant fractions (CSF) derived from marine-derived *Penicillin* sp. were assessed to inhibit the biofilm formation of *S. mutans*. The minimum inhibition concentration assay indicated that among the CSF (B1 to B7 fractions), B7 fraction significantly interfered the growth of *S. mutans*. Additionally, biofilm formation of *S. mutans* was inhibited by B7 fraction treatment. The amount of ATP efflux leaked from biofilm cells of *S. mutans* increased in a dose-dependent manner when the biofilm of *S. mutans* was treated with B7 fraction, suggesting that B7 fraction disrupted the membrane of the biofilm cells. Furthermore, it was also observed that B7 fraction inhibited biofilm formation of *S. mutans* by assessing confocal microscopy and scanning electron microscopy. These results suggest that B7 fraction of CSF from marine-derived *Penicillium* sp. can be potent for the control of the formation and development of *S. mutans* biofilm that may be used for the prevention of dental plaque.

D010

Inhibitory Effect of Lipoteichoic Acid from Probiotic *Lactobacillus* on Flagellin-induced IL-8 Production in Porcine Peripheral Blood Mononuclear Cells

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Probiotics in livestock feed supplements are considered as a replacement for antibiotics for the mucosal immunity of gastrointestinal tract. Although several effector molecules such as bacterial cell wall components have been proposed to be associated with the function of probiotics, little evidence is suggested that the effector molecules of probiotics are responsible for strain-specificity of probiotic functions. Lactobacilli are well known probiotic bacteria that are considered to be beneficial in the gastrointestinal tract of humans. This study demonstrated that a well-known virulence factor, flagellin of *Salmonella typhimurium* significantly induced IL-8 production in porcine peripheral blood mononuclear cells. However, lipoteichoic acid, one of the major cell wall components from *Lactobacillus plantarum*, but not heat-killed whole bacteria of *L. plantarum*, inhibited flagellin-induced IL-8 production. Dealanylated *L. plantarum* LTA inhibited flagellin-induced IL-8 expression, whereas deacylated *L. plantarum* LTA failed to inhibit flagellin-induced IL-8 expression, indicating that the lipid moieties of *L. plantarum* LTA were essential for the inhibition of flagellin-induced IL-8 expression. Taken together, lipoteichoic acid of *L. plantarum* acted on the effector molecule to confer anti-inflammatory response in porcine peripheral blood mononuclear cells.

D011

Anaerobic Growth Increases Colonization of *Vibrio cholerae* to Human Epithelial Cells by Enhancing Toxin Co-regulated Pilus Protein

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Cholera is an acute intestinal infectious disease caused by *Vibrio cholerae*. *V. cholerae* is endemic in many low-income countries, particularly in areas of inadequate sanitation and food hygiene practices. Up to date, two oral cholera vaccines, Dukoral and Shanchol, are qualified from WHO and licensed in several countries. Although the pathogenesis caused by *V. cholerae* takes place in the intestine, anaerobic environment, two oral vaccines are formulated with *V. cholerae* cultured in aerobic condition. Moreover, anaerobiosis-induced microbial gene regulation and virulence are not fully elucidated in *V. cholerae*. In this study, we investigated colonization of *V. cholerae* grown under anaerobic conditions in intestinal epithelial cells. Anaerobic growth of *V. cholerae* O1 significantly increased bacterial adhesion compared to aerobically grown bacteria in human epithelial cells. A similar adherent property of *V. cholerae* O1 was also observed in polarized Caco-2 cells. The expression of certain genes related to Toxin co-regulated pilus protein were increased during anaerobic growth. The *V. cholerae* deficient of these genes showed decreased colonization to intestinal epithelial cell. Collectively, our data indicate that *V. cholerae* upregulate expression of colonizing factors in anaerobic condition similar to intestinal environment.
[Supported by grants from NRF]

D012

Altered Immune Responses of the Porcine Macrophage Infected by a ppGpp Defective Enteropathogenic *Escherichia coli*

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Under nutrient-deficient environment, stringent response is triggered in microorganisms, which is mediated by an alarmone ppGpp (guanosine 3',5'-bispyrophosphate). During stringent response, ppGpp is rapidly synthesized by RelA and/or SpoT and subsequently alters various cellular processes such as growth, metabolism, motility and virulence. Our previous study showed that stringent response is essential for full virulence of enteropathogenic *Escherichia coli* (EPEC), a major etiological agent of post-weaning diarrhea in pigs. To assess host immune response toward ppGpp-defective ($ppGpp^0$) mutant of EPEC E2348/69 strain, a porcine alveolar macrophage cell line, 3D4/31, was infected with the $ppGpp^0$ mutant. Compared to the wild-type strain, the $ppGpp^0$ mutant displayed enhanced adhesion to 3D4/31 cells. Its susceptibility to phagocytosis was also increased. Interestingly, macrophage infection with the $ppGpp^0$ mutant boosted the expression of pro-inflammatory cytokines in 3D4/31 such as interleukin (IL)-6 and IL-8, was evident. Taken together, our results suggest that the $ppGpp^0$ mutant can augment the pro-inflammatory response in macrophage during a porcine infection.
[This study was supported by a grant from NRF (#2017R1A2B4013056) to J.W.Y.].

D013

High Serum Levels of Total IgE in Allergic Rhinitis Patient is Associated with Microbial Dysbiosis in Inferior Turbinate of Nasal Cavity

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Dysbiosis in the human microbiota are closely linked with the etiology of allergic diseases. Although disease site-specific microbiota may be associated with disease pathophysiology, the role of the nasal microbiota is unclear. We characterized the microbiota of inferior turbinate, the disease site of allergic rhinitis, in subjects with allergic rhinitis (n=20) and healthy controls (n=12). Microbial dysbiosis correlated with total IgE levels representing combined allergic responses. Compared to the populations with low total IgE levels (group IgE^{low}), low microbial biodiversity with a high relative abundance of *Firmicutes* (*Staphylococcus aureus*) and a low relative abundance of *Actinobacteria* (*Propionibacterium acnes*) was observed in individuals with high total serum IgE levels (group IgE^{high}). Microbial functional gene indicated an increase in signal transduction-related genes and a decrease in energy metabolism-related genes in group IgE^{high} as shown in the microbial features with atopic and/or inflammatory diseases. Thus, dysbiosis of the inferior turbinate mucosa microbiota, particularly an increase in *S. aureus* and a decrease in *P. acnes*, is linked to high total IgE levels in allergic rhinitis, suggesting that inferior turbinate microbiota may be affected by accumulated allergic responses against sensitized allergens and that site-specific microbial alterations play a potential role in disease pathophysiology.
[Supported by grant from NRF-2016R1E1A1A02921587]

D014

Genome-wide Transcriptional Networks of Iron Regulators in the Human Fungal Pathogen *Cryptococcus neoformans*

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Iron is an essential nutrient in both pathogens and vertebrate host, and contributes the elaboration of key virulence factors including melanin and capsule in the human fungal pathogen *C. neoformans*. Iron availability in the host is extremely low due to the sequestration of the free iron by the iron binding proteins such as transferrin. However, *C. neoformans* harbors sophisticated iron acquisition systems to adapt in the host niches. Previously, we revealed that iron responsive transcription factors Cir1 and HapX govern the genes responsible for iron acquisition and utilization in *C. neoformans*. However, the transcriptional regulatory mechanism by Cir1 and HapX remains elusive. We performed comprehensive analysis using the RNA-seq and ChIP-seq to identify the genes directly regulated by Cir1 and HapX in response to iron availability. These results showed that the subsets of genes encoding mitochondrial respiration, Fe-S cluster and heme biosynthesis were directly regulated by HapX in iron limited condition, whereas the genes including iron uptake and nitrogen metabolism were controlled by Cir1 in both iron replete and limited condition. In addition, our biochemical analyses revealed that Cir1 and HapX are iron-containing proteins, suggesting that the regulatory networks of Cir1 and HapX are influenced by incorporation of iron into the proteins. Taken together, our results lead new insight into the regulatory networks on iron acquisition and homeostasis in *C. neoformans*.

D015

Gastrin Promoter Activity Induced by *Helicobacter pylori*-induced HB-EGF Requires Sequential Activation of EGF Receptor, C-Raf, Mek1, and Erk2

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Background and aims: *Helicobacter pylori* is associated with hypergastrinemia, which has been linked to the development of gastric disease. Since this process is not fully understood, this study was designed to elucidate the molecular mechanism and signal pathways required for *H. pylori*-mediated changes in gastrin expression.
Methods: AGS cells were infected with G27, PMSS1 and K74 *H. pylori* strains and their isogenic mutants. Expression of gastrin and the EGF family members were measured and involvement of Raf, Mek and Erk isoforms in *H. pylori*-induced gastrin expression was examined by siRNA knockdown for each isoform.
Results: *H. pylori*-induced mRNA expression of amphiregulin, EGF, HB-EGF, and transforming growth factor-α. Of these, siRNA targeting HB-EGF significantly blocked *H. pylori*-induced gastrin expression. *H. pylori* also induced ectodomain shedding of HB-EGF. Knockdown of C-Raf, Mek1 and Erk2 in MAPK pathway inhibited *H. pylori*-induced gastrin expression.
Conclusion: Hitherto, EGF was the only EGF family member known to induce gastrin expression. Herein we show that expression of HB-EGF and its ectodomain shedding are critical for *H. pylori*-induced gastrin expression. Thus, we describe a novel role for mature HB-EGF in gastrin mRNA expression and delineate the signal cascade underlying *H. pylori*-induced gastrin expression as the sequential activation of HB-EGF, EGF receptor, C-Raf, Mek1 and Erk2 in MAPK pathway.

D016

Adenosylhomocysteinase Like 1 Interacts with Nonstructural 5A and Regulates Hepatitis C Virus Propagation

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Hepatitis C virus (HCV) is a hepatotropic RNA virus that causes progressive liver damage, including liver cirrhosis and hepatocellular carcinoma. HCV depends on host cellular proteins for its own propagation. With the aim to identify host factors involved in HCV propagation, we previously performed protein microarray assays using the HCV nonstructural 5A protein (NS5A) protein as a probe. Of ~9,000 human proteins immobilized in a microarray, approximately 90 cellular proteins were identified as HCV NS5A interacting partners. Among these candidates, adenosylhomocysteinase like 1 (AHCYL1) was selected for further characterization. Protein interaction between NS5A and AHCYL1 was verified by *in vitro* pulldown assay and further confirmed by coimmunoprecipitation assay. We showed that AHCYL1 interacted with domain I of NS5A. Silencing of AHCYL1 decreased both RNA and protein expression levels of HCV, whereas overexpression of AHCYL1 increased viral propagation. By using both subgenomic replicon and pseudoparticle entry assay systems, we demonstrated that AHCYL1 was not involved in entry and replication steps of the HCV life cycle. Employing HCV IRES-mediated translation assay, we concluded that AHCYL1 was involved in the translation step of the HCV life cycle. The precise involvement of AHCYL1 in the HCV life cylcle is currently under investigation.
[This work was supported by Ministry of Science, ICT and Future Planning (MSIP) (2016R010886).]

D017

Pseudomonas aeruginosa Genes Involved in Polymicrobial Interaction with *Staphylococcus aureus*

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Microorganisms usually coexist as a multifaceted polymicrobial community at mucosal sites of the human body as well as on abiotic surfaces in the natural habitats. More and more attention has been recently paid to the complicated interactions that might occur between microorganisms in the polymicrobial communities especially during infection. The two opportunistic human pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus* commonly coexist in the bacterial infections for hospitalized and/or immunocompromised patients. It is known that *P. aeruginosa* produces 4-hydroxy-2-heptylquinoline-N-oxide (HQNO) that inhibits the growth of *S. aureus*. In this study, we observed that the *P. aeruginosa* mutant (*pqsA*) defective in HQNO synthesis displayed residual killing activity against *S. aureus* strains, which was evident against the *S. aureus* mutants (*m6*) for respiratory activity. *lasR* and *lasI* mutants were newly isolated from about 6,000 random transposon insertion clones: LasR encodes the master quorum-sensing transcriptional regulator that responds to the N-(3-oxododecanoyl)-homoserine lactone signal produced by LasI. These finding may provide an insight to the previously unveiled molecular mechanisms by which *P. aeruginosa* outcompetes *S. aureus* during the polymicrobial interaction *in vivo*.
[Supported by grants from the National Research Foundations of Korea (NRF) (NRF-2017R1A2B3005239)]

D018

A Reverse Genetic System Reveals Critical Residues for Infectivity of the *Pseudomonas aeruginosa* Leviphage, PP7

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PP7 is a leviphage with single-stranded RNA genome that can infect *Pseudomonas aeruginosa* PAO1. Unlike other RNA viruses, however, not much comprehensive reverse genetic approach has been performed for PP7 due to the lack of infectious cDNA clones. Here we have created the reverse genetic system for PP7 using two full-length PP7 cDNA clones (PP7-S and PP7-O) linked to the T7 promoter: PP7-S was chemically synthesized by using the sequence deposited in the database; PP7-O was generated by RT-PCR using the isolated PP7 RNA. PP7-O and PP7-S displayed 19 nucleotide differences. Infectious PP7 virions were produced not from PP7-S, but from PP7-O, whereas the comparable amount of RNA-containing viral particles was observed for PP7-S. Reverse genetic analyses of the 19 different nucleotides revealed that 2 nucleotide differences for PP7-S (G1125A and C1157G) were critical in the infectivity in PP7, both of which resides in the maturation protein (R349Q and S360C). Further point mutation and complementation analyses showed that the two nucleotide differences of PP7-S resulted in the dysfunction of the maturation protein. These results substantiate the roles of maturation protein in phage infectivity and, more importantly, provide a lesson that the viral RNA genome sequencing needs functional verification possibly by a reverse genetic system.

D019

In vitro Comparison between Acid- and Alkali-induced *Listeria monocytogenes* Bacterial Ghosts (LMGs)

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To compare the efficiency of chemically induced *Listeria monocytogenes* ghosts (LMGs), various chemicals were treated with *L. monocytogenes* (*Lm*) culture using their MIC. When these chemically induced LMGs were inoculated onto selected medium, no viable colonies were detected within 5-15 min treatment depending upon chemicals. SEM showed that both acid- and alkali-induced LMGs form trans-membrane lysis tunnels on the cell envelopes. Acid-induced LMGs showed more damages of cell envelopes and bigger size of lysis tunnels than that of alkali-induced LMGs. There was a big difference in protein profiles on SDS-PAGE gel between acid- and alkali-induced LMGs. The only HCl-induced LMGs showed completely DNA-free which was confirmed by qPCR. The biofilm mass was markedly reduced in the NaOH- and HCl-induced LMGs when compared with that in wild-type *Lm*. Murine macrophages (RAW.264.7) exposed to the NaOH-induced LMGs at 3.7×10^6 CFU per ml showed higher cell viability than HCl-induced LMGs and wild-type *Lm*. Except for IL-1β mRNA, TNF-α, iNOS, IFN-γ, IL-6 and IL-10 mRNAs were more induced in the RAW.264.7 cells exposed to the NaOH-induced LMGs than HCl-induced LMGs. Both the HCl- and NaOH-induced LMGs were showed their capacities as potential vaccine candidates to prevent listeriosis.

[This work was supported by the Human Resource Tranining Program for Regional Innovation and Creativity through the Ministry of Education and National Research Foundation of Korea (2015035949)]

D020

Protective Immunity against Listeriosis in Rats, Provided by Chemically-induced *Listeria monocytogenes* Bacterial Ghosts (LMGs)

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Listeria monocytogenes (*Lm*) bacterial ghosts (LMGs) were generated by chemically induced lysis and the method is based on MIC of HCl and NaOH, respectively. To investigate the chemically induced LMGs as potential vaccine candidates, mice were divided into three groups such as PBS buffer injected group A (negative control) and subcutaneously immunized groups B (HCl-induced LMGs) and C (NaOH-induced LMGs). Animals were immunized three times at two-week intervals. Two weeks after the last immunization, all animals were challenged orally with 2×10^7 CFU of virulent *Lm*. Significantly higher levels of IgG antibody were induced in groups B (3.7 fold) and C (3.8 fold) on week 8 when compared with that of Group A. The highest percentage of $CD4^+$ T-cell populations were observed in group B mice, while the highest percentage of $CD8^+$ T-cell populations were observed in group C mice. However, the number of $CD4^+$ and $CD8^+$ T-cell populations were not significantly different between groups B and C. Most importantly, bacterial loads in both of LMGs-immunized groups were significantly lower than non-immunized control group after virulent *Lm* challenge. These results suggest that immunization with LMGs induces both humoral and cell-mediated immune responses and provides protection against virulent *Lm*.

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D021

Differences of Biofilm Formation in Soft Contact Lenses of *Staphylococcus epidermidis*

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Contact lens related bacterial infection may increase the risk of eye diseases including keratitis, corneal ulcer. Biofilm formation is important factor affecting the pathogenicity of bacteria related to these diseases, and biofilm formation is influenced by various environmental conditions. In this study, we investigated differences in biofilm formation between two materials of soft contact lens of *Staphylococcus epidermis*. The material of contact lenses was used as etafilcon A and hilafilcon B material. The XTT assay and dry weight measurement were used to compare the amounts of biofilm formation. Reverse transcription PCR and real time PCR were used to compare the expression level of genes related to biofilm formation. The adhered *S. epidermidis* was observed by scanning electron microscope. The biofilm formation was higher on the surface of etafilcon A than on the surface of hilafilcon B when measured by the XTT assay and by dry weight measurement. In the comparison of gene expressions of icaA, icaB, icaC, icaD, and arcA was higher on etafilcon A than on hilafilcon B. When observed with a scanning electron microscope, it was confirmed that *S. epidermidis* was more prolific and formed thicker layers on the surface of etafilcon A than on the surface of hilafilcon B. This suggests that *S. epidermidis* is more active in the formation of biofilm than etafilcon A material than hilafilcon B material, which may affect the pathogenicity of soft lens related eye disease.

D022

Effects of Antibiotic Treatment on the Host Immune Responses via the Alteration of Gut Microbiota

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Nowadays, antibiotics are widely used to control various infectious diseases. However, antibiotics could occur the significant dysbiosis of normal flora on the host gut because of their strong antibacterial effects. Here, to investigate the changes in host gut microbiota and immune responses caused by the treatment of antibiotics, we administered vancomycin, known as the effective antibiotic to the wide spectrum of bacteria, to mice for 14 days and analyzed microbial communities and immunological markers including *Tbet*, *Ifng*, *Gata3*, *IL4*, *Rorgt*, *Il17f*, *Foxp3*, and *Tgfb1* in gut samples. Therefore, we confirmed that the diversities of gut microbiota were significantly decreased in the vancomycin-treated group. Moreover, the abundance of *Akkermansia* sp., the dominant species in this group, showed significant correlations with the expression of Th17 cell related cytokine *Il17f*. These results suggest that the treatment of antibiotics could affect host immune responses strongly because of the drastic alteration in gut microbiota.

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D023

Salt-resistant Antimicrobial Peptide with Potent Antibacterial and Antibiofilm Activity against Multidrug-resistant *Acinetobacter baumannii*

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The emergence of multidrug-resistant pathogens is a serious public health concern. Increased antibiotic resistance requires the development of new antibacterial agents. Antimicrobial peptides are excellent candidates for the development of therapeutic agents. We investigated magainin 2, a compound derived from African clawed frogs (*Xenopus laevis*) that is known to have antimicrobial activity. Magainin 2 showed potent antibacterial activity against *Acinetobacter baumannii* strains and exhibited high stability at physiological salt concentrations. In addition, this peptide showed no toxicity against mammalian cells. Magainin 2 inhibited biofilm formation and eliminated formed biofilms. Increased permeability of the outer and cytoplasmic membranes of *A. baumannii* was confirmed by NPN uptake and DisC3-5 depolarization assays. These results indicate that magainin 2 has potential as an effective therapeutic agent against multidrug-resistant *A. baumannii*.

D024

Immuno-modulation Effects of Probiotics on Colonic Inflammation Using the Preventive Animal Model

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Inflammatory bowel disease (IBD) is a disease described by prolonged inflammation and malfunction of the gastrointestinal (GI) tract. The use of probiotics has become more popular in improving GI functions. Still, their effects on preventing GI disease, including IBD, have not been fully studied. In this study, to evaluate the preventive effects of probiotics on colitis, we initially administered 1 x 10^9 CFU/ml of each probiotic species (*Lactobacillus rhamnosus*, *Lactococcus lactis*, and *Bifidobacterium longum*) to female C57BL/6 mice for 7 days. We then induced the acute colitis in the mice using dextran sulfate sodium (DSS) and continued to treat probiotics until we sacrificed the mice on day 20. Phenotypic changes of mice, such as the weight and the colon length, were monitored during the experiment. Expression of intestinal inflammatory cytokines was measured by Real Time-PCR. We observed a noticeable alteration in gene expression of pro- and anti-inflammatory cytokines in probiotics-treated mice groups. Especially, *B. longum*-treated group showed significant reduction in TGF-β1 and Foxp3 gene expression compared to the others. These results suggest possible immune-modulation effects of probiotics on hosts, and the potential use of probiotics for prevention of colitis.

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D025

Virulence Profiles in *Pseudomonas aeruginosa* Blood Isolates

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Pseudomonas aeruginosa is a notorious pathogen in clinical settings for its virulence and multidrug resistance. Virulence of *P. aeruginosa* is known to be caused by multiple virulence factors and this study was to evaluate virulence profiles of *P. aeruginosa* blood isolates collected from six general hospitals participating in Korea Global Antimicrobial Susceptibility System (Kor-GLASS) between May 2016 and April 2017. A total of 126 *P. aeruginosa* blood isolates were assessed for the 20 virulence factors by real-time PCR array and ability to form biofilm was evaluated by using crystal violet. The capacity to form biofilm was grouped into four: 24 isolates (19.5%) had relatively high ability to form biofilm, 52 isolates (42.3%) form the biofilm in a moderate level, 28 (22.8%) isolates form less biofilm, and 19 (5.4%) isolates had no ability to form biofilm. Among the tested virulence factors, almost all the isolates harbored lasB (98.4%, n = 124), rhamnolipid B (93.7%, n = 118), type II secretion system (92.9%, n = 117), exoenzyme T (92.9%, n = 117), and siderophore (91.3%, n = 115), while few isolates carried type III secretion system (9.5%, n = 12). The virulence profiles are now assessed for correlation with outcomes of patients with *P. aeruginosa* bloodstream infection.

D027

NR1C1 Activation Promotes Lipid Catabolism and Mitochondrial Respiratory Function in Macrophages during Mycobacterial Infection

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The role of peroxisome proliferator-activated receptor a (PPAR-α, also known as NR1C1) in innate host defense is largely unknown. In this study, we show that PPAR-α is essential for antimycobacterial responses via activation of transcription factor EB (TFEB) transcription and inhibition of lipid body formation. PPAR- agonists promoted autophagy, lysosomal biogenesis, phagosomal maturation, and antimicrobial defense against *Mycobacterium tuberculosis* or *M. bovis* bacillus Calmette-Guérin. PPAR-α agonists regulated multiple genes involved in autophagy and lysosomal biogenesis, including *Lamp2*, *Rab7*, and *Tfeb* in bone marrow-derived macrophages. Silencing of TFEB reduced phagosomal maturation and antimicrobial responses during mycobacterial infection. Moreover, PPAR-α activation promoted lipid catabolism and fatty acid β-oxidation in macrophages during mycobacterial infection. Taken together, our data indicate that PPAR-α mediates antimicrobial responses to mycobacterial infection by inducing TFEB and lipid catabolism.

D028

Oral Administration of *Lactobacillus crispatus* Isolated from Human Vagina Attenuates Allergic Responses in a Mouse Model of Asthma

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Development of asthma is affected by the microbiota modulating immune and metabolic functions of host. Epidemiological evidences showed frequency of asthma was less in infants delivered through vaginal tract, which is enriched with *Lactobacillus* spp. in healthy women, than ones by Caesarean section. In this study, we hypothesized that exposure to vaginal *Lactobacillus* spp. can be helpful to alleviate asthma and investigated the effect of *Lactobacillus crispatus* (Lc) on mitigating asthmatic responses induced by ovalbumin (OVA) in a mouse model. Isolated Lc strain from a healthy Korean woman was orally gavaged to mice for four weeks (approximately 1 x 10^9 CFU/mouse/day). All mice were sensitized with OVA and alum through i.p. (day 7 and 21), followed by OVA challenge through nasal injection (day 28–30). The airway hyper-responsiveness to methacholine was assessed to measure lung function. After sacrifice, lung microbiome and immune cell profile in lung tissue were analyzed by 16S gene sequencing and flow cytometry. Lung tissue was stained with H&E and eosinophil infiltration around bronchioles was decreased in Lc-administered group. The relative expression levels of *Il-5*, *Il-13*, *Siglec-F* and *T-bet* decreased in lung tissue of Lc-administered mice. Taken together, Lc alleviated asthmatic responses provoked by OVA in a mouse model and can be applied to a microbiome-based therapy for asthma.

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D029

Diversity of Tetracycline Resistance Gene and Genotyping of Isolated *Paenibacillus larvae*, the Cause of American Foulbrood in Honey Bees

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Center for Honeybee Disease Control, Animal and Plant Quarantine Agency

American Foulbrood (AFB) disease caused by the spore-forming bacterium *Paenibacillus larvae* is a highly contagious disease affecting the larval and pupal stages of honey bees (*Apis mellifera* L.). AFB is the most widespread and destructive of the brood diseases, and possesses unique problems for prevention and control because the spores can remain viable for long periods and survive environmental adversities. In addition, *P. larvae* has been increasingly resistant to tetracycline. Strains were isolated from AFB-positive samples detected by polymerase chain reaction (PCR) with AFB primer set. Five strains designated RDA, 1-3, 18-1, 19-1, and 19-2 were identified *P. larvae* and ATCC 9545 was used as reference strain of *P. larvae* in this study. We analyzed the tetracycline-resistant genes and genotyping of *P. larvae* in Korea for the first time. We confirmed 6 of 16 tetracycline resistance genes: *tet(B-2), tet(C-2), tet(G-1), tet(L), tet(M), tet(K)* resistance in 5 strains. Genotyping of isolates was performed by PCR with primers corresponding to enterobacterial repetitive intergenic consensus (ERIC) element. Using ERIC-PCR technique different genotypes could be differentiated. These genetic analyses will be very helpful for understanding the relationship between genotypes and phenotypes of tetracycline resistance and transmission in farm, local, national, and international levels of AFB caused by *P. larvae*.

D030

Evaluation of the Efficacy of RNA Interference against Korean Sacbrood Virus

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Sacbrood virus (SBV), a causative pathogen of larval death in honeybees, is one of the most devastating diseases in bee industry throughout the world. Since 2010, the Korean Sacbrood virus (KSBV) caused great losses in Korean honeybee (*Apiscerana*) colonies. Sacbrood virus disease was caused by infection of Sacbrood virus, and providing sufficient and good dietary, enhancer of honeybee immune system and inactivator of Sacbrood virus have been applied to prevent and control SBV infection. RNA interference (RNAi) is a gene-silencing technology by which small double-stranded RNAs are used to target the degradation of RNA with complementary sequence. In this study, we found the prevention of SBV infection by feeding with double-stranded RNA both in laboratory and in the apiary for preventive purpose. dsVP1 was ingested with sugar solution by feeding biweekly on the apiary near a SBV-occurred apiary. The mortalities and viral loads were observed and analyzed by real-time PCR at RNAi injection period. As a result, once the typical SBV infection symptoms are confirmed, immediate burning or other control measures to prevent the spread of this devastating SBV virusare the best measure to minimize the loss of bee hives in SBV-infected apiaries and prevent the spread of the apiaries near SBV-infected apiaries of this devastating SBV virus.

D031

Genomic Analysis of Ketoconazole Resistance in *Malassezia restricta*

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Malassezia restricta is an opportunistic fungal pathogen on the human skin and associated with various skin diseases including seborrheic dermatitis and dandruff, which are usually treated with ketoconazole. In this study, we clinically isolated ketoconazole resistant *M. restricta* strains (named KCTC 27529 and KCTC 27550) from dandruff patients. To understand the ketoconazole resistance in the isolates, their genomes and transcriptomes were sequenced and compared with the susceptible reference strain *M. restricta* KCTC 27527. Comparative genome analysis identified 2 and 4 point-mutations in the coding region of the *ERG11* homolog in *M. restricta* KCTC 27529 and KCTC 27550, respectively. In addition, comparative transcriptome analysis revealed that the *ERG11* homolog is upregulated in *M. restricta* KCTC 27550, and the *PDR5* homolog is upregulated in both *M. restricta* KCTC 27529 and KCTC 27550. Our biochemically analysis revealed that ergosterol contents of the resistant strains were not altered suggesting no contribution of upregulation of the *ERG11* homolog. However, drug efflux was highly activated in *M. restricta* KCTC 27529 implying that upregulation of the *PDR5* homolog may contribute ketoconazole resistance of the resistant strain. Although further analysis is required, our data implies that mutations of the *ERG11* homolog or alteration of expression of the *PDR5* homolog is one of the main mechanisms of ketoconazole resistance in *M. restricta*.

D032

The Small Molecules Suppress the Pathogenesis through Inhibiting Morphogenesis in *Candida albicans*

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As an opportunistic human pathogen *Candida albicans* colonizes the human mucosal surface and skin. The morphogenesis is regarded as a profound virulence factor. This ability which is changing its morphology from yeast to hyphae in various environmental conditions is highly valuable when it penetrate to human host and overcome against human immune system. As a chronic drug therapy used for many patients, antifungal drug tolerance is magnified over the past few years. The development of new antifungal drugs which has different action mechanisms from pre-existing drugs is required to solve antifungal drug resistance problem. Here, we demonstrate the novel small molecules which are derived from a V-ATPase inhibitor Bafilomycin A1 as putative antifungal drugs for *C. albicans.* The inhibition of growth and suppression of morphological transition in various hyphae inducing conditions were shown when small molecules treated to *C. albicans.* Moreover, in the candidiasis murine model, molecule C treated mice have shown 100% survival from *C. albicans* infection. From the series of tests to estimate antifungal activity shown the evidence that the small molecules can be used for antifungal drugs.

D033

Subunits of Vacuolar ATPase (Vma4 and Vma10) are Involved in Hyphal Morphogenesis and Virulence in *Candida albicans*

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It is well known that hyphal morphogenesis of *Candida albicans*, a human opportunistic pathogenic fungus is important for the onset of *C. albicans* pathogenesis. In this study, it is shown that the filamentous growth critical for the pathogenesis of *C. albicans* requires vacuolar H^+-ATPase functions. Using biochemical analyses, it was determined that the Vma4p and Vma10p were increased in hyphal growing cells compared to that in yeast growing cells. *VMA4* and *VMA10* encode putative E and G subunits of a V-ATPase complex. Deleting *VMA4* or *VMA10* abolished vacuolar functions, such as endosomal acidification and trafficking. Moreover, Vma4 and Vma10 are important for morphological conversion. These deletion mutants were also characterized as avirulent in a mouse model of systemic infection. Furthermore, *VMA4* and *VMA10* deletion strains showed hypersensitivity in the presence of antifungal drugs such as fluconazole, terbinafine and amphotericin B. Based on these findings, Vma4 and Vma10 are not only involved in vacuole biogenesis, hyphal formation, as well as pathogenesis, but also are good targets for antifungal drug development in *C. albicans.*

D034

Antibiotics Susceptibility Test of *M. pneumoniae* for Standardization

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Mycoplasma pneumoniae is a bacterial respiratory pathogen that infected to children in their school year. Recently, emerging and expansion of resistant strains to antibiotics is an important issue in East Asia region.
Resistant strains on macrolides are usually detected by sequence variations of target genes but their susceptibility range also have to be verified. For this, we have performed susceptibility test by published standardized method using microdilution assay. 96-well plate was divided into drug test zone and control zone. In drug test zone, 4 drug was simultaneously tested as dulication until 1:128 dilution. In control zone, 4 control factors were included. Drug test range for macrolides was 1–0.004 μg/ml and for quinolone/tetracycline was 16–0.06 μg/ml. Susceptibility result is observed when color of growth control well was changed to yellow. MIC value was determined as the drug concentration of the well showing no color change right before well showing yellow color change.
After susceptibility tests, 12 M. pneumoniae resistant strains were selected. MIC on macrolides of susceptible strains was below 0.0001 μg/ml. However, MIC of 12 resistant strains showed more than 10 μg/ml. It was confirmed that all the resistant strains showed A2063G type of gene mutation in 23S rRNA gene domain V. In the case of quinolones, there were no resistant strain.
[This study was supported by intramural grant of Korea Centers for Disease Control and Prevention (4800-4845-300)].

D035

MLVA Typing and Analysis of *Bordetella pertussis* Strains

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Pertussis, known as whooping cough, is the respiratory disease caused by infection of *Bordetella pertussis*. In Korea, the incidence of pertussis is highly increased from 2009 and showed periodic increase pattern with every 3 or 4 years interval. One of the reasons of pertussis increasing is that currently distributing strain showed different genotypes with vaccine strain. Therefore, the genotype monitoring of current strain is important to evaluate current condition. In this study, we have performed MLVA typing and compared with previous MLST data. Finally, MLVA-MLST-P3 combined DB was constructed and anlayzed. As the results, 23 MLVA types were confirmed. Major MLVA types were MT27 (46%) and MT29 (22%). MT29 type was mainly isolated in 2000–2009 but MT27 was recently isolated type from 2010. As compared to MLST data, MT29 was AST type 1 with ptxP1 type and MT27 was AST-2 type with ptxP3 type. When we integrated these DB in parallel (HAMP DB), 26 combined types and one major type 13 were confirmed. As the conclusion, we have updated the genotyping DB of *B. pertussis* isolates using MLVA type analysis. As compared to previous MLST DB, diversity index of MLVA typing DB increased to 0.73. When combined to integrated parallel DB, the number of types were enlarged to 26 types and diversity index was also increased to 0.79.
[This study was supported by intramural grant of Korea Centers for Disease Control and Prevention (4800-4845-300)].

D036

Analysis of Pertactin Deficiency in *Bordetella pertussis* Strains

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Pertactin is an cell surface protein of *Bordetella pertussis* and an important component of acellular vaccine. The major role of pertactin is adhesive of bacterial cell to epithelial cells in respiratory tract. Recently pertactin-deficient isolates have been reported from France, Italy, Japan, Finland and USA. From these reports, it was assumed that pertactin-deficient strains are more selectable among vaccinated population because pertactin mediated immune response does not occur. Therefore, this study is performed to determine the distribution status of pertactin-deficient strain in Korea. For these, western analysis on pertactin protein and sequencing analysis of pertactin gene was performed. As the results, pertactin-deficient strains were also confirmed in Korean isolates. The observed sequence variations were G insertion at 1,180 bp, A insertion at 1,341 bp, C insertion at 253 bp and G insertion at 1,322 bp. As the further study, sequence variation study should be enlarged to 2.7 kb of whole pertactin gene.
[This study was supported by intramural grant of Korea Centers for Disease Control and Prevention (4800-4845-300)].

D037

Inhibition of *Pseudomonas aeruginosa* Biofilm Formation by Combination Treatment with Linoleic Acid and Tobramycin

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P. aeruginosa is known to cause serious chronic lung infections in cystic fibrosis patients. Moreover, *P. aeruginosa* biofilms are critical medicinal problems on the medical devices, contact lens, and artificial implants. However, the biofilm cells are 10–1,000 times more resistant to antibiotics than planktonic counterparts and it is extremely difficult to eradicate biofilms from infected patients. In addition, excessive amounts of antibiotics or antimicrobial agents may exhibit antibiotic resistance, and application of these compounds below the inhibitory level may cause biofilm stimulation. In this regard, new strategies have been tried to inhibit biofilm development mechanisms without affecting bacterial growth. Linoleic acid is one of the unsaturated fatty acids. And it has been found in plant, oil etc. Chemical structure of linoleic acid is a polyunsaturated omega-6 fatty acid, an 18-carbon chain with two double bonds in cis configuration. Our results showed that linoleic acid inhibited bacterial biofilm formation under static and flow conditions via quorum sensing (QS) interference and reduction of a secondary messenger, cyclic diguanylate (c-di-GMP) without affecting bacterial growth. In addition, combination treatment with linoleic acid and tobramycin more effectively inhibited bacterial biofilm formation than single treatment. This study investigating a molecular mechanism of biofilm inhibition can be beneficially applied to systems of medicinal biofilm problem.

D038

Prostate Tumor Induced Uremia and Secondary Gas-forming Bacterial Infection in a Wild Boar (*Sus scrofa*)

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One male wild boar (*Sus scrofa*) appeared in the hiking trail of the Bukhansan National Park, which was fully grown weighing 200 kg, panting severely, and bleeding from nose, mouth and anus. During the rescue, the dead wild boar was transferred to the Pathology Laboratory for full autopsy. When thoracic and peritoneal cavities were opened, the strong ammoniac odor came up, which is one of typical characteristics of the patient with uremic syndrome. Afterwards, the huge bulged bladder caught the eyes, which there was a massive prostate tumor around the prostate urethra. The volume of urine reached 1.75L. The prostate tumor appeared to be an active working gland producing a large amount of secretions.
Morphologically, the tumor is “acinar type” where tumor is thought to arise from or recapitulate prostatic acini. The acinar type (usual type) is characterized by back-to-back proliferation of small to intermediate sized tumor acini with scant to moderate intervening stroma.
Blood was not clotted, all organs were necrotic and the texture was like sponges containing gas, which were lesions suspicious of infection by gas-forming bacteria. The bacterium was identified *Clostridium novyi* after microbiology tests; bacterial culture (aerobic/anaerobic), serotyping, differential PCRs, and 16s rRNA sequencing. The etiology is presumed to be the uremia caused by the prostate tumor, which weakened blood vessels and organs, resulting in secondary infection. To the best of the authors’ knowledge, this is the first description of the prostate tumor induced uremia and a secondary bacterial infection resulting in severe internal organ malfunction and death in a wild boar in Korea.

E003

Profiling the Anthranilate Production of *Pseudomonas aeruginosa* along Long-term Growth

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Pseudomonas has remarkable metabolic versatility and colonizes a variety of habitats. They are of great interest because of their metabolic potential and importance in the pathogenic infection. *P. aeruginosa,* an opportunistic human pathogen, produces various metabolites, such as phenazines, pyocyanin, quinolones, acyl-homoserine lactones, anthranilate, and so on, and most of them are secreted and accumulated during growth. One of them, anthranilate is an important intermediate for the synthesis of tryptophan and *Pseudomonas* quinolone signal, and affects biofilm formation of various bacteria. We therefore investigated the production and accumulation of anthranilate along long-term growth of *P. aeruginosa*. The secretion of anthranilate remains low until *P. aeruginosa* reaches stationary phase, but it begins to secrete and accumulate to a high level as the stationary phase continues. Interestingly, the level of anthranilate rapidly dropped again when the stationary phase persisted longer. Since anthranilate can be degraded via the tri-carboxylic acid (TCA) cycle in *Pseudomonas* spp. by the function of anthranilate dioxygenase complex encoded by antABC operon, we investigated the role of antABC in controlling the anthranilate level. For this, we measured the expression of antABC throughout the growth of *P. aeruginosa*, and investigated the anthranilate production pattern in antABC mutant and qscR mutant that expresses antABC to high level.

E004

Quorum Sensing-mediated Post-secretional Activation of Extracellular Proteases in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa secretes multiple proteases as virulence factors, and most of them are quorum sensing regulated, for examples, protease IV (PIV, a lysyl-endopeptidase), elastase A (LasA), and elastase B (LasB). We found the activity of PIV which overexpressed in QS mutant was inhibited by its own propeptide, and LasB counteracted this inhibition by degrading the propeptide. LasA is a zinc metallopeptidase specifically disrupt the glycine-glycine peptide bonds and also with a low elastinolytic activity. Present study found that LasA expressed in QS mutant also has severe reduction of activity. Like PIV, LasA purified from the QS mutant (M-LasA) had much lower activity than LasA purified from wild type (P-LasA). We found that most of LasA from QS mutant was in the form of pro-LasA, suggested that some QS-dependent factors are related to the extracellular maturation of LasA by cutting off the propeptide. We tested QS-dependent proteases of *P. aeruginosa* and found that LasB and PIV can cutting off the propeptide from LasA and restore its activity. We suggested that the post-secretional activation of PIV and LasA in a cascade form, LasB, as a trigger, firstly activate PIV by degrading its propeptide, then the activated PIV facilitate the maturation of LasA by cutting off and degrading its propeptide. Meanwhile, LasB also can activate the LasA, directly. More interestingly, human elastase B also can restore LasA activity which overexpressed in QS mutant.

E005

Bacillus licheniformis Uses an Unusual SufU Paralog with High-affinity Zn Binding for the Biogenesis of Iron-sulfur Cluster under Zn-depleted Growth Conditions

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Iron-sulfur clusters are ubiquitous inorganic cofactors which are employed to fulfill a variety of essential cellular functions. It is now appreciated that *Bacillus subtilis* engages only SUF system (SufCDSUB) as a [Fe-S] biogenesis machinery, and SufU has been characterized as a Zn-utilizing sulfur transferase. In this study, our initial genome-wide analysis data indicate that the industrially important strain *B. licheniformis* contains an additional *sufU* gene (*sufU2)* in addition to authentic *sufU* (*sufU1*). Interestingly, the expression of *sufU2* was specifically induced under Zn-limited growth conditions, suggesting the hypothesis that SufU2 has evolved fine-tuned to strongly bind Zn. As expected, SufU2 showed remarkably higher affinity for Zn and less vulnerability to oxidation by H_2O_2, when compared to those of SufU1. Inversely, SufU2 exhibited relatively lower sulfur transferase activity and impaired complementation effect in *sufU1sufU2* null mutant cell. Together, those results imply the fact that the increase in Zn-binding affinity could negatively affect the sulfur transferase activity of SufU. Further site-directed mutagenesis studies of putative Zn-binding ligands provide an evidence that the N-terminal Cys residue of SufU is mainly involved in the role of sulfur-receiving but not in control of Zn-coordination.
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E006

Functional Analysis of Cytosolic Monothiol Glutaredoxin Grx4 in *Schizosaccharomyces pombe*

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Grx4 in *Schizosaccharomyces pombe* is a multidomain monothiol gluta-redoxin. It consists of two major domains; a thioredoxin (TRX)-like domain near N-terminus and a glutaredoxin (GRX)-like domain near C-terminus. The TRX-like domain contains a WAAPC^{35}K sequence which is similar to thioredoxin active site motif WCGPCK and the GRX-like domain contains a ^{172}CGFS active site motif. In case of active Cys in GRX-like domain, it is known to serve as a Cys ligand for [2Fe-2S] in grx4 homodimers. Well known function of grx4 is to regulate iron homeostasis via two repressor type transcription factors, Fep1 and Php4. Grx4 protein serves as an inhibitory partner for Fep1 in response to iron deficiency, whereas it is required for the inhibition of Php4 under iron-replete conditions. In this study, we isolated grx4 deletion mutant under anaerobic condition and performed phenotypical analysis. Grx4Δ strain was only able to grow in the absence of oxygen. Next, mitochondrial morphology was observed using mitotracker. Mitochondria in grx4Δ was severely fragmented under anaerobic condition. Based on a previous report that cytosolic protein aggregants can induce mitochondrial fragmentation, we examined whether overexpression of deaggregase Hsp104 could rescue the defects of grx4Δ. Surprisingly, Hsp104 overexpression overcame the sensitivity to aerobic growth of grx4Δ. How Hsp104 alleviates the air-sensitive phenotype of grx4Δ requires further investigation.
[Supported by NRF]

E007

Crystal Structure of YpgQ in Complex with Metal and Nucleotide

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The histidine-aspartate (HD) domain is characterized by a metal-coordination motif of two histidine residues and two aspartate residues and functions in the nucleotide metabolism and signal transduction. YpgQ is a bacterial protein that contains the HD domain. Here, we report the crystal structure of YpgQ in complex with nucleotide. YpgQ simultaneously accommodates a metal ion and a nucleotide molecule in a cavity, which is created between the two lobes of YpgQ. The metal ion is coordinated by histidine and aspartate residues and is closely positioned to the β-phosphate group of the nucleotide potentially to facilitate nucleophilic attack on the β-phosphorus of the nucleotide. Moreover, our modeling and mutational studies on YpgQ orthologs unraveled the significance of peripheral substrate-binding site in substrate preference, providing an enzyme engineering platform to modulate substrate specificity.

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E008

Pathogenicity Control of *Staphylococcus aureus* Using the Transcriptional Regulation Mechanism of Peroxide Sensor, PerR

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Staphylococcus aureus is a life-threatening pathogenic bacterium that causes severe human diseases. The cells of human immune system secrete high concentration of H_2O_2 to cope with *S. aureus* infection. PerR is a homodimeric transcription factor that plays a role in H_2O_2 resistance by up-regulating antioxidant enzymes which defend against the oxidative stress exerted by human immune system. PerR is also related to virulence in *S. aureus*: many studies show that *S. aureus perR* null mutant strain has decreased virulence in various animal models when compared to wild type. Thus, we utilized dimerization domain (DD) of PerR to inhibit the repressor activity of PerR. We show, by using bacterial two hybrid assay system, that $PerR_{DD}$ specifically interacts with PerR, but not with Fur or Zur. This result indicates that DD can efficiently be used as a specific antirepressor. Indeed, the expression of $PerR_{DD}$ in *S. aureus* led to the up-regulation of PerR regulon genes such as *mrgA*, *ahpCF*, and *katA*. Interestingly, *S. aureus* wild type strain expressing $PerR_{DD}$ showed the reduced virulence when compared with that of *S. aureus* wild type in an invertebrate animal *C. elegans* model. These results suggest that PerR is an attractive target to reduce the virulence of *S. aureus*.

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E009

Effect of Outer Membrane Porins on Antibiotic Resistance in *Escherichia coli*

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The recent emergence of carbapenem-resistant Gram-negative pathogens, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, poses a serious threat to public health worldwide. Unlike Gram-positive bacteria, Gram-negative bacteria have an additional membrane (outer membrane), which offers a strong barrier to block the transport of antibiotics. Porins are outer membrane proteins associated with modulating cellular permeability and antibiotic resistance. Some porins is associated with antibiotic resistance, whereas loss of other porins lead to decreasing the minimal inhibitory concentrations (MICs) of several antibiotics. To understand the relationship between porins and antibiotic resistance, we examined the MIC change of various antibiotics by deletion of porins in *Escherichia coli*. Liposome swelling assay and envelope integrity analysis showed that each antibiotic may be mainly transported by a specific porin and three porins (OmpA, OmpC, and OmpF) are associated with membrane integrity as well as transport of antibiotic. These results indicate that various porins of outer membrane have each specific feature and three major porins, including OmpA, OmpC, and OmpF, play an important role in the regulation of membrane integrity.

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E010

Activation of Isocitrate Dehydrogenase in the Citric Acid Cycle by Enzyme IIA^{Ntr} of the Nitrogen PTS

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In addition to the canonical phosphoenolpyruvate (PEP) phosphotransferase system (PTS) for sugar transport, many *Proteobacteria* possess the so-called nitrogen PTS that transfers a phosphate group from PEP over enzyme I^{Ntr} (EI^{Ntr}) and NPr to enzyme IIA^{Ntr} ($EIIA^{Ntr}$). Because the nitrogen PTS lacks membrane-bound components, it seems to function exclusively in a regulatory capacity. $EIIA^{Ntr}$ has been implicated in a variety of cellular processes, such as potassium homeostasis, nitrogen metabolism, carbon metabolism, and poly-β-hydroxybutyrate accumulation in many *Proteobacteria*. In this study, we provide another role related to $EIIA^{Ntr}$. Cells lacking $EIIA^{Ntr}$ exhibited the significant growth defect in minimal medium containing TCA cycle metabolites as a sole carbon source. The $EIIA^{Ntr}$ was found to be necessary to the normal growth of cells in this medium. To search for a factor that is regulated by $EIIA^{Ntr}$, we used the *in vivo* ligand fishing using formaldehyde crosslinking. The binding partner was identified as Icd, an isocitrate dehydrogenase in the TCA cycle. Through bacterial two-hybrid analysis and pull-down assay, we found that $EIIA^{Ntr}$ forms a complex with Icd. In addition to, we found that $EIIA^{Ntr}$ activates Icd activity. Thus, our data suggest that the TCA cycle is regulated by $EIIA^{Ntr}$ through direct interaction with Icd.

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E011

Structural and Biochemical Insights into the Molecular Mechanism of an Archaeal-like Chaperonin from a Thermophilic Bacterium

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The chaperonins (CPNs) are a ubiquitous megadalton sized hollow complex with two rings, each forming an inner cavity that opens and closes to encapsulate non-native proteins. CPNs are assigned to two groups that share conserved sequence motifs but have distinct allosteric mechanisms and co-chaperones. In Group I CPNs a detachable co-chaperone, GroES, closes the chambers whereas in Group II a built-in lid closes the chambers. Group I CPNs have a bacterial ancestry, whereas Group II CPNs are archaeal in origin. Here, we describe open and closed crystal structures of a novel CPN representing a deep branch of the CPN phylogenetic tree, which we refer to as Group III. Group III CPNs are divergent in sequence and structure from both Group I and Group II, but are closed by a built-in lid like Group II CPNs. The nucleotide-sensing loop, present in both Group I and Group II CPNs, is notably absent. We identified inter-ring pivot joints that articulate during ring closure. These Group III CPNs likely represent a relic from the ancestral CPN that formed distinct bacterial and archaeal branches. These results strengthen our inference that the Group III CPN clade represents an evolutionary relic from the divergence of the ancestral CPN into three paralogous groups.

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E012

CRP-mediated Regulation of *bipA* Expression at Low Temperature

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BipA is a widely conserved ribosome-associated GTPase and shares structural similarity with translational GTPases, including EF-Tu, EF-G, and EF4. In spite of its conservation in bacteria, *bipA* is not essential for cell growth under normal growth condition, but, at 20°C, deletion of *bipA* causes not only growth defects but also several phenotypic changes, such as capsule production, motility and ribosome assembly. Furthermore, in recent study, it was observed that expression of *bipA* was stimulated by low temperature, which could explain the cold-sensitivity of the *bipA*-deletion strain.

In this study, we aimed to elucidate how the expression of *bipA* is regulated at 20°C. We found a putative CRP-binding site through analysis of *bipA* gene upstream region and CRP directly bound to this site. In *crp*-deletion strain, expression of *bipA* didn't increase at 20°C. Furthermore, in *cyaA*- or *crr*-deletion strain, expression level of *bipA* didn't increase at 20°C. To determine whether CRP and/or cAMP are also cold-inducible, we measured change in cAMP level and expression level of *crp* at 20°C and found that the expression of *crp* gene was stimulated by low temperature, while cAMP level was not changed. Our findings demonstrate that expression of *bipA* is positively regulated by cAMP-CRP complex at low temperature and that increased level of *bipA* at 20°C is due to over-expression of *crp*, not increase in cAMP level.

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E013

Crystal Structure of Thioredoxin-fold Protein from *Deinococcus radiodurans*

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The redox proteins with thioredoxin-fold have diverse properties and functions. The bacterial DsbA protein, the most oxidizing protein among the thioredoxin family, catalyzes disulfide-bond formation during the folding of secreted proteins. *Deinococcus* species are well-known for their extraordinary resistance to ionizing radiation (IR), ultraviolet radiation (UV) and desiccating conditions. Due to their high level of resistance to radiation and desiccation, *Deinococcus* species have been studied for understanding the biochemical mechanisms responsible for their stress resistance. The effects of radiation or desiccation on cells, including DNA damage, are mainly caused by ROS. In fact, the damaging effects of ROS are not limited to DNA but oxidation and inactivation of proteins. Interestingly, while DNA from both radiation-sensitive and -resistant organisms is equally susceptible to radiation or desiccation, there is much less damage of proteins in *Deinococcus* compared to sensitive organisms. This shows that the resistance of *Deinococcus* species to high doses of radiation and other ROS-inducing conditions is largely related with the protection ability for protein damage. As a first step toward elucidating the relationship between protein disulfide-bond formation and radiation-resistance in *D. radiodurans*, we determined the crystal structure of thioredoxin-fold protein from *D. radiodurans* at 2.35 Å resolution.

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E014

Insights into the Inhibition of Class C β-Lactamases through the Adenylylation of the Nucleophilic Serine from Crystallographic and Kinetic Studies

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β-Lactamases hydrolyzing β-lactam antibiotics contribute to the antibiotic resistance. The use of combination with β-lactam antibiotics and β-lactamase inhibitors (BLIs), clavulanate, sulbactam, tazobactam, and avibactam, is an effective strategy to cope with β-lactamase resistance. BLIs are especially active against class A enzymes, displaying much less or no effect on other classes of β-lactamases except for avibactam, which also inhibits class C and some class D β-lactamases. In addition, the emergence of novel β-lactamases that are not inactivated by existing BLIs highlights the need of novel BLIs. One approach to reach the goal is to secure new chemical scaffolds that fit into the active site of β-lactamases in a different way from existing BLIs. Based on structural and biochemical observation, we demonstrate that the adenylylation of the nucleophilic serine residue is an operative strategy to inhibit the enzymes. These findings clearly indicate that acAMP is a promising lead compound to be exploited for the development of non-β-lactam irreversible covalent inhibitors with therapeutic efficacy. The atomic details about the interactions of AMP with the active sites of AmpC BER and CMY-10 will play an invaluable platform for structure-based optimization of the nucleotide scaffold for developing BLIs.

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E015

Mono-ubiquitination of Rps3 Mediated by Ubc4, Hel2 and Ubp3 Regulates Protein Quality Control in *Saccharomyces cerevisiae*

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In eukaryotes, when a ribosome complex is stalled during translation process, mono-ubiquitination of several ribosomal proteins has been recently known to be critical for ribosome quality control. Here, we report that the mono-ubiquitination of yeast Rps3 is tightly modulated by the reciprocal action between Hel2 E3 ligase and Ubp3 de-ubiquitinase for protein quality control. First, we corroborate that Rps3 is a substrate for ubiquitination by a specific E3 ligase Hel2 *in vivo* and *in vitro*. Through *in vitro* ubiquitination assays and gene knockout experiments, we found that Hel2 was a major E3 ligase targeting K212 of Rps3 on ribosome complex. We also found that Ubp3 was a de-ubiquitinase (DUB; ubiquitin specific protease) which could modulate Hel2 mediated Rps3 mono-ubiquitination. In addition, we found that Hel2 and Ubp3 appeared to be differently localized in ribosome complex after UV irradiation. However, rapamycin induced Rps3 mono-ubiquitination was found to be caused by Ubp3 sequestration into the autophagosome. Together, our results support a model in which coordinated ubiquitination and de-ubiquitination activities can finely balance the level of Rps3 mono-ubiquitination in ribosome associated quality control (RQC) and autophagy processes.

E016

Gcn4 Protein Which is a Critical Factor for Quality Control is Regulated by Ssb2 in *Saccharomyces cerevisiae*

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Yeast cells confront environmental stress by inducing the core regulator Gcn4 to control other genes involved in biosynthesis of amino acids and ribosomes. Gcn4 is a typical member of the bZIP family and binds directly as a homodimer to a conserved regulatory region of its target genes. Ssb2 was discovered as a suppressor for the mutant gcn4 with a decreased DNA-binding ability of target genes. Ssb2 is one of the Hsp70 family proteins which are responsible for protein quality control. It assists the proper folding of nascent polypeptide chains translated from the ribosomes. To characterize the mechanism of suppression for the mutant gcn4, we performed a multi-copy suppression screening using the yeast genomic library cloned in a high copy plasmid. We found that Ssb2 is shown to improve the expression of Gcn4 target genes by increasing the DNA-binding affinity of gcn4 mutants to their promoters under conditions of amino acid starvation. Protein level of Gcn4 was also increased at both the translational and post-translational level by overexpressed Ssb2. These findings suggest that Ssb2 is a critical factor in the first line of a protein quality control system that manages the synthesis and degradation of the Gcn4 protein.

E017

The SufS-SufE Complex of the Extremophilic *Fervidobacterium islandicum* AW-1 Plays an Important Role in Keratin Degradation

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The extremophilic eubacterium *Fervidobacterium islandicum* AW-1 can degrade native chicken feathers at 70°C under anaerobic conditions. However, its degradation mechanism remains unclear. Herein we compared the genome sequence of *F. islandicum* AW-1 with that of the closest strain *F. nodosum* Rt17-B1, revealing cysteine desulfurase (*Fi*SufS) and sulfur acceptor protein (*Fi*SufE) presumably involved in keratin degradation. Indeed, expression levels of the genes encoding *Fi*SufS and *Fi*SufE were >2-fold higher when cells were grown on native feathers than on glucose. The recombinant *Fi*SufS exhibited maximal activity at 90°C and pH 8, and its sulfur transfer activity was considerably increased in the presence of *Fi*SufE through the SufS-SufE complex formation. Site-directed mutagenesis revealed that two cysteine residues in SufS (Cys372) and SufE (Cys34) are essential role in catalysis. The three-dimensional structures of *Fi*SufS, *Fi*SufE and *Fi*SufS/SufE complex were resolved at 2.1Å, 2.1Å, and 2.5 Å resolutions respectively. Furthermore, both enzymes accelerated keratin degradation by the whole cell extract of *F. islandicum* AW-1, suggesting that the Suf system of *F. islandicum* AW-1 may contribute to the cleavage of cysteine disulfide bonds in recalcitrant feather keratins, which is prerequisite for sulfur metabolism under anaerobic conditions.

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F001

Comparative Genomic Analysis of *Geosporobacter ferrireducens* and Its Versatility of Anaerobic Energy Metabolism

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Members of the family *Clostridiaceae* within phylum *Firmicutes* are ubiquitous in various iron-reducing environments. However, genomic data on iron-reducing bacteria of the family *Clostridiaceae*, particularly regarding their environmental distribution, are limited. Here, we report the analysis and comparison of the genomic properties of *Geosporobacter ferrireducens* IRF9, a strict anaerobe that ferments sugars and degrades toluene under iron-reducing conditions, with those of the closely related species, *Geosporobacter subterraneus* DSM 17957. Putative alkyl succinate synthase-encoding genes were observed in the genome of strain IRF9 instead of the typical benzyl succinate synthase-encoding genes. Canonical genes associated with iron reduction were not observed in either genome. The genomes of strains IRF9 and DMS 17957 harbored genes for acetogenesis, that encode two types of Rnf complexes mediating the translocation of H^+ and Na^+ ions, respectively. Strain IRF9 harbored two different types of ATPases (Na^+-dependent F-type ATPase and H^+-dependent V-type ATPase), which enable full exploitation of ion gradients. The versatile energy conservation potential of strain IRF9 promotes its survival in various environmental conditions.

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F002

Function of SigR1, an ECF Sigma Factor, in *Streptomyces venezuelae* ATCC15439

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Widely known for producing diverse antibiotics and secondary metabolites, Streptomycetes are well studied microorganisms. *S. venezueale* ATCC15439 has special features; not only it produces a polyketide pikromycin and sporulates in liquid culture to near-completion, it also grows faster than *S. coelicolor*. Here, we studied further on ECF sigma factors of *S. venezueale* ATCC15439 to understand the complex relations between sigma factors. *S. venezueale* ATCC15439 is predicted to encode 43 sigma factors with conserved domain 2 and 4: 1 house-keeping sigma factor HrdB, 2 Group 2 factors (HrdA & HrdD), 6 Group 3 factors, and 34 Group ECF sigma factors. SigR, an ECF sigma factor that governs the thiol-oxidative stress response, is also known to be induced by translation-inhibiting antibiotics. In *S. venezueale* ATCC15439, SigR has two homologues: SigR1 and SigR2. Unlike SigR1, SigR2 was hard to investigate due to its low level of expression. Therefore, we chose to compare the functions of SigR1 and SigR whether they share common functions or have different functions. SigR and SigR1 have a protein sequence correlation exceeding 90% and have similar anti-sigma factor RsrA and RsrA1, respectively. However, our results show that they are induced by different stresses and the expression of *sigR1* is not regulated by *sigR*. In this respect, we conclude that SigR1 and SigR may have different functions, requiring more thorough and in-depth investigations.

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F005

Analysis of Dhh1 Functional Motifs in Pseudohyphal Growth and Ste12 Expression in *Saccharomyces cerevisiae*

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In response to nitrogen limitation, diploid cells of the yeast *Saccharomyces cerevisiae* undergo a dimorphic transition to pseudohyphal growth. The dimorphic transition is regulated by the mitogen-activated protein kinase (MAPK) which activates the filamentous specific transcription factor Ste12. Dhh1 controls pseudohyphal growth by regulating the STE12 expression at the translation level. Dhh1 is a DDX6-like RNA helicase which is known to be a component of P-bodies, a discrete cytoplasmic site for mRNA degradation. Dhh1 contains the DEAD box motifs and putative phosphorylation sites. The ATP hydrolysis and RNA unwinding activities have been shown to be important for Dhh1 function. We are interested in the question whether the functional motifs of Dhh1 affect yeast pseudohyphal growth by regulating STE12 translation. In order to understand how the translation of Ste12 is regulated by Dhh1 during pseudohyphal growth, the dhh1 deletion in Σ1278b background and the mutant strains of each domain were constructed. Site-directed mutagenesis was carried out at Thr10, Ser14 and Thr16 phosphorylation sites of Dhh1. The Q motif for RNA binding, ATPase A (Motif I), ATPase B (Motif II), RNA binding motif HRIGR and C-terminus of Dhh1 were also included in these mutational analysis. The phenotypes of Dhh1 mutations in pseudohyphal growth and Ste12 exprssion will be presented.

[Supported by grants from NRF]

F006

The Genetic and Functional Characteristics of *Lactobacillus plantarum* by Clusters

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Lactobacillus plantarum is a lactic acid bacterium that may promote animal intestinal health and is found in a wide variety of habitats. We investigated the genetic features different clusters based on SNPs of *L. plantarum* strains via pan-genomic methods. We compared the genomes of 108 *L. plantarum* strains that were available from NCBI's GenBank database. On the bases of SNPs from the core gene, 108 strains were clustered into five major group. Certain origins are weakly associated with SNP based group, however, each group have specifically enriched or depleted genes. We identified that there are critical differences in gene content and survival strategy among genetically clustered *L. plantarum* groups regardless of habitats. For example, the group with poor utilization of carbohydrate showed higher expression of genes involved in apoptosis and self defense mechanism.

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F007

Genomics Analysis of *Lactobacillus plantarum* Associated with Kimchi/Piglet Origin

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Lactobacillus plantarum is a member of the genus *Lactobacillus* and commonly found animal gut or fermented food. Antimicrobial substances from *L. plantarum* effect on Pathogenic bacteria. This is why it is useful as probiotics in livestock industry South Korea. While biological functions of probiotics are well known, their depending on the difference in origin genetic functions are still unknown. In this study we performed genomics analysis of *L. plantarum* with kimchi/pig origin through HTS (High Throughput Sequencing), we sequenced genome of 10 *L. plantarum* from kimchi and 9 *L. plantarum* from piglet. All Kimchi/Piglet genome sequences were annotated by RAST (Rapid Annotation using Subsystem Technology) and summarized their subsystems. Kimchi/Pig groups of genome size no observed significant difference. But Kimchi/Piglet groups observed significant difference in subsystems. To subsystems we identified significant difference in Kimchi/Piglet groups. Moreover we will be SNP analysis genome of *L. plantarum* with kimchi/Piglet origin.

[This study was supported by a grant from the Strategic Initiative for Microbiomes in Agriculture and Food, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea as part of the multi-ministerial Genome Technology to Business Translation Program (Grant ID : 914005-04) and Brain Korea 21 Plus Project.]

F008

Identification of Genetic Element behind the Formation of Multiple *cagA* Genotypes in *H. pylori*

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Helicobacter pylori is one of the most genetically diverse bacteria causing various gastric diseases. CagA is known to be one of the major virulence factor associated with gastric carcinoma and it has been recently found that some of the *H. pylori* strains including PMSS1 consist of a heterogeneous population in terms of *cagA* copies enabling them to express different virulence characteristics. Moreover, it has been suggested that the *cagA* homologous area (CHA) located both the upstream and downstream of *cagA* (CHA-ud, 449 bp) would be likely important for this numerical gene variation but the mechanism has yet to be revealed.

Hence, this study is conducted with the objective of generating isogenic mutants to identify the involvement of CHA-ud on the formation of multiple *cagA* genotypes. Initially, the loss of function of CHA-ud was postulated by generating PMSS1 mutants containing only a single CHA-ud at either upstream (SF) or downstream (SL) of *cagA*. The gain of function was postulated by generating G27 mutant with two CHA-ud at both upstream and downstream of *cagA* with contrast to its wild type (G27) which was reported of having a single *cagA* gene. Then single colonies were isolated and PCR-based screening was carried out for the detection of multiple *cagA* genotypes. Results suggested that the presence of CHA-ud at both upstream and downstream of *cagA* plays a critical role in this numerical gene variation of *cagA*.

F009

Comparative Genomic Analysis of Radiation Resistant Bacteria Isolated in Korea

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The genus *Deinococcus* was known for its remarkable resistance to a range of damage caused by the ionizing radiation, desiccation, and UV radiation. Similarly, the species of *Hymenobacter* and *Spirosoma* isolated from a soil sample collected in Korea showed resistance to gamma irradiation. In this study, strains *Hymenobacter sedentarius* DG5B^T, *Spirosoma montaniterrae* DY10^T and *Spirosoma radiotolerans* DG5A^T were compared with *Deinococcus radiodurance* R1 and the comparative genomic analysis of radiation-resistant bacteria such as *Hymenobacter*, *Spirosoma,* and *Deinococcus* was done compared with the non-radiation resistant bacterium *E. coli* K12. The genome was annotated by NCBI Prokaryotic Genome Automatic Annotation Pipeline and deeper protein analysis was done by PSI-BLAST. The genomes of radiation-resistant bacteria such as *Hymenobacter* and *Spirosoma* have the genes of DNA repair enzymes such as RecA, DNA gyrase, and Excinuclease ABC complex. The genome of resistant bacteria showed the presence of chaperonin proteins which involved in the protein repair. We expect that the comprehensive understanding of the mechanisms of damage repair in radiation resistant bacteria will provide the tool for better biotechnological application.

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F010

Regulation of Rsv1, an Important Regulator for Cell Survival under Glucose-starved Condition in Fission Yeast

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Nutrient starvation stress is one of the main stresses cells encounter, and cells can activate various signaling mechanisms to trigger transcriptional action for responding the starvation condition. Rsv1 is the zinc-finger transcription factor important for maintaining cell viability under glucose starvation condition in *Schizosaccharomyces pombe*. The transcription of *rsv1* gene increases after glucose deprivation, and Rsv1 protein level also increases after starvation in nucleus. To understand the regulation mechanisms for Rsv1, we immunoprecipitated Rsv1 protein in fission yeast for LC-MS/MS. We could find that Rsv1 has the phosphorylated serine residues of Sty1 MAP kinase. We could find that *rsv1* transcript level decreases in *sty1 ⊿* cells. Atf1 is the main effector transcription factor of Sty1, and we found that *rsv1* induction is positively regulated by Atf1, and that Atf1 can directly bind to the regulator region of *rsv1*. PKA pathway is another important pathway under starvation condition. We verified that the induction of *rsv1* is under the regulation of PKA pathway. Also, Rst2, the main effector of PKA pathway in starved condition, can bind to *rsv1* regulator region specifically in starvation condition. To further understand the downstream regulators of Rsv1, we performed the RNA-sequencing and ChIP-sequencing analyses. Target genes of Rsv1 will reveal mechanisms of cell survival under glucose starvation condition.

[Supported by grants from NRF]

F011

Genome Sequencing of *Ottowia* sp. nov., Isolated from Andong Sikhye, a Korean Traditional Rice

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Sikhye is a famous traditional rice beverage in Korea. It is made by mixing steamed rice, malt extract and water. Many kinds of sikhae are being produced according th the regions. Among them, Andong sikhye has a peculiar taste as the broth is made with red pepper, radish and ginger. During the fermentation and storage periods, diverse microorganisms are habitted in. Strain KADR8-3 is one of the microorganisms habitted in Andong sikhye. Result of 16S rRNA gene sequencing of strains revealed high sequence similarity with genus of *Ottowia* and confirmed this isolates is novel species. To figure out distinct characteristics, whole genome sequencing was conducted. It has a single circular DNA chromosome consisting of 3,901,011 bp and dose not contain any plasmid DNA. The chromosome is consisted of 3,523 CDS, 58 tRNA and 6 rRNA genes. The genomic DNA G+C content of this strain was 66.80 mol%. This results propose strain KADR8-3 belongs to a new species of the genus *Ottowia*.
[This study was carried out with the support of "Research Program for Agricultural Science & Technology Development (Project No. PJ013549), National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea]

F012

Endoribonucleases Regulateenolase Expression in *Escherichia coli*

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Previous studies have shown that RNase G negatively regulate enolase gene expression in *Escherichia coli*. We observed that enolase expression is also positively regulated by RNase III. Primer extension and S1 nuclease mapping analyses of *eno* mRNA *in vivo* identified one major and two minor *eno* mRNA transcripts generated by RNase III and an RNase G-targeted mRNA species that is processed by RNase III. We found that RNase III-mediated processing of primary *eno* mRNA enhances production of enolase protein; this processing appears to require the involvement of an antisense RNA that is transcribed from the coding region of *eno* to that of *pyrG* in the opposite direction of mRNA synthesis of these genes. Our results reveal a coordinated action of endoribonucleases that posttranscriptionally modulate expression of enolase, which is one of key enzyme in carbon metabolism.

F013

Genome-encoded Divergent rRNAs Regulate Gene Expression in *Vibrio vulnificus* CMCP6

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The wide use of rRNA sequences in quantifying evolutionary relationships among organisms is based on the assumption that each organism is assumed to have evolved to possess a unique ribosomal RNA (rRNA) species that is optimal for its physiological needs. However, some organisms express genome-encoded heterogeneous rRNAs, whose functional roles remain unknown. Here, we show that ribosomes containing the most variant rRNAs encoded by the *rrn*I operon (herein designated I-ribosome) contribute to differential protein synthesis in *Vibrio vulnificus* CMCP6. This study identifies genome-encoded heterogeneous rRNAs for the first time as regulators of gene expression.
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F014

Superoxide Dismutase Genetically Interacts with Rad51 for Genomic Stability and Oxidative Homeostasis in the Budding Yeast

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Targeting and inhibiting SOD1, an antioxidant enzyme scavenging reactive oxygen species (ROS), has been considered as a prospective approach for selective killing of RAD54B-deficient colorectal cancer cells based on the genetic analysis of synthetic lethal (SL) interactions in yeast revealing that mutation of SOD1 impaired growth of DNA double-strand break (DSB) repair pathway mutants. However, we show that deletion of RAD51 and SOD1 is not synthetic lethal but displays substantially slow growth and hypersensitivity to both ROS- and DSB-inducing drugs in *Saccharomyces cerevisiae*. The function of Sod1 in regard to Rad51 is dependent on Ccs1, a copper chaperone for Sod1. Sod1 deficiency exacerbates genomic instability in the absence of Rad51. Inversely, lack of Rad51 aggravates Sod1 deficiency-derived elevation of intracellular ROS level. Taken together, our results indicate that there is a significant genetic interplay between two major cellular damage response pathways, ROS signaling and DSB repair.
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F015

The Antimicrobial Peptide HPA3P^{His} Delivered by Gold Nanoparticle-DNA Aptamer Conjugates Effectively Eliminates *Vibrio vulnificus* Cells from Mammalian Cells

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Vibrio vulnificus causes fatal infections in humans. In this study, we demonstrated that an antimicrobial peptide (AMP), HPA3P^{His}, loaded onto a gold nanoparticle-DNA aptamer (AuNP-Apt) conjugate (AuNP-Apt-HPA3P^{His}) is an effective therapeutic tool against *V. vulnificus* infection *in vivo* in mice. This study demonstrated the potential of an AMP delivered by AuNP-Apt as an effective and rapid treatment option against infection caused by a major pathogen in humans and aquatic animals.

F016

RNase G Controls *hns* mRNA Abundance in *Salmonella* Typhimurium

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RNase G (Rng) is an endoribonuclease, which is involved in rRNA processing and degradation of a subset of mRNAs in *Escherichia coli*. However, its physiological role remains largely uncharacterized in *Salmonella* Typhimurium. Here, we report that RNase G controls expression levels of histone-like nucleoid structuring protein (H-NS) encoded by *hns*, which were strongly associated with the pathogenicity of *S*. Typhimurium cells in both epithelial cells and mice. We validated that *hns* mRNA abundance is mediated by RNase G, where 5′-UTR of *hns* mRNA was directly cleaved by RNase G *in vivo* and *in vitro*. We suggest that RNase G-mediated modulation of *Salmonella* pathogenicity island 1 type III secretion system involves H-NS as a key factor for the survival and virulence of *S*. Typhimurium in host cell.
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F017

Hexameric Assembly of RraA Proteins is Required for Their Inhibitory Effects on the Ribonucleolytic Activity of RNase E-like Enzymes

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RNase E plays a pivotal role in the degradation and processing of RNAs in *Escherichia coli*, and protein inhibitors RraA and RraB control its enzymatic activity. The halophilic pathogenic bacterium *Vibrio vulnificus* also expresses orthologs of RNase E and RraA- VvRNase E, VvRraA1, and VvRraA2. In this study, we report that the oligomer formation of VvRraA proteins affects binding efficiency to VvRNase E as well as inhibitory activity on VvRNase E action. We suggest that hexameric assembly of RraA homologs may well be required for their action on RNase E-like proteins.

F018

Regulation of Cdc42 Localization by the NDR Kinase Cbk1 in *Candida albicans*

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Cell polarity is defined as asymmetry in cell shape, protein distributions and cell functions. Cell polarity is important for the morphogenesis of *Candida albicans* and associated with its virulence. Cdc42, a Rho family GTPase, plays a central role in the establishment and maintenance of cell polarity by controlling the growing site selection and actin dynamics. And the NDR kinase Cbk1 is also essential for polarized growth of *C. albicans*. Although Orb6, the ortholog of *C. albicans* Cbk1 in *Schizosaccharomyces pombe*, is known to regulate Cdc42 localization through phospho-regulation of Gef1, Cdc42 GEF, it remains unclear whether Cbk1 is involved in the regulation of Cdc42 in *C. albicans*. Here, we show that Cbk1 is required for Cdc42 localization to growing sites in *C. albicans*. We found that Cdc42 was not localized to the growing sites in the *cbk1 Δ/Δ* mutant, unlike in the WT. Interestingly, however, a constitutive form of Cdc42, Cdc42^{G12V}, was correctly localized at the septum and growing tips in both WT and *cbk1 Δ/Δ* mutant strains, which suggests that Cbk1 may be involved in the activation and localization of Cdc42 in *C. albicans*. In addition, we will discuss how Cbk1 regulates Cdc42 activity for polarized growth of *C. albicans*.

F019

Genomic and Transcriptomic Landscapes of Multidrug Resistant *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa, an opportunistic pathogen of clinical significance, is notorious for its antibiotic resistance. Here, we sought to understand genomic and transcriptomic landscapes of *P. aeruginosa* isolates that are highly resistant to multiple antibiotics. To address this issue, we sequenced the whole genomes and profiled RNA transcripts of three multi-drug resistant (MDR) isolates and one antibiotic-susceptible isolate. Comparative genomic analysis reveals that MDR strains harbor well-defined genetic markers for antibiotic resistance. RNASeq analysis demonstrates that Rhl and Pqs quorum sensing (QS) and type 6 secretion system are remarkably downregulated in MDR strains. Interbacterial competition assays further confirm their defective capabilities to kill competing microbes, leading us to hypothesize that MDR strains may have evolved to attain antibiotic resistance in exchange for virulence. When MDR strains were treated with antibiotic cocktail (Imipenem, Ciprofloxacin, Tobramycin), expression of genes involved in general stress responses was increased, while that of genes involved in cell motility and energy metabolism was decreased. In contrast, no significant changes in expression of antibiotic-resistance genes were detected. Together, our results show that the antibiotic resistance of *P. aeruginosa* is more likely to be contributed by the canonical expression of previously acquired genes than by the genes that immediately respond to antibiotic stress.

F020

Crosstalk between Hog1 and Mpk1 MAPK Pathways Coordinately Regulate the Cell Wall Integrity of *Cryptococcus neoformans*

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Mitogen-activated protein kinases (MAPK) play pivotal roles in growth, stress response and adaptation, and differentiation of eukaryotic organisms. In *Cryptococcus neoformans*, which causes fatal meningoencephalitis in both immunocompromised and immunocompetent individuals and is responsible for more than 600,000 deaths annually in a global scale, three MAPK pathways play critical roles in growth, differentiation, stress responses and pathogenicity. In spite of extensive researches in these MAPK pathways, it still remains elusive how MAPKs crosstalk with each other and their downstream transcription factors still need to be uncovered. We focused on characterizing how Mpk1 and Hog1 MAPK pathways crosstalk and elucidating their downstream transcription factors in *C. neoformans*. Here we found that the phosphotyrosine phosphatase, Ptp2, plays a key role in coordinating Mpk1 and Hog1 MAPK pathways to respond to the cell wall damaging stresses.

F021

Comparative Genomic Analysis of Four Probiotic *Lactobacillus* Species, *L. acidophilus*, *L. helveticus*, *L. rhamnosus*, and *L. casei*

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Lactobacillus species are members of lactic acid bacteria and are widely used as a starter culture in the manufacture of fermented food products or probiotics for health improvement, because of its high metabolic function to produce various bioactive compounds. Here, we report the genome sequence of *Lactobacillus helveticus* LH5 and *Lactobacillus rhamnosus* LR5, each of which had been isolated from a healthy Korean adult. DNA sequencing was performed using the PacBio platform to assemble the genomes and Illumina MiSeq to improve the sequence accuracy. The genome sequences of *L. helveticus* LH5 and *L. rhamnosus* LR5 were compared with those of other strains in the species, along with those of *Lactobacillus casei*, *Lactobacillus acidophilus*. Using the genomic information, we underwent a comparative genomic analysis of factors that might be involved in adaptation to the host intestinal conditions, S-layer proteins, antioxidants, and bacteriocins. We found that strains possessing a high ratio of strain-specific genes tend to have more genes for adaptation in different environments. In the same context, S-layer gene clusters and antioxidant genes were more wildly distributed among habitat-versatile strains. A number of bacteriocin gene clusters were identified using a combination of *in silico* prediction tools.

F022

Genomic Analysis of a Pathogenic Bacterium, *Paeniclostridium sordellii* CBA7122 Containing the Highest Number of rRNA Operons, Isolated from a Human Stool Sample

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Paeniclostridium sordellii is mainly related to trauma, toxic shock, and gynecologic infections. Despite the result of serious infections caused by *Paeniclostridium sordellii*, it is difficult to treat because it is rapidly progressing in the recognition of first symptoms to death. In this study, genome sequencing and genomic analysis of strain CBA7122 belonging to *Paeniclostridium sordellii*, isolated from the stool sample were performed. The size of *Paeniclostridium sordellii* strain CBA7122 genome was 3,550,411 bp long and consisted of 3 contigs. The number of total predicted genes were 3,459 based on NCBI PGAP, including 17 rRNA operons, 105 tRNAs, and 4 ncRNAs. The genome of the strain CBA7122 has the most known number of rRNA operons in bacterial strains, the 17 rRNA operons. Number of rRNA operons in the genome is known to be connected with growth rate and growth efficiency. The phylogenetic tree constructed based on orthoANI values show that the strain CBA7122 is closely related to *Paeniclostridium sordellii*. The core genome was consisted of 2,481 POGs and the genome of strain CBA7122 have only 145 POGs as singletons, based on the pan-genome analysis. Although the several known virulence factors was found in the genome of strain CBA7122, there were no large clostridial cytotoxin genes. Our data based on genome analysis provides basic information on *Paeniclostridium sordellii* and will be a useful reference for detailed studies.

F023

Molecular Surveillance for Tick-borne Diseases in Dogs in Republic of Korea, 2017

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Tick-borne disease has given a fatal damage to both humans and animals and caused large economic losses to livestock industry worldwide. Many of rickettsial and protozoal species, including *Ehrlichia* spp., *Anaplasma* spp., and *Babesia* spp. have been transmitted by tick. Climate change making a habitat more favorable for vectors, may have raised the risk of potential introduction of exotic tick-borne diseases into the Republic of Korea. Many epidemiological surveys of tick-borne diseases reported in many countries. In the Republic of Korea, molecular surveillance was carried out for the cattle, human and tick. However, the surveillance of tick-borne infectious diseases in dogs is still insufficient. Therefore, in this study was to investigate the current situation through genetic testing in dogs about tick-borne infections by. A total of 1,462 dog blood samples were collected. Whole bloods' DNAs were extracted. PCR reaction was carried out by previously described methods and analyzed 16S rRNA gene. A total of 8 blood samples were positive for *Anaplasma phagocytophilum* (0.55%). *Ehrlichia* spp. and *Babesia* spp. was not detected in this study. Molecular surveillance of tick-borne rickettsial and protozoal infectious diseases is very useful for investigating the infectious status in Republic of Korea. Further investigation of tick-borne rickettsial and protozoal infectious diseases of dogs will be needed to prevent these neglected tick-borne zoonoses.

F024

The Phase Variation of *Salmonella* Typhimurium Occurs under Phagocytosis in Mouse Macrophages

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Salmonella Typhimurium which belongs to enterobateriaceae is the rod shaped, flagellate, aerobic, Gram-negative bacterium and the reason that induce food poisoning, nausea, diarrhea, fever, vomiting. *S.* Typhimurium is able to infect human as well as rodent, cattle, swine, sheep. One of the important traits that *S.* Typhimurium have is evasion of immune system, Toll-like receptor 5, by means of phase variation. Phase variation is the specific method as breakthrough under limited condition without requiring random mutation. *S.* Typhimurium has two types of flagellin which is made up of either type 2 FljB or type 1 FliC. At the general environment, FljB and FljA are generated and FljA served as a repressor for the *fliC* gene. In contrast to previous environment, FliC is generated by switching a *hin* domain. Along with this reason, the direction of *fljB* promoter that belongs to *hin* domain is switched and FljA is not able to interrupt *fliC* expression. Especially, Type 1 FliC differs from type 2 FljB in that the former is performed at the time that site-specific inversion occurs at the region of *hin* domain and later is intact flagellin. We showed here that *S.* Typhimurium replaces type 2 FljB with type 1 FliC under macrophage phagocytosis.

F025

The Velvet Regulators in *Aspergillus flavus*

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Fungal development and secondary metabolism are intimately associated via the activities of novel regulators, called the Velvet proteins that are highly conserved in filamentous fungi. Here we investigated the roles of the *velvet* genes in *Aspergillus flavus*, a fungus that produces the most potent naturally occurring carcinogen, aflatoxin. To study the function of the *velvet* genes, we generated individual deletion mutants in *A. flavus*. The mutant strains were examined for asexual spore production and sclerotia formation. The *Δ velB* strains, similar to the *Δ veA* strains, showed a reduction in fungal growth and conidiation along with a complete loss of sclerotia formation. The deletion of *vosA* and *velB* caused reduced trehalose amounts and increased sensitivity to thermal stress on conidia. We also examined aflatoxin production and found that the *Δ velB* and *Δ velD* mutant strains were found to be deficient in aflatoxin production. These results are consistent with the current model of the *velvet* genes in *A. nidulans*, indicating that their function may be highly conserved among the Aspergilli.

F026

The MAP Kinase Signal Pathway Dependent Asexual and Sclerotial Development in *Aspergillus flavus*

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The sexual development process of fungus is guided by signaling the external stimulus to the inside of the cell through MAP kinase pathway. *Aspergillus nidulans* MAP kinase, encoded by *mpkB* was known to coordinate sexual development as well as secondary metabolism. *Aspergillus flavus* also reported that sexual sclerotia are formed when cultured with mating type partner under experimental conditions for a long period of time, and then the asci and ascospore are produced therein. In previous experiments, we have identified the phenotype of *mpkB* knock-out strains in *A. nidulans and A. flavus. The ΔAfl_mpkB* showed that MpkB MAP kinase of *A. flavus* plays central roles in the asexual and sclerotial development but didn't affect biosynthesis of aflatoxin. In this study, we investigated the function of the MkkB MAPK kinase gene, a part of MpkB MAP kinase cascade in *A. flavus*. The production of sclerotia is fully blocked in *ΔAfl_mkkB* and all the phenotypes are identical with *ΔAfl_mpkB* just as we expected. Our results are expected to be an important hint to grasp the relationship between the sexual development pathway and the mycotoxin biosynthetic pathway of the filamentous fungi.

F027

Spatial Mapping of *Lactobacillus rhamnosus* GG in Mouse Gut

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Lactobacillus rhamnosus GG (LGG) is one of the most studied probiotics in the world. Since its discovery in 1985, growing body of evidence has shown that the symptoms of metabolic diseases can be alleviated by oral administration of LGG. In general, it is known that probiotics, including LGG, attach themselves in the intestinal tract of the host to produce beneficial effects in the host's intestines by interacting directly with host cells or intestinal bacteria. However, most of these studies have been *in vitro* experiments using Caco-2 cell lines, or sequencing of human colon tissues through biopsy. The design of these studies did not allow the comprehensive examination of LGG habitation in the intestine. To overcome these limitations, our study uses LGG labeled with fluorescent protein, mCherry. The recombinant LGG was orally administered to mice once a day for 2 weeks; then the intestine was removed and the fluorescence signal was observed through IVIS imaging system. To confirm whether the observed fluorescence signal was actually from recombinant LGG, the intestine was divided into five regions (duodenum, jejunum, ileum, cecum and colon) and the presence of LGG was confirmed using qPCR. In this poster, we show *in vivo* distribution of LGG in mouse gut and discuss about interactions between other microbes and host cells that might cause positive effect to human intestinal health.
[This work was supported by Holzapfel Effective Microbes (HEM).]

F028

Spore-specific Gene Expression in *Aspergillus nidulans*

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Homothallic model filamentous fungus *Aspergillus nidulans* develops sexual and asexual spores by environmentally and genetically regulated manners. Although asexual spores or conidia differentiation is well-characterized by analyzing important genes for controlling orchestrated developmental pathways, including the *brlA* gene-mediated conidiophore and conidia morphogenesis, only a few genes such as *nsdD*, *nsdC* and *veA* have been elucidated for playing important roles in sexual development and ascospore formation. Unlike conidia, however, physiological and genetic studies of ascospores are remained to be characterized. To know more about the ascospores biology as well as conidia, we performed RNA-seq analysis from from *A. nidulans* conidia and ascospores RNA samples. Comparative analysis of transcription profiles of conidia and ascospores revealed many genes that are expressed differentially in both spores. Detailed investigation of the differentially expressed genes is in progress.

F029

The *mgtA* Gene is Controlled by Elongation Factor P during Salmonella Infected in the Host

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Elongation factor P (EF-P) is essential to translation of ribosome stalling. The stalling is when the ribosome pauses translation in the presence of a continuous proline. Therefore, EF-P helps to smooth translation for the ribosome stalling which are caused by consecutive proline. Here we show that EF-P controls translation of *mgtA* gene in *Salmonella enterica* serovar Typhimurium. EF-P acts on the 550th and 551th proline codons of the amino acid sequence of *mgtA*. Therefore, the substitution of those proline codons does not affect the intrinsic activity of *mgtA* and induces smooth translation. The Pro 500, 551-substitution of the *mgtA* showed increased intramacrophage survival and mouse virulence, suggesting that EF-P mediated translation of *mgtA* is important for the pathogenesis of *Salmonella*.

G001

Optimization of Medium for Industrial Application by *Bacillus subtilis* SRCM102064 Using Response Surface Methodology

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Previously, *Bacillus subtilis* SRCM102064 having various benefits for industrial application such as biogenic amines degradation, extra cellular enzyme activity and high γ-PGA (gamma polyglutamic acid) productivity was isolated from Doenjang (Korean traditional soybean paste). *Bacillus subtilis* SRCM102064 was identified by analysis of 16S rRNA sequencing and biochemical characterization. In this study, we carried out the medium optimization for industrial application of *Bacillus subtilis* SRCM102064 by response surface methodology (RSM) based on Plackett-Burman design (PBD) and central composite design (CCD). Four factors molasses, sucrose, GOS (galacto-oligosaccharides) and peptone as medium constituent improving cell growth finally were selected by PBD. And then, we designed the central composite design in response surface methodology in order to find out optimal concentration of selected medium constituent. Finally, Optimum conditions for increasing biomass of *Bacillus subtilis* SRCM102064 by CCD was predicted to be molasses of 7%, sucrose of 7%, and peptone of 2% respectively. R^2 (correlation coefficient) of CCD model was 0.9755 and predicted the biomass was 22.0275 g/L.

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G002

A Study of Microalgae-mediated Process for Treating Radioactive Cesium (Cs)

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This present study investigated the feasibility of using an isolated novel microalga, *Desmodesmus armatus* SCK, for treating cesium (Cs^+) and radioactive ^{137}Cs from aqueous solution with effects of different temperature, potassium (K^+)-starvation of seed cells, and organic substrate including volatile fatty acids (VFAs) on bioaccumulation of the Cs^+. The obtained results revealed that *D. armatus* SCK accumulated a relatively higher Cs^+ than other green microalgae, yielding uptake of 2.08 nmol Cs^+ 10^6 $cells^{-1}$ at 25°C under illuminated condition. At lower temperature, 3.7-fold higher Cs^+-accumulation than that observed in 25°C was also obtained, probably due to excessive accumulation phenomena of exterior K^+ by inhibitory effect of chilling stress. The biologically engineered *D. armatus* SCK grown in K^+-depletion condition allowed to increase 26% of its maximum uptake capability observed in K^+-sufficient condition. Furthermore, the capability of Cs^+-uptake by *D. armatus* SCK was significantly enhanced when the VFAs are added as organic substrates in algal culture. The result of radionuclide experiment in this study also indicated that this strain can eliminate a wide level of the radioactive ^{137}Cs ranged from very lower (10 Bq/ml) to higher level (1000 Bq/ml) of its radioactivity.

[This work was supported by the Nakdonggang National Institute of Biological Resources grant funded by the Ministry of Environment, Republic of Korea].

G003

Characterization of a Novel SGNH Hydrolase (*Nm*Est) from *Neisseria meningitidis* 053442

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*Nm*Est, a novel SGNH-hydrolase from *Neisseria meningitidis* 053442 was identified, purified, and characterized by biochemical and biophysical methods. Multiple sequence alignment of *Nm*Est with other SGNH family member proteins confirmed a putative catalytic triad (Ser29-Asp169-His172), and a conserved sequence motif of $Ser(S)^{29}$-Gly $(G)^{87}$-Asn $(N)^{89}$-$His(H)^{172}$. Here, biochemical properties of *Nm*Est were surveyed with fluorescence analysis, dynamic light scattering (DLS), electron microscopy, and time of flight (TOF) mass spectrometry. The investigation of the factors affecting a substrate specificity of *Nm*Est revealed that Leu92 is responsible for the stabilization of hydrophobic moiety in substrates. At last, immobilization of *Nm*Est exhibited good durability after repeated usages and improved catalytic efficiency. Collectively, we characterized a novel SGNH-hydrolase, *Nm*Est and analyzed structural factors determining the substrate specificity. These studies provide a deeper insight at substrate specificity of SGNH-hydrolase.

G004

Characterization of a Novel Serine Hydrolase (Lg35) from *Lactococcus grarvieas* Lg2

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Hydrolase receive much attention because of their wide distribution in biological systems and their importances for chemical synthesis. Here, a novel hydrolase gene, Lg35, was isolated form *Lactococcus grarvieas* Lg2. The gene encoded a protein of 314 amino acids with a molecular mass of 35,306 Da. Lg35 showed the highest identity of 55% to ADP, which is an alkaline $_D$-peptidase from *Bacillus cereus* DF4-D. The Lg35 primary structure showed a putative motif of S-x-x-K motif, which is conserved in both family VIII carboxylesterase and class C β-lactamase. Biochemical assay confirmed hydrolase activity of Lg35 and revealed a preference for short chain p-nitrophenyl esters, particularly p-nitrophenyl acetate. In addition, Lg35 preferred an *S*-enantiomer, such as (*S*)-methyl-3-hydroxyl-2-methylpropionate, as a substrate. Finally, this work may provide potential advantage in the use of the enzyme in biotechnological processes.

G005

Characterization of a Novel Family VIII Carboxylesterase from *Pseudomonas fluorescens* KCTC 1767

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A novel family VIII carboxylesterase (*pf*EstA) from *Pseudomonas fluorescens* KCTC 1767 was identified, purified, and characterized by biochemical and molecular simulation methods. Multiple sequence alignment of *pf*EstA with other related enzymes confirmed a putative catalytic serine (Ser79) and the three conserved motifs. Molecular simulation data suggested Phe66 and Leu223 residues might play role in the enantioselectivity of *pf*EstA toward roche esters. Site-directed mutagenesis analysis confirmed that Phe66 and Leu223 interact with methyl group of (R)- and (S)-roche esters, respectively. Accordingly, both F66A and L223F mutants presented strongly enhanced (*S*)-roche ester specific activity. Furthermore, to elucidate a catalytic promiscuity of family VIII esterases, a catalytic pocket of *pf*EstA was compared with other related enzymes using a docking simulation. Then, their pockets and mouths were compared by calculating using CASTp. The results revealed that a catalytic pocket of *pf*EstA was too small to accommodate to bulky antibiotics, such as, cephalothin.

G006

Systems Metabolic Engineering of *Escherichia coli* for the Production of Acrylic Acid

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Producing industrial chemicals from renewable resources to replace petroleum-based chemical industry has been spotlighted as a promising alternative to solve recent environmental issues. Acrylic acid (AA) is an important industrial chemical widely used in polymers, coatings, and adhesives. Although dehydrating bio-based 3-Hydroxypropionic acid (3-HP) to replace current propylene-derived process is underway, cost problems arising from purification, separation, and catalysts need to be improved. Here we first report a new one-step route for the production of AA from glucose in metabolically engineered *Escherichia coli* through the β-alanine (BA) route. We partitioned the AA production pathway into two modules: a BA forming upstream pathway and an AA forming downstream pathway. First, the downstream pathway was validated *in vivo* and *in vitro*. Candidate enzymes for each step were screened and the best combination was selected. The newly constructed pathway was introduced into BA producing *E. coli* strains and the strain utilizing aspartate ammonia-lyase (aspA) route resulted in the highest AA titer. This is the first report to produce acrylic acid through BA route.

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G007

Production of 1,3-Diaminopropane in Metabolically Engineered *Escherichia coli*

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Biological chemical production is essential for sustainable chemical industry. Here, *Escherichia coli* is metabolically engineered to produce 1,3-diaminopropane (1,3-DAP). Comparison of heterologous C4 and C5 pathways for 1,3-DAP production by *in silico* flux analysis revealed that the C4 pathway using *Acinetobacter baumannii dat* and *ddc* genes was more efficient. In a strain harboring feedback resistant aspartokinases, the *ppc* and *aspC* genes were overexpressed to increase flux towards 1,3-DAP synthesis. Also, *pfkA* deletion was found to increase 1,3-DAP production by applying 128 synthetic small RNAs. Overexpression of the *ppc* and *aspC* genes in the *pfkA* deleted strain resulted in even higher production of 1,3-DAP. Fed-batch fermentation of the final strain achieved 13 g/L of 1,3-DAP production in a glucose minimal medium.

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G008

Systems Metabolic Engineering of *Escherichia coli* for 3-Aminopropionic Acid Production: a Precursor for Nylon-3 Synthesis

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3-Aminopropionic acid (3-AP) is an important platform chemical for manufacturing acrylamide and acrylonitrile. Using a previously developed fumaric acid producing *E. coli* strain as a host, the *C. glutamicum panD* gene (encoding L-aspartate-α-decarboxylase) was overexpressed and the native promoter of the *aspA* gene was replaced with the strong *trc* promoter. The *aspA* and phosphoenolpyruvate carboxylase (*ppc*) genes were additionally overexpressed, and the ammonium sulfate was supplemented in the medium, which resulted in the production of 3.49 g/L 3-AP. Optimization of PPC expression level by using synthetic promoter and RBS sequences and replacement of native promoter of the *acs* gene led to produced 32.3 g/L of 3-AP in 39h with an overall yield and productivity of 0.135 g 3-AP/g glucose and 0.828 g/L/h, respectively, in fed-batch fermentation.

[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science and ICT (MSIT) through the National Research Foundation (NRF) of Korea (Grant Nos. NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)].

G009

Production of Putrescine and L-Proline by Metabolically Engineered *Escherichia coli* Using Synthetic Regulatory Small RNA

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Fine-tuning of gene expression is essential for optimization of metabolic and genetic networks, but conventional methods are time-consuming and laborious. Here, we report development of a fine-tunable knockdown system by modulating synthetic small RNA (sRNA) expression levels in *Escherichia coli*. A library of 75 synthetic sRNAs-promoter combinations was constructed for the enhanced production of putrescine, an engineering plastic monomer. Optimal repression of *argF* and *glnA* using sRNAs led to rapid development of an engineered *E. coli* strain capable of producing 43.0 g/L of putrescine by fed-batch cultivation. Similarly, through fine-tuned repression of *argF* and *glnA* by applying 25 sRNAs-promoter combinations, an engineered *E. coli* strain capable of producing 32.7 g/L of L-proline by fed-batch culture could be developed. The fine-tuning system of modulating synthetic sRNA expression levels reported here will be useful for optimal and rapid design of microbial strains through simultaneous optimization of multiple gene expression levels at systems level. This strategy will serve as a good complementing alternative to individually re-designing sRNAs in sRNA-based gene expression knockdown system. [This work was supported by MSIT (Grant Nos. 2011-0031963; NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)].

G010

Development of CRISPR/Cas9-coupled Recombineering Tool for *Corynebacterium glutamicum*

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Corynebacterium glutamicum is an important industrial microorganism for amino acids production. Genome engineering of this important organism, however, currently relies on random mutagenesis and inefficient double crossover events. Here we report a rapid genome engineering tool for scarless and iterative knockout of multiple genes in *C. glutamicum*. In this system, recombinase RecT incorporates synthetic single-stranded oligodeoxyribonucleotides into the genome and CRISPR/Cas9 counter-selects unedited organisms. To generate the final strain free of CRISPR/Cas9 and RecET vectors after iterative engineering, plasmids harboring CRISPR/Cas9 and RecET systems were engineered to be curable. The performance of the rapid and iterative genome engineering system was demonstrate by generating seven different mutants within two weeks to study the combinatorial deletion effects of three different genes on the production of γ-aminobutyric acid, an industrially relevant chemical of much interest. This genome engineering tool will expedite metabolic engineering of *C. glutamicum*.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) of the Ministry of Science and ICT of Korea.]

G011

Characterization and Immobilization of a Novel Esterase (LBA21) from *Lactobacillus acidophilus*

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A novel esterase (LBA21) from *Lactobacillus acidophilus*, 186 amino acids with a molecular mass of approximately 21kDa, was identified, expressed and characterized. Sequence analysis of LBA21 revealed a putative catalytic triad of Ser^{10}-Asp^{161}-His^{164} and a conserved sequence motif GDSL. The optimal pH of LBA21 was slightly alkaline (pH 8.0), and LBA21 was active toward short and medium chain esters such as, p-nitrophenyl acetate, butyrate, hexanoate and octanoate. Furthermore, this enzyme efficiently hydrolyzed glyceryl tributyrate, α-$_D$-glucose pentaacetate and *t*-butyl acetate. Cross-linked enzyme aggregates of LBA21 were prepared by precipitating the enzyme with ammonium sulfate, and adding L-arginine and finally cross-linking with glutaraldehyde. Interestingly, L-arginine can improve significantly CLEA-LBA21 activity. Higher thermal and good durability after repeated use of CLEA-LBA21 highlight its potential applicability as a biocatalyst in the pharmaceutical and chemical industries.

G012

Enhancement of Gene Expression in *Clostridium acetobutylicum* by Engineering 5' Untranslated Region

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Overexpression of genes is one of the most fundamental strategy used in metabolic engineering. However, mechanisms of regulating gene expression levels in *Clostridium* spp. has been poorly studied. In this study, we observed decreased gene expression level with a short single-stranded 5' untranslated region (UTR) in *Clostridium acetobutylicum*. *In vitro* enzyme assay and reverse transcription-quantitative PCR further revealed that a stem-loop at 5' UTR increases mRNA and protein expression levels up to 4.6-fold, possibly protecting the mRNA from exonuclease attack. The stability of the stem-loops calculated in Gibbs free energy had no correlation with the expression level of the modified genes, inferring the existence of a stem-loop itself is more important factor for the mRNA stability. These findings were applicable to enhancing the expression level of *adhE1* and *adhE2* genes in *C. acetobutylicum* and *C. beijeinckii*. Applying these findings, more reliable regulation of gene expression for metabolic engineering of *Clostridium* strains will be possible.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) of the Ministry of Science and ICT of Korea.]

G013

Enhanced Production of Sterol Precursors in Yeast by Modulating Negative Feedback Regulation of Ergosterol Biosynthesis Pathway

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The biosynthesis pathway of ergosterol, the major fungal membrane sterol, has been exploited for the engineered biosynthesis of natural terpenoid and sterol products in various yeast species. There are several regulatory mechanisms to maintain ergosterol homeostasis, including the degradation of proteins and the transcriptional inactivation of genes involved in the ergosterol biosynthesis. In an effort to develop *S. cerevisiae* strains overproducing dammarenediol, a precursor for ginsenoside production, we constructed a set of Erg1 variants to avoid ubiquitin-mediated degradation in the ER. The single mutant, $Erg1_{K311R,}$ was shown to improve most efficiently the stability of Erg1. The additional expression of $Erg1_{K311R}$ in the *S. cerevisiae* strain producing dammarenediol showed the increased production yield by more than 2 fold. We also constructed several mutants of Upc2, a key transcription factor of ergosterol pathway, to ablate the feedback inhibition by ergosterol. By modulating the expression levels of Upc2 variants, we observed that Upc2-1, carrying G888D mutation in the ligand-binding site, directed the most enhanced production of sterol precursors. Further improved production of dammarenidol, about 2 fold, was obtained by Upc2-1 expression in the *S. cerevisiae* strain carrying the $Erg1_{K311R}$ variant. Altogether, we demonstrated that modulating the feedback inhibition by engineering Erg1 and Upc2 is an efficient strategy for the enhancing of sterol precursors.

G014

Characterization and Immobilization of a Novel Esterase (HA29) from *Halocynthiibacter arcticus*

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A novel esterase (HA29) from *Halocynthiibacter arcticus*, which is composed of 186 amino acids with a molecular mass of approximately 29kDa, was identified, expressed and characterized. Sequence analysis of HA29 revealed a putative catalytic triad Ser^{10}-Asp^{161}-His^{164} and a conserved sequence motif GHSAG. The optimal pH for enzyme activity was slightly alkaline (pH 8.0) and HA29 was active toward short chain esters such as, p-nitrophenyl acetate. Furthermore, this enzyme efficiently hydrolyzed glyceryl tributyrate, α-$_{D}$-glucose pentaacetate and *t*-butyl acetate. Cross-linked enzyme aggregates of HA29 were prepared by precipitating the enzyme with ammonium sulfate, followed by adding L-arginine as an additive and finally cross-linking with glutaraldehyde. Interestingly, L-arginine can improve significantly CLEA-HA29 activity. Higher thermal and good durability after repeated use of CLEA-HA29 highlight its potential applicability as a biocatalyst in the pharmaceutical and chemical industries.

G015

Improved Indigo Productivity of FMO (T424A) Mutated Recombinant *E. coli*

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Indigo is one of the most widely used blue dyes and was traditionally extracted from plants. Recently, a method of producing indigo in through microorganisms has been studied. Previously, we reported that 370 mg/L Indigo produced in flavin containing monooxygenase (FMO) recombinant *E. coli* derived from *Celeribacter* sp. TSPH2 isolated from marine environment. To further improve indigo production, FMO mutated recombinant strains were produced. In the newly constructed FMO mutated vector, the threonine at position 424 was changed to alanine (T424A). Also, a portion of the T vector used for sub cloning, 34 bp, is included after the *fmo* termination codon. In the same flask culture conditions, the production ability of FMO mutated recombinant strain was improved by 40% compared to the original FMO recombinant strain. In the 5 L jar culture, 633 mg/L Indigo was produced within 24 hours and 934 mg/L Indigo was produced in 50 L. Another important feature is that the FMO mutated strain, indigo production is possible even at 37°C.

Keywords: Indigo, Mutated flavin containing monooxygenase (FMO), *Celeribacter*.

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G016

2DE GEL Analyses of Intact *Bacillus subtillis*, *Weissella kimchii* and Their Bacterial Ghosts (BSGs, WkimGs)

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The differences of protein pattern and profiles between the non-treated intact cells and the chemically induced BGs were mainly observed. The 2DE lysis solution was directly added to quantify the extracted protein, 2DE was developed using 200 μg per sample and stained with alkaline silver. On average, about 840 spots were observed in the images. Image analysis was performed on spots that showed a tendency to increase or decrease more than three times quantitatively in chemically-introduced BGs based on non-treated cells samples. Based on the normalized spot intensity, we selected spots (342 spots) that showed an increase or decrease. Of those spots, 8s pots (7113, 7210, 8417, 8808, 9205/BSGs and 3205, 5308, 5311/WKimGs) that showed the most increase in amounts were reselected for analyzing PMF (Peptide Mass Fingerprinting)/MALDI-TOF.

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G017

Development of Immunity Enhancer Using Chemically Induced *Bacillus subtilis* and *Weissella kimchii* Bacterial Ghosts

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B. subtillis and *W. kimchii* bacterial ghosts (BGs) were made by chemical induction under the treatments of MIC. Chemicals used to produce BG consist of groups of acids, alkalis and alcohols including HCl, H_2SO_4, HNO_3, CH_3COOH, $C_6H_8O_7$, C_4H_4O4, NaOH, KOH, Na_2CO_3, n-butanol, 2-propanol, ethanol and methanol. Of BGs produced by chemical treatments, only BGs of HCl-induced *Bacillus subtilis* and NaOH-induced *Weissella kimchii* were completely nonliving and DNA-free. A SEM showed that both acid and alkali induced BGs formed a membrane-membrane lytic tunnel structure in the cell envelope. For the analysis of cytotoxicity in marcrophages cells exposed to BGs, BGs were treated to murine macrophages and their viabilities were determined by using Cell Counting Kit–8. Compared the cytokine expressions of the macrophage cell (control) with those of macrophage cells exposed to WKimgGs (*Weissella kimchii* ghosts) and to BSGs (*Bacillus subtilis* ghosts), most of macrophages exposed to WkimGs, BSGs induced IL-1β,IL-6 and TNF-α higher than control macrophages non-exposed to BGs. In the neural stem cells exposed to BGs, differentiation was strongly induced by means of the high expression of NT3, NT4/5 and Tuj1.
[This work was supported by the Human Resource Training Program for Regional Innovation and Creativity through the ministry of Education and National Research Foundation of Korea(NRF-2015H1C1A1035949)]

G018

Research of Immunoadjuvant Using Chemically-induced *Weissella koreensis* and *Pediococcus pentosaceus* Bacterial Ghost (BG)

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W. koreensis LKS42 bacterial ghosts and *P. pentosaceus* KA94 bacterial ghosts were produced by means of chemically-induced lysis through the membrane and the cell wall and this method is based on MIC. WKorGs and PPGs were observed membrane transverse dissolution tunnel structures on the envelope by means of SEM. Both the acid- and alkali-treated cells retained the whole cellular morphology, but produced a trans membrane lysis tunnels on the cell surface structures. Next, cytotoxicity upon murine macrophage Raw 264.7 cells was measured by exposing to the HCl-induced WKorG and PPG that were manufactured on the MIC values. The viability of macrophage cells treated with HCl-induced WKorG and PPG for 60min showed 90.4% and 99.6%, respectively. The macrophages exposed to WKorGs and PPGs demonstrated relatively higher induction of pro- or anti-inflammatory cytokines including IL-6, IL-10, TNF-α, IL-1β, iNOS and IFN-γ, except for COX-2. Compared with macrophage cells exposing to the intact and living cells, macrophage cells exposed to WKorG showed a higher induction of TNF-α and iNOS expression, and macrophage cells exposed to PPG showed differently higher stimulation on IL-1β.
[This work was supported by the Human Resource Training Program for Regional Innovation and Creativity through the ministry of Education and National Research Foundation of Korea(NRF-2015H1C1A1035949)]

G019

Human Skin Microbiome, *Epidermidibacterium keratini*, a Novel Actor for Skin Barrier Development

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The human skin is home to provide the habitats to various microorganism, and stably maintain the composition of the skin microbial communities by commensal relationship. This symbiotic relationship between the skin and the microbiome constitute complex protective barriers against external environment factors. Various metabolites generated by the skin microbiome extensively affect beneficial effects to human skin. In this study, we isolated a novel bacterium stain, *Epidermidibacterium keratini*, called as EPI-7, from human skin. Analysis of 16S rRNA gene sequences showed that the newly found bacterium shares 93.4% homology with the genus *Sporichthya*, thus corroborating the discovery of a novel genus.
Here, the metabolites of the EPI-7 were treated in keratinocytes to evaluate the skin barrier and hydration function. The mRNA expression levels of skin moisturizing factors, such as filaggrin, claudin, aquaporin 3, and HAS3, were significantly increased in the EPI-7 derived metabolites treated cells.
To observe the anti-inflammatory properties of metabolite products on skin, the keratinocytes were treated with IL-4/poly (I:C) to induce the inflammatory reaction. The metabolites of EPI-7 significantly down-regulated the mRNA expression levels of chemokines and pro-inflammatory cytokines, including IL-6, TNF-α, TSLP, and TARC.
Taken together, these results show that the metabolites of EPI-7 are effective to improve the skin barrier function and the resistance against external environment factors.

G020

Characterization of a Novel GDSL Family Carboxylesterase from *Halocynthiibacter arcticus*

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A novel GDSL family (*Ha*Est1) from *Halocynthiibacter arcticus*, which is composed of 200 amino acids with a molecular mass of 21.0 kDa, was identified, expressed and characterized. *Ha*Est1 displayed a significant sequence similarity with family II lipolytic hydrolases that are also known as a GDSL family. Further sequence analysis revealed a conserved motif of Ser^{18}(S)-Gly^{82}(G)-Asn^{83}(N)-His^{174}(H). Finally, cross-linked enzyme aggregates (CLEAs) of *Ha*Est1 was prepared to investigate potential biotechnological applications.

G021

Characterization of a Novel Family V Carboxylesterase from *Shewanella frigidimarina*

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A novel esterase (*Sf*AcE) from *Shewanella frigidimarina*, which is composed of 279 amino acids with a molecular mass of 31.0 kDa, was identified, expressed and characterized. *Sf*AcE displayed a significant sequence similarity with S-formylglutathione hydrolases. Further sequence analysis of *Sf*AcE revealed a conserved pentapeptide of G-X-S-X-G and a putative catalytic triad of Ser148-Asp224-His257. *Sf*AcE hydrolyzed short-chain esters such as *p*-nitrophenyl acetate, butyrate, hexanoate and octanoate. Taken together, this study will contribute to improve our understanding for thermostable hydrolase, providing useful information in sequence, biophysical properties and catalytic activity.

G022

Characterization of *Weissella Koreenis* is Producing β-Glucosidase for Bioconversion of Isoflavone

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β-Glucosidase (EC 3.2.1.21) plays an important role in the removal of non-reducing terminal glycosyl residues from glycosides. *Weissella koreenisis* MBE2467 producing high levels of β-glucosidase were isolated from Kimchi to convert isoflavone glycosides into isoflavone aglycones. When cultivated in MRS medium containing glucose as carbon source, *W. koreenisis* MBE2467 showed the highest specific growth rate of 0.61 ± 0.03 1/h at 40°C and pH 6.5. The optimum reaction conditions for *W. koreenisis* MBE2467 β-glucosidase activity was pH6.5 and 45°C to show maximum enzyme activity of 2.02 ± 0.03 U/mg.

G023

Characterization of *Bacillus subtilis* MBE/L3395 that Produces in *Cordyceps militaris* Extracts

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Bacillus subtilis that has 5'-nucleotidase activity was isolated from Korean traditional foods. As the 5'-nucleotiase (EC. 3.1.3.5) activity plays an important role in producing an isomer of quinic acid such as cordycepin from adenosine monophosphate. Twenty strain from the 132 bacterial strains isolateds high 5'-nucleotidase activity were used for producing the cordycepin using cordyceps militaris extract. The MBE/L3395, which was identified as *Bacillus subtilis*, showed a maximum 5'-nucleotidase activity of 0.277±0.021 mU/mg. Specific growth rates of the MBE/L3395 was determined in various temperatures and medium acidities. The MBE/L3395 showed highest specific growth rate of 1.91 ± 0.03 1/h at 55°C, pH 6.5.

Keywords: 5'-Nucleotidase, *Bacillus subtilis*, Cordycepin, Fermented foods, *Cordyceps militaris*

G024

Microbial Carbohydrate Resource Bank

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Microbial carbohydrates have a variety of characteristics and original functionalities comparing with carbohydrates produced by animals and plants. Microbial carbohydrates are natural, non-toxic and biocompatible polymers, GRAS (Generally Regarded As Safe) and their structural diversities lead to a variety of functions. Recently, many novel applications have been developed using microbial polysaccharides such as drug delivery systems, hydrogels, nanoparticles, and materials for tablet-pressing process in the pharmaceutical and biomedical industries as well as in the bio-nano engineering. The Microbial Carbohydrate Resource Bank (MCRB) was established to investigate and collect various functional polysaccharides and microorganisms in order to widely utilize the microbial resources. MCRB will contribute the advancement of industrial fields using carbohydrates and provide microbial resources into various researchers to encourage basic and applied researches.

G025

Self-healable and Highly Stretchable Agarose-based Gel by Cyclodextrin Functionalization with Poly(acrylic acid-b-styrene)

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Agarose-based hydrogels are used in biotechnology because they are non-toxic, stable at room and body temperature, and relatively inexpensive. However, agarose-based hydrogels are biologically inactive, highly rigid, and not much stretchable. Here, we developed a double network hydrogel that can self-heal itself and stretch significantly using cyclodextrin-functionalized agarose (CFA) and poly(acrylic acid-*b*-styene) (PAS). Although the cyclodextrins (CD) grafted on agarose (AG) weaken the gelation property of AG by interrupting the formation of double helices at low temperatures, it grants the gel high flexibility and host-guest complexability with benzene groups of PAS network instead. The CFA/AG/PAS gel, which is a mixture of CFA, AG and PAS, showed self-healing property and the self-healing efficiency was better than one of the conventional AG gel.

G026

Substitution of the Native Promoter of an rRNA Operon in *Escherichia coli* to an rRNA Promoter of *Vibrio natriegens* for Accelerated Growth

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The bacterium *Escherichia coli* is one of the most widely used microorganisms for producing various bioproducts such as amino acids and nucleic acids. *E. coli* is used as a model organism for scientific research and a platform cell factory for industrial application, because it grows quickly and is easy to edit the genome. *Vibrio natriegens* is a fast-growing microbe that can double with a generation time of 9.8 min. A previous study that characterized the rRNA operons of *V. natriegens* suggested that a high protein synthesis rate of *V. natriegens*, which may contribute to the rapid growth, could be due to the extremely high promoter activities of rRNA operons and the high copy number of the rRNA operons. Here, in an attempt to accelerate the growth of , we substituted the natural promoters of rRNA operons in *E. coli* K-12 MG1655 with the *rrnA* promoter of *V. natriegens*. The lambda red recombination system was used to introduce the *rrnA* promoter of *V. natriegens* to *E. coli*: it was recombined to the promoter sites of *rrnA, rrnB, rrnG, rrnH, rrnA* and *rrnG, or rrnB and rrnGrrn* promoter-substituted *E. coli* strains did not change significantly in M9 minimal medium supplemented with 4g/L glucose, except for one that has the *V. natriegens rrnA* promoter in *rrnG*. It grew 23.3% faster than the wild-type strain of *E. coli*. Quantitative measurement of the rRNA transcript from each *rrn* operons to verify the effect of promoter substitution is in progress.

G027

Construction of Foreign Gene Expression System in a *Aspergillus oryzae* Strain Isolated from Korean Traditional Fermented Foods

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Protein expression systems that produce the heterologous gene products using fungi are important for various aspects and it is recommended to use the GRAS strain as a host to ensure product safety. Therefore, here we constructed a heterologous gene expression system for producing foreign gene product including bacterial origin one such as bacterial β-glucosidase by using a GRAS fungus *Aspergillus oryzea*. The produced β-glucosidase is a hydrolytic enzyme and the expression of the gene was stimulated by placing it under the control of the constitutively activated *gpdA* gene promoter or threonine-inducing *alcA* gene promoter of *Aspergillus nidulans*. The pyrithiamine-resistant gene, *ptrA,* was used as the selection marker for *Aspergillus* transformation. The signal peptide of *A. oryzae* α-amylase AmyB was linked to the N-terminus of the bacterial β-glucosidase protein, and 3X FLAG was tagged at the C-terminus. *A. oryzae* transformants successfully overexpressed the β-glucosidase gene, and expression level was monitored by western blot analysis with anti-FLAG antibody. The functional activity of the protein was detected by esculin hydrate converting test and pNP-β-D-glucopyranoside (pNP-Glc) measurement assay. The expression system of *A. oryzae* could be beneficial for industrial applications.

G028

Development of a Cell Growth-based High-throughput Screening System for Engineering of the Substrate Specificity of Sugar Isomerase

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Broad substrate specificity of sugar isomerases provides novel biological catalysts to produce rare sugars. L-Arabinose isomerase (AI) can convert D-galactose to D-tagatose. Hence AI with higher specificity toward D-galactose is prefer to produce D-tagatose. Here we constructed a *de novo* D-tagatose catabolic pathway in *Escherichia coli* BL21 (DE3) lacking D-galactose utilization by the implementation of a D-tagatose 1,6-bisphosphate aldolase from *Bacillus licheniformis* ATCC 14580^{T}. Subsequently, expression of the *araA* gene enabled the D-tagatose auxotroph to grow on D-galactose, which was converted to D-tagatose through isomerization. To further investigate the efficacy of enzyme activity-based cell growth, we generated an AI mutant library (×10^{9} mutants), which was expressed in the D-tagatose auxotroph. After several rounds of sub-cultures on D-galactose as the sole carbon source in minimal media, cells with higher growth rates were dominant over cells containing proto-type enzyme activity. Detailed biochemical and biophysical analyses with purified AI variants indicated that higher affinity of AI toward D-galactose accelerated the growth of its expression host, suggesting that such an *in vivo* screening system can be a powerful tool for directed evolution of sugar isomerases. [Supported by the National Research Foundation of Korea (NRF) (grant ID: 2017R1A2B4005051 and 2017M3A9F3043852)]

H001

Effects of Feeding Methods on Ruminal Fermentation Characteristic and Microbial Community Change

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This study was conducted identification of various microbial community change in rumen according to the feeding methods. The experiment was conducted using three castrated steers, 0.9 kg of roughage, 2.7 kg of concentrate were fed twice a day. Feeding methods was CON (Feed concentrate and after 40 minutes later feed roughage), TRT1 (Feed roughage and after 40 minutes later feed concentrate), TRT2 (Mixed feed by adding little amount of water with roughage and concentrate) and performed in 3 x 3 Latin square design. pH value was lower in all TRT group including the CON group at 3 hours after feeding than before feeding. Total VFA was higher after feeding TRT1 group, however, not differences observed. VFA group did not different between all TRT group and CON group. Comparing ruminal microbial community results, At phylum level, *Bacteroidetes* and *Proteobacteria* were different between CON group and TRT group. At species level, *Prevotella ruminicola* ratio was highest, ratios of *Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens* were 0.3, 0.2 and 0.1% respectively. However, there are microorganisms such as fungi, yeast and protozoa in rumen as well as bacteria. Therefore more screening experiments are needed for other microorganisms.
[This work was carried out with the support of the Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ012664)]

H002

A Study on Changes in Microbial Community in Rumen after Feeding

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Ruminants are different with monogastric animals that can gain energy from plant-based food by fermentating it in 4 specialized stomach. The first stomach called rumen, which is many microorganisms exist it helps to digest plant-based food very quickly. This study was carried out to observe microbial community changes in rumen during the fermentation time after feeding. The feed consists of 2.7 kg concentrate and 0.9 kg roughage. The rumen fluid was collected each fermentation time 2, 4, 6 h before feeding. And then analyzing rumen fluids to observe microbial community. At phylum level, *Bacteroidetes* was the most abundant in microbial community. The *Aquificae* community was not found in 2 and 4h of fermentation time, but at 6 h 0.01% very small amount was found. At genus level. *Ruminococcus* which is one kind of rumen microorganism was no significant difference in rumen microorganisms at all fermentation time. However, *Butyricicoccus* was found in rumen fluid before feeding, but 6h it disappeared. At Species level, *Prevotella ruminicola* occupied the highest proportion of all fermentation time and unclassified species were approximately 4.9%. *Ruminococcus albus* was respectively accounted for about 0.1%. Especially we found that *Methanobrevibacter boviskoreani* was not found in rumen fluid before feeding.
[This work was carried out with the support of the Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ012664)]

H003

Synergistic Effect of Antimicrobial Substances Mixture Active against *Staphylococcus aureus*

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Microbial Safety Team, National Institute of Agricultural Sciences

Staphylococcus aureus is an important pathogen implicated in various diseases including food poisoning. *S. aureus* has rapidly developed resistance to multiple antibiotics and has become a global public health concern. Therefore, increasing attention has focused on identifying alternative antimicrobial substances as antibiotic substitutes. Bacteriocins are considered safe and effective antimicrobial substances for the prevention of pathogenic bacteria. In this study, 3 antimicrobial substances active against *S. aureus* were isolated from *S. pasteuri, S. epidermidis* and *S. gallinarium*. These 3 antimicrobial substances were stable at heat treatment (100°C) and 2 of 3 substances were also stable at wide pH range (2–10). The host range against *S. aureus* was wider when 3 antimicrobial substances were treated with mixture because those 3 substances have different host range. A treatment of mixture of 3 antimicrobial substances showed synergistic antimicrobial effect compared with treatment of a single substance. The mixture tested in this study has potential to use alternative antibiotics against *S. aureus*.
[Supported by grants from RDA]

H004

Optimization of Antimicrobial Compound Production by *Bacillus* sp. TGW6 Isolated from Nakdong River

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Forty two strains were isolated from freshwater of the upper region of the Nakdong River and screened for antibacterial activity against methicillin-resistant *Staphylococcus aureus*. The active strain TGW6 was identified as *Bacillus* sp. using 16S rRNA phylogenetic analysis. Optimization and production antibacterial compound were checked antimicrobial activity according to culture medium, temperature and pH. The antimicrobial activity was determined by an *in vitro* bioassay on R2A agar. Optimal conditions for growth and antimicrobial activity in strain TGW6 were found to be: YPD medium, 20-25°C, 6.5-7.0. Culture filtrate of strain TGW6 showed antimicrobial activity against MRSA strains, *Bacillus cereus* and *Candida albican* with inhibition zones from 1 to 3 mm. Optimal reaction time in 2 L-scale fed-batch fermentation process was 48 h in YPD medium, 100 rpm and 0.3 vvm. Culture optimization of strain TGW6 can be improved on antimicrobial activity.

H005

Detection of *Staphylococcus aureus* and Enterohemorrhagic *Escherichia coli* (EHEC) by PCR on Fresh-cut Produce

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The fresh-cut or ready-to-eat produce is not heated before eating, so there is concern about foodborne pathogens. Therefore, development effective detection method for foodborne pathogens is important as well as hygienic management. The purpose of this study is determination of detection limit of conventional PCR detection method for target pathogens (*S. aureus* and enterohemorrhagic *E. coli* (EHEC)). To determinate PCR for the limit of detection (LOD) of genomic DNA of *S. aureus* and enterohemorrhagic *E. coli*, species-specific and single-copy genes were used. The selected genes were thermonuclease (*nuc*) for *S. aureus* and β-D-glucuronidase A (*uidA*), shiga toxin genes (*stx1* and *stx2*) for enterohemorrhagic *E. coli*. The LOD was 5pg for *nuc* gene, corresponding to *S. aureus* genomic copies of 10^3 CFU. The LOD was 10, 1 and 1 pg for *uidA*, *stx1* and *stx2* genes, corresponding to *E. coli* genome copies of 10^3, 10^2, and 10^2 CFU, respectively. Since plants are not hosts, foodborne pathogens generally exist in fresh-cut produce at very low concentrations. The goal of the further study is to optimize PCR detection method and link with sample preparation method optimized for each type of produce in order to establish an effective detection method.

[Supported by grants from RDA]

H006

Microbial Reduction Effect and Color Change of Red Pepper Powder by Sterilization Methods

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Red pepper powder is a popular seasoning in Korea. The red pepper powder in non-heated cooking foods such as kimchi and jeot-kal may affect microbiological safety. Several countries which imports Korean red pepper powder require less than 10^3 CFU/g of aerobic bacteria. Contamination of red pepper powder by microorganisms can occur through cultivation, harvesting and processing. Therefore, this study was conducted to investigate the microbial contamination from the harvest of pepper to the red pepper powder production and to optimize sterilization condition using UV and hot air proposed in HACCP system. The microbial contamination level of harvested red pepper was 4.64 ± 0.45 log CFU/g and washing process was not effective to reduce microorganism level. However, microorganism level was reduced 2.57–2.76 log CFU/g (99.73–99.83%) after drying process. Depending on the drying conditions, color level of red pepper powder could be changed. The various drying conditions were tested to maintain red color and reduce microorganism level. UV (40.40 MW/cm^2/sec) and hot air (70°C) was tested. Aerobic bacteria and *E. coli* were reduced by 0.49 log CFU/g (67.78%) and 0.95 log CFU/g (88.91%) in 60 min. The hot air decreased 0.21 log CFU/g (50.50%) of aerobic bacteria in 60 min, and 3.92 log CFU/g (99.99%) of *E. coli* in 30 min.

H007

Bre1 Regulates Lge1 Protein Stability to Precisely Control H2B Ubiquitination

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H2B ubiquitination is crucial for regulating the stability and reassembly of the nucleosome. *In vitro* ubiquitination assay demonstrated that H2B ubiquitination is mediated by the Rad6/Bre1 complex of *Saccharomyces cerevisiae.* However, additional proteins other than Rad6 and Bre1 are required for the ubiquitination of H2B *in vivo*. To understand the difference between *in vitro* and *in vivo* mechanisms for the ubiquitination of H2B, we explored proteins related to Rad6/Bre1 complex. Interestingly, we observed that the stability of Lge1, which was reported to be Bre1's cofactor, was greatly reduced in the absence of Bre1. The stability of Lge1 did not require the E3 ligase activity of Bre1, but did require Bre1's middle fragment containing a coiled-coil structure. Additionally, we found that Lge1 was involved in the "writing" step of H2B ubiquitination by constructing an Lge1-knockout mutant bearing the deubiquitinase deletion. Our data suggest that Bre1 mediates H2B ubiquitination more precisely by maintaining the stability of Lge1 as well as its role as a known E3 ligase.

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H008

The Arginine Residues of Histone H4 Tail are Important for the Matintenance of HM Silencing in S. *cerevisiae*

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Transcriptional gene silencing is one of the important concepts for the epigenetic gene expression. Histone modifications are critical factors for the maintenance of gene silencing in eukaryotic systems. However, none of the histone modifications as epigenetic silencing markers is conserved in *Saccharomyces cerevisiae*. To identify global epigenetic silencing marker from yeast to human, we developed screening method using the system for the maintenance of yeast mating type. We used yeast histone library, which is a collection of strains containing alanine-substituted histone residue, and yeast single-gene knockout library. From this screening, we found histone arginine residue is required for the maintenance of HM silencing in *S. cerevisiae.* Among these histone residues, two basic residues of histone H4 tail including H4R17 and H4R19, are critical for the HM silencing. These basic amino acids are also known to be important for the telomeric silencing by recruiting Dot1, which is a specific methyltransferase for histone H3 lysine 79. We found that these two amino acids can maintain HM silencing with their positive charge, but their regulation for the HM silencing is not mediated by Dot1.

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H009

CDC73, a Subunit of Paf1 Complex, is Important for Factor Expression Required for Yeast Mating

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Epigenetic gene silencing plays an important role in regulating gene expression.
However, none of the histone modifications as epigenetic silencing markers is conserved in *Saccharomyces cerevisiae*.
We performed screening using yeast's property for the maintenance of mating type to find a well preserved epigenetic gene silencing marker from yeast to human. Screening using yeast single gene knock-out library was performed. This screening revealed that the deletion of *CDC73*, a subunit of Paf1 complex, did not maintain HM silencing in *S. cerevisiae*. However, the relationship between Paf1 complex and silencing has not been reported.
Based on these results, we confirmed localization of the SIR complex which takes responsibility for the silencing of the genes within hidden mating (HM) type locus. Unexpectedly, the localization of Sir2 in Ddc73 deleted strain is very similar that in wild-type. The transcriptomic analysis in the absence of cdc73 through RNA-seq revealed that the expressions of a factor and α-receptor are much lower than that of wild-type.
Therefore, we confirmed that the phenotype such as the failure to maintain HM silencing in *⊿cdc73* resulted from the decreased expression levels of a factor and α-receptor.
Thus, Cdc73 is critical for the expression of the mating pheromone and its receptor required for yeast mating.
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H010

The Histone Deacetylase Rpd31 and Rpd32 is Important for Virulence of *Candida albicans*

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Rpd3 is a well-known Class I histone deacetylase in *Saccahromyces cerevisiae*. In *Candida albicans*, there are two proteins as the orthologue of Rpd3, Rpd31 and Rpd32. Previous studies have focused on the physiological roles of Rpd31 and Rpd32 in hyphal formation, white-opaque switching, and drug resistance. However, in order to show these phenotypes, it is not known which genes were transcriptionally regulated by Rpd31 and Rpd32. In this study, we performed the RNA-sequencing of WT and ⊿rpd31/32 mutant in serum condition. The genes associated with biofilm formation and adhesion are down-regulated in *⊿rpd31⊿rpd32* mutant. Especially, various transcription factors related to morphogenesis were down-regulated in *⊿rpd31⊿rpd32* mutant. Indeed, we observed that the morphology of *⊿rpd31/32* mutant series were resembled with an opaque cell and forms a pseudohyphae. Surprisingly, we found that the *⊿rpd31/32* strains were avirulent in mice. These results show that the Rpd31 and Rpd32 are required for the survival in host cells by regulating the expression of genes involved in morphogenesis required for virulence.
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H011

Characterization of Lytic Bacteriophages Specific against Multidrug-resistant *Salmonella* Typhimurium

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The use of bacteriophages has received great attention as an alternative over conventional antibiotics due to their specificity for pathogenic bacteria and no serious side effects. This study aimed to evaluate the lytic activity of bacteriophages (P22-B1, P22, PBST10, PBST13, PBST32, and PBST35) against *Salmonella* Typhimurium ATCC 19585 (ST^{WT}), ciprofloxacin-induced *S*. Typhimurium ATCC 19585 (ST^{CIP}), *S*. Typhimurium KCCM 40253 (ST^{KCCM}), and *S*. Typhimurium CCARM 8009 (ST^{CCARM}). Compared to ST^{WT}, the susceptibilities of ST^{CIP} to cefotaxime, ciprofloxacin, meropenem, and norfloxacin were decreased by 32, 32, 16, and 64-folds, respectively. The adsorption rates of PBST35 ranged from 82% to 95% against ST^{WT}, ST^{CIP}, and ST^{CCARM}. The bacteriophage-binding protein bands between 24 and 36 kDa were not observed in ST^{CIP}, showing low adsorption rates of P22 and PBST10. The outer membrane-related genes (*btuB*, *ompC*, and *tolC*) and flagellar-related genes (*fliC*, *fljB*, and *fliK*) were suppressed in ST^{CIP}, resulting in the low adsorption rate. The results provide new insights for effective treatment for antibiotic-resistant bacteria. The results provide useful information to design new therapeutic strategy for the control of antibiotic-resistant pathogens.
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H012

Korea Mushroom Resource Bank

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The Korea Mushroom Resource Bank (KMRB) was launched as a national research resource bank in 2015 by the Ministry of Science, ICT and Future Planning. The main goal of the KMRB is to secure important biological resources, mushroom-forming basidiomycota, significant sources of fundamental and novel substances and materials, as dried specimen, cultures, and genomic DNA. For wider application of fungal resources in education, medicinal and industrial uses, the KMRB will undertake following tasks: 1) Survey natural environments across Korea to catalogue mushroom diversity, 2) Establish resource management system based on accurate identification of mushroom, 3) Evaluate the usefulness of the discovered mushroom, 4) Create a secure preservation and loan system. With a global focus on utilizing natural resources, mushroom resources provide excellent opportunities for academic research, and discovering novel substances for use as medicine and energy.

H013

Development of Middle East Respiratory Syndrome Coronavirus Vaccine Using a Biodegradable Adjuvant

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Middle East respiratory syndrome coronavirus (MERS-CoV) has emerged as a new pathogen that can transmit between humans as well as animals and humans. Despite high mortality rates in human and pandemic MERS cases, no licensed MERS vaccines is available. In this study, we aimed to design MERS vaccine using a biodegaradable nano-adjuvant. As MERS antigen, MERS-CoV Spike protein fused with human Fc4 (S1-Fc4) was expressed in baculovirus/insect cell system. Purified S1-Fc4 MERS antigen was intramuscularly administered to mice at a dose of 10 μg with or without biodegaradable nanoparticulate adjuvant. Vaccination was done twice with an interval of 2 weeks. Serum samples and spleens were collected two weeks after the second immunization. As compared to the other groups, the group co-treated with biodegradable nano-adjuvant provided higher titers of specific IgG antibody and neutralizing antibody. Interferon gamma secretion, an indicator of cellular immune response, was also higher in the peptide group than in the other groups. Taken together, these results suggest the potential of S1-Fc4 MERS vaccine with biodegaradable nano-adjuvant as a MERS prophylactic vaccine candidate. [Supported by grants from KHIDI (No. HI15C2842)]

H014

Development of Therapeutic Antibody Platform Using Phage Display Antibody Library against Middle East Respiratory Syndrome Coronavirus

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MERS-CoV (Middle East Respiratory Syndrome Coronavirus), a novel coronavirus causing critical respiratory diseases which shows about 37% of mortality worldwide, first appeared in Saudi Arabia in 2012. As it is well known, a spike(S) protein of the MERS-CoV has an important role in viral entry to host cell via its receptor CD26, known as a human dipeptidyl peptidase 4 (hDPP4). Thus this characteristic has led researchers to develop vaccines and therapeutic treatments. In this study, we expressed a recombinant MERS-CoV S partial protein (rS) by using *E. coli* overexpression system, and selected the antibodies which can capture the MERS-CoV by screening the rS antigen with phage antibody library. These candidates were tested by ELISA and Western blotting to figure out their binding activity to either the rS antigen or MERS-CoV S-pseudotyped virus (SPV). As a result, selected phage 4C3 and 4H3 has shown highest reactivity to both rS antigen and SPV. Following these results, further studies will show that the anti-MERS-CoV scFvs detached from the selected phage libraries have virus neutralizing activity either *in vitro* or *in vivo*. After that, this can be expected to apply one of the platforms for research to develop the therapeutic antibodies against MERS-CoV infections.

H015

Antiviral Activity of Poncirus Trifoliate Seed Extract against Oseltamivir-resistant Influenza

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The emergence of oseltamivir-resistant variants of influenza virus has led to the need for the development of novel and effective antiviral drugs. Numerous studies have focused on developing antiviral drugs using natural resources such as traditional herbal medicines. *Poncirus trifoliate* has been widely used in oriental medicine as a remedy for gastritis, dysentery, inflammation, and digestive ulcers. In this study, we investigated the antiviral effect of *Poncirus trifoliate* orange seeds extract against influenza virus. An ethanol extract of the *Poncirus trifoliate* seeds (PTex) inhibited influenza viruses and especially oseltamivir-resistant strains in Madin-Darby canine kidney cells. Unlike oseltamivir, PTx exerted greater inhibitory effect on the cellular penetration pathway of influenza rather than HA receptor binding. The potent antiviral effect and the new mode of antiviral working mechanisms suggest that PTx may be further developed as a new natural antiviral drug with a wide spectrum against influenza and oseltamivir-resistant virus.

H016

Efficacy of a Recombinant Baculovirus FMDV Type O Vaccine Delivering FMDV Capsid Genes in Mice

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The foot-and-mouth disease virus (FMDV), a picornavirus, the prototypical member of the Aphthovirus genus, is one of the most contagious and economically devastating viruses that affect cloven-hoofed livestock. Current vaccines with inactivated FMDV have several limitations including efficacy, safety, and escaping of the live virus. To overcome these limitations, we developed FMDV vaccine using that recombinant baculovirus encoding FMDV Type O capsid and capsid-processing proteins. A baculovirus has known as a safe viral vector which does not replicate in mammalian cells. In this study, we constructed a baculoviral DNA vaccine delivering FMDV P1-3D gene or VP1 gene. The transduction efficacy in HEK293 cells was analyzed by western blotting. In immunized mice with AcHA2-FMDV, we confirmed that AcHA2-FMDV induced strong IgG antibodies as well as IFN-γ. The level of IgG and IFN-γ from immunized mice was dramatically boosted after the three times of immunization. This study supports that the recombinant baculovirus vaccine, AcHA2-FMDV, may serve as a potential FMDV vaccine which can provide both humoral and cell-mediated immune responses.

H017

Development of a Middle East Respiratory Syndrome Coronavirus DNA Vaccine Using a Baculoviral Delivery System

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Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel betacoronavirus that has been emerging infectious disease in human. In 2015, the MERS-CoV outbreak has been occurred in the Republic of Korea. In order to aid prophylactic strategies and control of MERS-CoV outbreak in future, we have developed a MERS-CoV DNA Vaccine using baculoviral delivery system. For enhancing cellular delivery, we constructed a non-replicating recombinant baculovirus coated with human endogenous retrovirus envelope (AcHERV). First, we constructed a recombinant baculovirus encoding each of S, S1, RBD genes under the control of the AcHERV system. We confirmed MERS-CoV S, S1 and RBD genes expression levels by western blot in Huh7 cell. To investigate the efficacy of vaccine, we immunized with each of recombinant baculoviruses in Balb/c mice. We found that a recombinant baculoviruses encoding each of MERS-CoV S, S1 and RBD genes elicited high level of IgG, IgG1, IgG2a. Further studies, we will check the humoral and cellular immune response. In conclusions, AcHERV baculoviruses could serve as a potential prophylactic vaccine against MERS-CoV.

H018

Immunogenicity of Baculovirus Based on DNA Vaccine for Zika Virus

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Zika virus has been spreading widely by mosquito, causing a serious after-effect named microcephaly. ZIKV is an enveloped, positive-sense, single stranded RNA virus that is a constituent of the *Flaviviridae* family, *Flavivirus* genus. The researches of the ZIKV have been discussed actively since the linkage between the virus and the fetus microcephaly was discovered. However, there is no commercial vaccine and antiviral drugs. It is important to develop a safe and efficient vaccine for the public health. We generated that recombinant baculovirus encoding human endogenous retrovirus (HERV) envelope protein for ZIKV DNA vaccine (AcHERV-SV40 CMV ZIKV prM/E), expressing the prM/E protein of ZIKV under the control of cytomegalovirus promoter (CMV). In this study, we transduced recombinant baculovirus with or without HERV in mammalian cells and confirmed higher expression of target DNA in with HERV. Mice immunized with AcHERV-SV40 CMV ZIKA prM/E induced high level of ZIKV specific antibodies. These data show the potential that AcHERV-SV40 CMV ZIKA prM/E can be used as a safe and efficacious vaccine against the ZIKA virus. We are going to study the additionally animal experiments, and these findings may present a new avenue for developing ZIKA virus vaccine development.

H019

Identification of Toll-like Receptor Gene from *Macrobrachium nipponense*

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According to the outbreak of the infectious disease still occurs in shrimp agriculture have become a serious threat in globally. To control for infectious disease, shrimp immunity has been studied a lot of research. It has been reported that shrimp immunity is innate immunity and there is no adaptive immunity. Toll-like receptors, which are known to play an important role in innate immunity, have been investigated. In this Study, we have newly identified Toll-like receptors in *Macrobrachium nipponense* (MnToll). MnToll is 935 residues and contains 10 leucine rich repeats (LRRs) domains, LRR CT (leucine rich repeat C-terminal) domain, and TIR (Toll/interleukin-1 receptor). MnToll gene showed high similarity to MrToll (*Macrobrachium rosenbergii*) with 90% identity and other specious shrimps with about 40% identity, respectively. The transcripts of MnToll are distributed in various tissues of the heart, gills, stomach, digestive gland, ventral nerve cord, antennal gland and muscle. To analyze the Toll-like receptor characteristics, the expression level of the MnToll gene during white spot syndrome virus (WSSV) infection was monitored by qPCR for 12, 24, 48 and 72 hours. As a result, the amount of MnToll gene expression was significantly upregulated from 12 hours to 72 hours. Therefore, this suggests that the identified MnToll gene belongs to the other class of Toll receptors in shrimps and MnToll might be involved in innate host defense, especially against the WSSV.

H020

Screening of Non-catalytic Integrase Inhibitor for Anti-HIV Drug Development

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Integrase (IN) is an essential protein for HIV replication that has a role in integrating synthesized viral DNA into the host genome. For this reason, many integrase inhibitors (INIs) were developed for AIDS treatment known as highly active antiretroviral therapy (HAART). Catalytic integrase inhibitor (CINI) and Non-catalytic integrase inhibitor (NCINI) are the latest developed antiviral drugs. In this study, we compared antiviral activity of CINI and NCINI by p24 ELISA and TZM-bl cell system which expressing luciferase by HIV-1 infection. Since this TZM-bl luciferase system is the easiest assay method, it is used for antiviral primary screening. For analysis, Sup T1-CCR5 and TZM-bl cell lines were infected with M tropic HIV-1 strains. Prior to the infection, each anti-HIV drug candidates were serially diluted by 3-fold from 50 μM to 0.01 nM, and then treated to cells. p24 ELISA was performed with culture media after 5 days post infection. TZM-bl luciferase assay was performed at 2 days after infection. Strangely, commercial CINI and NCINI showed high p24 reduction at SupT1-CCR5 cell, but NCINI did not show antiviral activity at TZM-bl luciferase system. We have confirmed that CINI and NCINI have different inhibition mechanism. This is presumably due to the characteristic of NCINI, which working at second round of HIV infection. If people do not want to miss NCINI during the screening process, they have to do other analysis methods rather than TZM-bl luciferase system.

H021

Global Transcriptional Repression Memory is Mediated by H3K4me3-Rpd3L HDAC Pathway

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Transcriptional memory is critical for the faster reactivation of necessary genes upon environmental changes and requires that the genes were previously in an active state. However, whether transcriptional repression also displays "memory" of the prior transcriptionally inactive state remains unknown. In this study, we show that transcriptional repression of approximately 540 genes in yeast occurs much more rapidly if the genes have been previously repressed during carbon source shifts. This novel transcriptional response has been termed transcriptional repression memory (TREM). Interestingly, Rpd3L histone deacetylase (HDAC), targeted to active promoters induces TREM. Mutants for Rpd3L exhibit increased acetylation at active promoters and delay TREM and RNA PolII dissociation significantly. Surprisingly, the interaction between H3K4me3 and Rpd3L via the Pho23 PHD finger is critical to induce histone deacetylation and TREM by Rpd3L. Therefore, we propose that an active mark, H3K4me3 enriched at promoters, instructs Rpd3L HDAC to induce histone deacetylation and TREM.

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H022

The Antagonistic Function of NuA3 HAT and Rpd3S HDAC Fine-tunes mRNA and lncRNA Dynamics

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Histone acetylation controlled by the antagonistic function of HATs and HDACs directly activates RNA PolII transcription. NuA3 HAT is known to bind to active promoters via interaction between Yng1 PHD finger and H3K4me3 to acetylate histone H3K14 and activate transcription. However, the role of this complex in global transcription remains elusive. To scrutinize the function of NuA3 HAT in gene expression, genome-wide transcription was analyzed in cells undergoing a series of carbon source shifts. Loss of NuA3 has no effect on basal transcription levels but delays or reduces gene induction. Interestingly, N-terminal region of Yng1 and Nto1 PHD finger but not Yng1 PHD finger are important for optimal induction of NuA3 target genes. We also find that these two binding modules activate mRNA and cryptic promoters repressed by the Set2-Rpd3 HDAC pathway. Chromatin immunoprecipitation assay reveals that NuA3 directly acetylates histones at Rpd3S-repressed promoters to activate mRNA and lncRNA transcription. These findings suggest that the balance between NuA3 and Rpd3S fine-tunes mRNA and lncRNA expression dynamics upon environmental changes.

H023

Exocyclic GpC DNA Methyltransferase from *Celeribacter marinus* IMCC12053

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DNA methylation is involved in diverse processes in bacteria, including maintenance of genome integrity and regulation of gene expression. CcrM, the DNA methyltransferase conserved in Alphaproteobacterial species, has N6- adenine or N4-cytosine methyltransferase activities using S-adenosyl methionine as a co-substrate. *Celeribacter marinus* IMCC12053 and *Novosphingobium pentaromativorans* US6-1 isolated from the marine environment are alphaproteobacteria. Both strains replace the methyl groups of the exocyclic amines of CpG and GpC cytosines to produce N4-methyl cytosine. Using single molecule real-time sequencing method (SMRT), methylation patterns of *C. marinus* IMCC12053 and *N. pentaromativorans* US6-1 were compared using Gibbs motif sampler program. Both strains showed conversion of adenosine of 5'-GANTC-3' to N6-methyladenosine, and N4-cytosine of 5'-GpC-3' (IMCC12053) and 5'-CpG-3' (US6-1) to N4-methylcytosine. Exocylic DNA methyltransferases from both of the species were chosen for cloning using phylogenetic analysis. Using dam-/dcm- *E. coli* as expression host. The genomic DNA and plasmid carrying methylase-encoding sequences were extracted and cleaved with restriction enzymes sensitive to methylation to confirm the methylation activity. These methylases protected the restriction enzyme site once methylases methylate the chromosome and plasmid of their. In this study, characteristics of cloned exocyclic DNA methylases were investigated for potential uses of novel type of GpC methylase for molecular biology and epigenetics.

H024

Immunomodulatory Effects of Five Novel Probiotic Strains Isolated from Infant Feces

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The purpose of this study is to isolate, identify and characterize novel strains of probiotics from infant feces, Various physiological features of the candidate probiotics isolates were preliminarily investigated, including gram-staining, API test, 16S rRNA sequencing, tolerance to stimulated gastric juice and bile salts, adherence ability, antibiotic resistance, and immunomodulation. Based on this morphological and biochemical chracteristics, five potential probiotic isolates (*Enterococcus faecalis, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus paracasei, Streptococcus thermophilus*) were selected. *Lactobacillus plantarum* and *Enterococcus faecalis* showed potential tolerance to stimulated gastric juice and bile salts. The two strains described above and *Streptococcus thermophiles* had higher adherent properties compared to *Lactobacillus rhamnosus* GG. All five strains inhibited LPS-induced pro-inflammatory cytokine, IL-6 and TNF-α, in RAW 264.7 macrophages. *Lactobacillus fermetum* and *Lactobacillus plantarum* enhanced anti-inflmmatory cytokine, IL-10. These results indicate that pretreatment with our strains could influence the immune-modulating activity. Overall, our findings suggest that the specific strains from infant stools should be considered as probiotic strains, and may be useful for the prevention and treatment of various immunologic disorders which are associated with abnormal inflammatory responses such as allergies and inflammatoy bowl diseases.

H025

Functional Divergence of Paralogous Cyclic AMP Receptor Proteins in *Mycobacterium smegmatis*

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Cyclic AMP receptor protein (CRP) is a transcription factor that recognizes intracellular cyclic AMP (cAMP) levels, thereby controlling gene expression in response to changes in cAMP levels. The genome of *Mycobacterium smegmatis* possesses two *crp* paralogous genes; *crp1* (*MSMEG_6189*) and *crp2* (*MSMEG_0539*). The sequence identity between CRP1 and CRP2 is 78% at the amino acid level. To assess the function of the two CRP paralogs, *crp1* and *crp2* mutants of *M. smegmatis* (Δ*crp1* and Δ *crp2*) were constructed. SEM analysis showed severe clumping of Δ *crp1* and Δ *crp2* cells. RNA sequencing analysis revealed that inactivation of Δ *crp1* or Δ *crp2* resulted in considerable changes in transcriptional profiles. A total of 633 genes were differentially expressed (*p*-value < 0.05, |Fold changel $\geq$ 2) among the 6938 genes in Δ *crp1* mutant relative to the wild type strain, of which 374 were induced and 259 were repressed in the mutant. There were 354 differentially expressed gene (DEG, *p*-value < 0.05, |Fold changel $\geq$ 2) in Δ *crp2* mutant relative to the wild type strain, of which 288 were induced and 66 were repressed in the mutant. Interestingly, the DEGs of Δ *crp1* overlap with 46% of the known dormancy survival regulator (*dosR*) regulon. Moreover, 63% of genes that are induced under starvation conditions overlap with the Δ *crp2* DEGs. These results imply that CRP1 and CRP2 have divergent function in gene regulation.

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H026

Upregulation of the SigF Regulon in an aa_3-Cytochrome *c* Oxidase Mutant of *Mycobacterium smegmatis*

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In *Mycobacterium smegmatis*, SigF, an alternative sigma factor, plays a role in adaptation to stationary phase and oxidative stress. The functionality of SigF is post-translationally regulated by the anti-sigma factor RsbW (MSMEG_1803). We identified two genes encoding the putative anti-anti-sigma factors, RsfA (MSMEG_1786) and RsfB (MSMEG_6127), based on their homology to *Mycobacterium tuberculosis* RsfA and RsfB. To examine the function of these anti-anti-sigma factors in *M. smegmatis*, we constructed *sigF*, *rsfA*, and *rsfB* mutant strains (Δ*sigF*, Δ*rsfA*, and Δ*rsfB*) of *M. smegmatis.* The expression of *MSMEG*_1777, which is known as SigF regulon, was abolished in the Δ*sigF* and Δ*rsfB* mutant strains, but not in the Δ*rsfA* mutant, suggesting that RsfB is required for SigF function. Interestingly, expression of the SigF regulon was significantly increased in an aa_3-cytochrome *c* oxidase mutant strain (Δaa_3) of *M. smegmatis*. Although expression of the SigF regulon was markedly increased in the Δaa_3 mutant, no significant changes in expression of *sigF* and *rsbW* occurred in the Δaa_3 mutant. To examine whether RsfA and RsfB are involved in upregulation of the SigF regulon in the Δaa_3 mutant, the Δaa_3 Δ*rsf*A and Δaa_3 Δ*rsfB* mutant strains were constructed. Upregulation of *MSMEG_1777* was abolished in the Δaa_3 Δ*rsfB* mutant, but not in the Δaa_3 Δ*rsf*A mutant, suggesting that RsfB is implicated in the upregulation of SigF regulon in the Δaa_3 mutant.

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H027

Characterization of Human Monoclonal Antibodies Neutralizing MERS-Coronavirus

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Neutralizing antibodies protect against viral infection by interfering with virions that bind to receptors, blocking virus entry into cells, resulting prevention of virus propagation and spread. The outbreak of MERS occurred in the Republic of Korea in 2015. Although high mortality (~35%) by MERS infection has been reported worldwide, unfortunately, there is currently no specific vaccine or treatment for MERS-CoV infection. In this study, we present seven human monoclonal antibodies (mAbs) isolated from peripheral blood mononuclear cells of Korean MERS patients. The mAbs specifically bind to MERS-CoV spike protein S1. The plaque reduction neutralization test was performed to quantify the neutralizing activity of the antibody against the virus. Out of 7 candidates, three antibodies, named 58, 68, and 72, are found to neutralize MERS-CoV infection efficiently. We also evaluated antibody affinity based on enzyme-linked immunosorbent assay and surface plasmon resonance using S1 antigen. Western blot analysis demonstrated that these mAbs can be applicable to detect S1 efficiently when compared with commercial antibodies. Immunocytochemistry assays showed that our mAbs have the specificity to MERS-CoV, not bind to other human coronaviruses. Further biophysical analysis reveal that the mAbs have structural stability. Therefore, we expect our mAbs to be potent molecules to use for MERS-CoV therapeutics or diagnostics.

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H028

Characteristics of Zika Korean Isolates in Mouse and Evaluation of Antiviral Compounds *in vitro*

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Zika virus (ZIKV) is an arbovirus belonging to the genus Flavivirus (family *Flaviviridae*) and has RNA as a genome. The ZIKV is caused mosquito-borne disease that characterized by fever, rash, arthritis, and myalgia symtoms in the patient. In 2016, microcephaly and Guillain-Barre syndrom have been reported to be associated with this virus. The ZIKV spread on the world wide, since then, there have been reports of 28 cases of infected patients from abroad with ZIKV in South Korea. In this study, we evaluated virulence within AG129 mice as an infectious animal model using the MR766 and the Korean isolates. Survivla of mouse was monitored, there was confirmed a difference virulence between each strain. Moreover, antiviral-agent studies have been conducting globally, commercialized antiviral-agent have not yet been developed. Herein, we founded two substances had antiviral effects *in vitro* in a concentration-dependent manner. These results might be applicable to the development of therapeutic measures against Zika virus infection.

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