**Adhaerimonas asanensis** gen. nov., sp. nov., a Novel Bacterium Isolated from a Tidal Flat in the Yellow Sea

Sophea Pheng1,2, A-young Park1, Yong-Jae Lee1, Kang Hyun Lee1, and Song-Gun Kim1

1 Korea Research Institute of Bioscience and Biotechnology, 2University of Science and Technology

A novel strain designated YelD216T was isolated from the tidal flat of Asan Bay in the Yellow Sea, South Korea. Strain YelD216T was facultative-anaerobic, Gram-negative, motile with a single polar flagellum, non-spore forming, sticky on an agar plate, short-rod-shaped, and required NaCl for growth. The optimal growth occurred at 25°C to 35°C, pH 7-8 and in the presence of 2% NaCl. The comparative phylogenetic analysis based on the 16S rRNA showed that the strain YelD216T belongs to the family Alteromonadaceae. Higher similarities of 16S rRNA gene sequences were found with *Bowmanella denitrificans* (93.2%), *Aestuaribacter salixigenus* (93.1%), *Salimonas changwensis* (92.8%), and *Alteromonas macaoledoi* (92.7%). Strain YelD216T contained Q-8 as a major isoprenoid quinone; polar lipid consisted of phosphatidylethanolamine, phosphatidylglycerol, and an unidentified amino lipid. The major fatty acids were C16:0, C17:0 10-methyl, summed feature 3 (C16:0 6c and/or C17:0 7c), and summed feature 8 (C18:1ω6c and/or C18:1ω7c). The DNA G+C content was 51.3 mol%. Based on the different polyphasic taxonomic and low 16S rRNA gene sequence similarities, we propose the strain forms a novel genus and species for which the name *Adhaerimonas asanensis* gen. nov., sp. nov. is proposed. The type strain is YelD216T (= KCTC 32984T = CGMCC 1.15039T).

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**Paenibacillus mobilis** sp. nov., a Gram-negative Motile Bacterium Isolated from Soil

Dahye Yang and Taegun Seo*

Dongguk University Biomedi Campus 32

A novel Gram-negative bacterium, designated strain S8T, was isolated from soil sample from Gyeonggi Province, South Korea. Cells of strain S8T were endospore-forming, motile by means of peritrichous flagella, and rod-shaped. Colonies were round, convex, wavy and white. Strain S8T grew optimally at 40°C, pH 6-9 (optimum, 8), and in the presence of 0-8.8% NaCl (optimum, 0.4%). Strain S8T contained a phosphatidylethanolamine, a diphosphatidylglycerol, and two unidentified aminophospholipids as the major polar lipids. The major fatty acids were C18:1ω6c and/or C18:1ω7c, and summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH). The predominant respiratory quinone was ubiquinone Q-8. DNA-DNA hybridization values of strain S10T with *Hydrogenophaga caeni* KCTC 12613T, *Hydrogenophaga atypica* DSM 15341T, and *Hydrogenophaga defluvii* DSM 15341 were 16.1 ± 4.8%, 49.0 ± 3.2%, and 21.9 ± 8.8%, respectively. Based on phylogenetic distinctiveness, DNA-DNA hybridization, and specific physiological and biochemical tests, strain S10T (=KCTC 52520T =JCM 31711T) represents a novel species of the genus *Hydrogenophaga*, for which the name *Hydrogenophaga soli* sp. nov. is proposed.

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**Flavobacterium communis** sp. nov., Isolated from Freshwater

Adaeze Precious Ekwe and Seung Bum Kim*

Department of Microbiology and Molecular Biology, Chungnam National University

A Gram-negative, yellow-pigmented, facultative-anaerobic, rod-shaped, non-spore forming bacterium designated PK15T was isolated from freshwater. Growth was observed at 4-40°C (optimum, 30°C), pH 6-9 (optimum, 8), and in the presence of 0-8.8% NaCl (optimum, 0.4%). Strain PK15T exhibited both catalase and oxidase activities and was able to reduce nitrate. On the basis of 16S rRNA gene sequence similarity, strain PK15T was shown to belong to the genus *Flavobacterium* with close similarities to *Flavobacterium palustre* S44T (97.9%) and *Flavobacterium sequolense* EM1321T (97.7%). Menaquinone-6 (MK-6) was the major respiratory quinone while the G+C content of the genomic DNA was 35.5 (±0.9) mol%. The major polar lipids were phosphatidylethanolamine, three unknown aminolipids, one unknown aminophospholipids and three unknown polar lipids. The predominant fatty acids were anteiso-C15:0 (17.3%), a summed feature comprising C16:1ω7c and/or C17:0 3-OH (15.1%) and iso-C15:0 2-OH (10.0%). Chemotaxonomic data supported the affiliation of strain PK15T to the genus *Flavobacterium*. The results of the physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain PK15T from closely related species. It is therefore evident that PK15T represents a new species, for which the name *Flavobacterium communis* sp. nov. is proposed with the type strain PK15T (=KCTC 52562T).

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**Hydrogenophaga soli** sp. nov., a Novel Species Isolated from Rice Field Soil in South Korea

Dahye Yang and Taegun Seo*

Dongguk University Biomedi Campus 32

A novel Gram-negative bacterial strain, designated strain S10T, was isolated from rice field soil collected in Goyang, South Korea. Cells of strain S10T were strictly aerobic, motile and rod-shaped. Colonies were round, convex, smooth and white. Strain S10T grew optimally at 37°C, at pH 7.0 and at 0% (w/v) NaCl. Phylogenetic analysis of the 16S rRNA gene sequence of strain S10T revealed that this bacterium was related to the family Comamonadaceae, and it is related to members of the genus *Hydrogenophaga*, with *Hydrogenophaga caeni* KCTC 12613T being its closest relative (97.9% sequence similarity). The DNA G+C content of strain S10T was 68.17 ± 0.03 mol%. Strain S10T contained a phosphatidylethanolamine, a diphosphatidylglycerol, and two unidentified aminophospholipids as the major polar lipids. The major fatty acids were C16:0 and summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH). The predominant respiratory quinone was ubiquinone Q-8. DNA-DNA hybridization values of strain S10T with *Hydrogenophaga caeni* KCTC 12613T, *Hydrogenophaga atypica* DSM 15341T, and *Hydrogenophaga defluvii* DSM 15341 were 16.1 ± 4.8%, 49.0 ± 3.2%, and 21.9 ± 8.8%, respectively. Based on phylogenetic distinctiveness, DNA-DNA hybridization, and specific physiological and biochemical tests, strain S10T (=KCTC 52520T =JCM 31711T) represents a novel species of the genus *Hydrogenophaga*, for which the name *Hydrogenophaga soli* sp. nov. is proposed.
**Deinococcus gammatolerans sp. nov., a Novel Bacterium Isolated from Soil, South Korea**

Sathiayar Srinivasan, Myung-Suk Kang, and Myung Kyum Kim

1 Department of Bio & Environmental Technology, College of Natural Science, Seoul Women’s University, 2 Microorganism Resources Division, National Institute of Biological Resources

A Gram-positive, catalase and oxidase positive short-rod-shaped bacterial strain designated as Ant2-2 was isolated from soil in South Korea. Cells showed high gamma-ray and UVC radiation resistance. The 16S rRNA sequences of the strain Ant2-2 was indistinguishable and showed 97.5~91.3% similarity levels with other Deinococcus species. The strain showed the typical chemotaxonomic characteristics of the genus Deinococcus, with the presence of respiratory quinone as menaquinone 8; the major fatty acids are summed feature 3 (composed of C16:1ω7c /C16:1ω6c, C17:0 3ωc, and C18:1ω7c). The DNA G+C content of the strain Ant2-2 is 57.3 mol%. The polar lipids profile included major amounts of phosphatidylglycerol, phosphatidylylycerol, and unknown aminolipid. On the basis of its phenotypic and genotypic properties, and phylogenetic distinctiveness, strain Ant2-2 should be classified in a novel species in the genus Deinococcus, for which the name Deinococcus gammatolerans sp. nov. is proposed.

This work was supported by a research grant from the National Institute of Biological Resources (NIBR201601113), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201601113).

**Xanthomonas arboricola pv. juglandis Isolated from Walnut Tree in Korea**

Hyun-sun Kim and Sang-tae Seo*

National Institute of Forest Science

Xanthomonas arboricola is a bacteria, Gram-staining-negative and rod-shaped. The bacteria can cause diseases in many plants and is divided into various pathovars according to the host. One of pathovars, Xanthomonas arboricola pv. juglandis cause walnut blight in walnut tree and reduces yields. The bacteria can infect many organs of walnut including catkins, green branches, leaves and nuts and infected sites changed to black. The disease was detected in all major walnut growing areas. But, the disease was not detected in Korea.

In 2016, we found walnut trees turned to black in Korea. The symptoms of trees were very similar to walnut blight. We isolated various bacteria from walnut trees and collected the colonies, mucoid and yellow morphology in NA (Nutrient Agar) medium. PCR was performed for 16S rRNA gene. As a result of BLAST, these colonies showed various bacteria including Pantoea, Pseudomonas, and Xanthomonas. Most of them were Xanthomonas arboricola pv. juglandis strain KJ 417(98.0% 16S rRNA gene sequence similarity). Phylogenetic analysis, based on 16S rRNA gene sequencing, showed that the bacteria was most closely related to Xanthomonas arboricola pv. juglandis NCPPB411 and LVMG747[100%].

[Supported by grants from National Institute of Forest Science]

**Deinococcus koreense sp. nov., a Novel Bacterium of Isolated from a Soil**

Myung Kyum Kim, Myung-Suk Kang, and Sathiayar Srinivasan*

1 Department of Bio & Environmental Technology, College of Natural Science, Seoul Women’s University, 2 Microorganism Resources Division, National Institute of Biological Resources

Strain KSM4-11, a red-colored, non-motile, catalase and oxidase positive short-rod-shaped, Gram-negative bacterium, was isolated from a dry soil sample collected at Seoul, South Korea. The isolate aerobically grew at 25–30°C (optimum 30°C), pH 7.0–8.0 (optimum pH 7.0) and in the presence of 0.0–0.5% (w/v) NaCl (optimum 0 % NaCl). Cells showed high gamma-ray and UVC radiation resistance. Phylogenetic analysis based on 16S rRNA gene sequence of strain KSM4-11 revealed that it belongs to the genus Deinococcus in the family Deinococcaceae. The highest degree of sequence similarities of 94.0% with Deinococcus radiorestris 8A. The strain showed the typical chemotaxonomic characteristics of the genus Deinococcus, with the presence of respiratory quinone as menaquinone 8; the major fatty acids are summed feature 3 (composed of C16:1ω7c /C16:1ω6c, C17:0 3ωc, and C18:1ω7c). The DNA G+C content of the strain KSM4-11 is 56.3 mol%. The polar lipids profile contained major amounts of phosphatidylglycerol and unknown aminolipids. By its phenotypic and genotypic properties, and phylogenetic distinctiveness, strain KSM4-11 should be classified as the representative of a novel species the genus Deinococcus, for which the name Deinococcus koreense sp. nov. is proposed.

This work was supported by a research grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201601113).

**Genome Comparison of 12 Lactobacillus Species for Species-specific Media**

Inhwan You, Jihyun Won, Gwi-Deuk Jin, Jongbin Park, and Eun Bae Kim*

1 Department of Animal Life Science, College of Animal Life Science, Kangwon National University, 2 Institute of Animal Resources, College of Animal Life Science, Kangwon National University, 3 Division of Applied Animal Science, College of Animal Life Science, Kangwon National University

Lactic acid 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. However, enrichment medium for Lactobacillus specific strain is not well established. In this study, we compared of 12 Lactobacillus species to obtain useful information for development of enrichment media for a specific Lactobacillus species. Using such information, we modified a pre-existing media to enrich a Lactobacillus species. These results indicates that comparative genomics is useful for the development of in vitro bacteria tools. This media will be helpful to isolate useful Lactobacillus strains for food and feed industry.

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Marinirhabdus citreus sp. nov., a Marine Bacterium Isolated from Tidal Flat Sediment

Sung-Hyun Yang, Hyun-Seok Seo, Jung-Hyun Lee, and Kae Young Kwon*
Korea Institute of Ocean Science & Technology

A gram-negative, aerobic, rod-shaped (1.3-1.9 μm×0.3-0.5 μm) and non-motile marine bacterium, designated as MEBiC09412\(^T\) was isolated from tidal flat sediment of the Yeonggwang County, South Korea. The 16S rRNA gene sequence analysis revealed that strain MEBiC09412\(^T\) showed high similarity with the Marinirhabdus gelatinyltica NH83\(^T\) (96.5%). Growth was observed at 17–38°C (optimum 30°C), at pH 4.0–8.5 (optimum pH 7.0) and with 0.5-6% (optimum 2.5%) NaCl. The predominant cellular fatty acids were iso-C\(_{16:0}\) (27.4%), iso-C\(_{15:1}\) G (9.6%), iso-C\(_{17:0}\) 3OH (13.2%) and summed feature 3 (comprised of C\(_{15:0}\)ω6c and/or C\(_{16:1}\)ω7c; 7.4%). The DNA G+C contents is 43.1 mol%. The major respiratory quinone is MK-6. Several phenotypic characteristics such as production of indole and Enzyme activities of α-chymotrypsin and α-galactosidase differentiate strain MEBiC09412\(^T\) from M. gelatinyltica NH83\(^T\). On the basis of this polyphasic taxonomic data, strain MEBiC09412\(^T\) should be classified as a novel species in the genus Marinirhabdus and it is proposed as Marinirhabdus citreus sp. nov. The type strain is MEBiC09412\(^T\) (=KCCM 43216\(^T\) =ICM 31588\(^T\)).

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Complete Genome Sequence of the Aneurinibacillus soli CB4\(^T\) from Soil of Mountain

Keun Chul Lee, Kwang Kyu Kim, Byungwook Lee, and Jung-Sook Lee*
Korea Research Institute of Bioscience and Biotechnology

Aneurinibacillus soli CB4\(^T\) is a gram-positive, motile rods and strictly aerobic bacterium. Here we present the 4.1-Mb genome sequence of the type strain of A. soli CB4\(^T\), which consists a chromosome for the total 4,116,770 bp with a G + C content of 45.9 mol%. Genes related to diverse secondary metabolites were detected in this genome. The genomic data is expected to understand the possibility of industrial and commercial use by strain CB4\(^T\).

[This work was supported by Mid-career Researcher Program through NRF grant funded by the Ministry of Science, ICT and Future Planning (MSIFP) of the Republic of Korea and a grant from the KRBEB Research Initiative Program.]

Isolation of Probiotic Leuconostoc gasicomitatum BB7 from Cabbage Kimchi

Byung-Min Lee\(^1\) and Oh-Sik Kwon* \(^2\)

\(^1\)Department of Biology, Graduate School, Keimyung University, \(^2\)Major in Biological Science, College of Natural Science, Keimyung University

Isolation of Leuconostoc species from fermented kimchi was carried out in order to study L. species as probiotics. After CFU was tested, each 10\(^5\) cells (BB7, KCTC 3525, KCTC 3527, and KCTC 3753) were to incubate at 25°C for carbohydrate fermentation tests. As a result Lc. carnosum KCTC 3525 showed big differences comparing to other tested species in fermentation of pentose, dextran and trisaccharide. In disaccharide tests BB7 revealed exactly same pattern of fermentation with L. gelidum subsp. gasicomitatum KCTC 3753. From NaCl tests they failed to grow over 4% NaCl containing MRS media except KCTC 3525. At 2% NaCl KCTC 3527 showed poor growth (O.D. 1.43±0.01) but BB7 (3.63±0.02), KCTC 3753 (4.27±0.02), KCTC 3525 (4.76±0.01). This tendency was repeated in 3% NaCl. In tests of bile salt tolerance all test strains could not grow with sodium glycocholate (SGC) however KCTC 3525 could grow with sodium taurocholate (1.16±0.01). From the results of acid tolerance tests BB7 grew very well in pH 3.0 and pH 3.5 MRS broth (0.12±0.01, 0.18±0.01, respectively) comparing to pH 4.0 and pH 4.5 (0.14±0.01, 0.08±0.01). Interestingly addition of NaCl (2%) to the broth, BB7 showed outmost growth in pH 3.5 (0.10±0.01). From antibiotic resistant tests, BB7 revealed outstanding results comparing to other test strains. BB7 was very resistant to ampicillin, kanamycin, streptomycin and vancomycin with concentration of 0.5 μg/ml. All test strains showed strong resistance to vancomycin.

Diversity of Macrofungi at the Royal Tombs of the Joseon Dynasty and Jongmyo Shrine near Seoul South Korea

Hae Jin Cho, Hyun Lee, Vladimir Li, Ki Hyeong Park, Nam Kyu Kim, and Young Woon Lim*
School of Biological Sciences, Seoul National University

Macrofungi play important roles in maintaining forest ecosystems via carbon cycling and the mobilization of nitrogen and phosphorus. The Royal Tombs (Donggureung and Seooreung) of the Joseon Dynasty and Jongmyo Shrine were targeted in this survey because the surrounding vegetation is well-preserved and they have been designated as World Heritage sites by UNESCO. During field surveys of the Royal Tombs and Jongmyo Shrine in 2015-2016, 882 macrofungi were collected and classified. A total of 250 species belonging to 142 genera and 58 families were identified by morphological characteristics and ITS sequence analysis. However, 111 species did not satisfactorily align with previously identified species. Thus, further study is needed to determine if these samples represent new species. Species diversity was highest at the Donggureung site and the lowest at the Jongmyo site. Diversity of the ECM community of Donggureung (49.5%) was much higher than at the other two sites while wood decay fungi was highest at the Jongmyo site (58%). This study identified 13 species as the first reports of these species in Korea: Agrocybe smithii, Cortinarius hinuleoarmillatus, Cruentomycena kedrovoa, Galertina sulciceps, Heloboloma arenorum, Hymenopellis chiaiangiae, Incocybe stellate, Leiotrametes lactinea, Parasola setulosa, Piptoporellus soloniensis, Pluteus longistriatus, Simocybe serrulata, Strobilomyces pteroceticulosporus.
Identification of Dextran Producing Lactic Acid Bacteria that Isolated from Kimchi

Bo-Hyun Hwang1 and Oh-Sik Kwon2,*

1Department of Biological, Graduate School, Keimyung University, 2Major in Biological Science, College of Natural Science, Keimyung University

Isolation of lactic acid bacteria from kimchi was done to study lactic acid bacteria (LAB) from kimchi that produced dextran. With the organisms comparative analysis was done with NaCl tolerance tests, carbohydrate fermentation tests and 16S rRNA sequence analysis. From NaCl tests JC2-3 revealed outmost cell growth (O.D. 8.5±0.01 in 1% NaCl) other than LAB. All strains could not grow in media containing higher salt concentrations such as 7% and 8% except JC2-3. From the results of hexose, JC2-3 fermented fructose, glucose and mannose excessively than others (O.D. 2.89±0.02, 3.65±0.01, and 4.06±0.03). In fermentation of disaccharide, all strains fermented sucrose, maltose, and trehalose with 5.4±0.01, 8.17±0.03, and 2.89±0.02. In fermentation tests and 16S rRNA sequence analysis. From NaCl tests JC2-3 revealed different pattern of fermentation comparing to JA1-3 and JB1-2. Same tendency of fermentation was found in triascaride. In case of complex sugar fermentation, all strains ferment very well with amygladin and salcin (O.D. 1.74±0.01* 2.03±0.01 and 1.97±0.02* 2.13±0.04). From 16S rRNA sequence analysis 2 strains were turned out to be Weissella cibaria (JA1-3 and JC2-3) and others were to be L. mesenteroides (JA2-3 and JB1-2) that were determined by 99% match in 16S rDNA sequences. Among them, the JC2-3 revealed outmost production of dextran in 3% sucrose media comparing to other strains such as JA1-3, JB1-2 and JB1-3. Thus it is concluded that the JC2-3 is valuable LAB in order to study dextran production in relating with Weissella cibaria.

Taxonomic and Genomic Analysis of an Extremely Halophilic Archaeon, Halostella salina gen. nov. sp. nov., Isolated from Solar Salt

Hye Seon Song1, Jin-Kyu Rhee1, Joon Yong Kim1, Yeon Bee Kim1, Changsu Lee1, Joseph Kwon1, Jin-Kyu Rhee1, Hak-Jong Choi1, and Seong Woon Roh2,*

1Microbiology and Functionality Research Group, World Institute of Kimchi, 2Biological Disaster Analysis Group, Korea Basic Science Institute, 3Department of Food Science and Engineering, Ewha Womans University

Extremely halophilic archaea, called halaarchaea, are adapted to hypersaline environments. In this study, a novel halophilic archaeon designated strain CBA1114T was isolated from solar salt. Strain CBA1114T, which is a cocoid and stained Gram-negative, grew in the presence of 15–30% (w/v) NaCl (optimum, 20%) and at 20–50°C (optimum, 40°C) and pH 7.0–9.0 (optimum, pH 8.0). The 16S rRNA gene sequence of strain CBA1114T showed a 91.7% similarity to that of Haloterrigena thermovorans PRL5. A phylogenetic tree generated from the results of 16S rRNA gene and MSLA of the five housekeeping genes showed that strain CBA1114T was closely related to the species of the genus Halorantiella in the family Halobacteriaceae. The draft genome sequence of strain CBA1114T contains 3,518,863 bases with G+C content of 67.1%, 4 rRNAs, 42 tRNAs and 3,915 CDSs. The annotated genome contains a number of genes associated with “Amino Acids and Derivatives”, “Carbohydrates”, and “Co-factors, Vitamins, Prosthetic Groups”. According to the results of phylogenetic, phenotypic, chemotaxonomic and genomic analyses, we designate strain CBA1114T as Halostella salina gen. nov., sp. nov., which represents a novel species of a novel genus within the family Halobacteriaceae. Their characteristic and functional gene information will be of importance for the halaarchaea researches and industries with extremozymes produced from the extremophiles.

Isolation and Identification of Purple Colored Pigment Producing Bacterium, Soonwoo sp. Strain I54 from River Water

Mercy Rose Stella Sirra and Jong-Chan Chae*

Division of Biotechnology, Chonbuk National University

A rod shaped, aerobic, non-motile, gram negative bacterium, strain I54 was isolated from water sample of river in Iksan, South Korea. Colonies of strain I54 on Trypticase soy agar were pale brown colored, round and convex with entire margins. Identification based on EZ BioCloud blast analysis of 16S rRNA gene sequence revealed that the strain I54 showed sequence similarity of 97.52% with Soonwoo buanensis HM00324T of the family Flavobacteriaceae. Neighbour joining phylogenetic analysis using MEGA6 software package showed that strain I54 clustered with Soonwoo buanensis KCTC 22689T with high bootstrap support. Aerobically grown culture of strain I54 produced a purple colored pigment after 48h of incubation. The major fatty acids (>5%) of strain I54 were iso-C15:0 anteiso-C15:0. Sum in feature 3 (comprising of C15:0, iso-C15:0, C16:0, C18:1ω9c, and C18:1ω7c) is 34.21 (± 0.3 mol%). The menaquinone was MK-6. Phosphatidylethanolamine, an aminolipid and two unknown lipids are the predominant polar lipids. On the basis of molecular and phenotypic characteristics, strain I54 is proposed as the representative of a novel species within the genus Soonwoo.

Pseudaeromonas gen. nov. in the Family Aeromonadaceae to Accommodate Pseudaeromonas sharmana comb. nov and Pseudaeromonas pectinilytica sp. nov.

Shalem Raj Padakandla and Jong-Chan Chae*

Division of Biotechnology, Chonbuk National University

A Gram-negative, rod shaped, facultatively anaerobic, motile bacterium strain AR1T was isolated from a fresh water stream in Jeonju, South Korea. Identification by 16S rRNA gene sequencing and BLAST analysis in EZ-Taxon server revealed that strain AR1T is closely related to Aeromonas sharmana GPTSA-6T with a sequence similarity of 96.83%, a value below the threshold for description of novel species. However, based on the type biochemical characters of Aeromonas sharmana GPTSA-6T to that of the other members of the genus Aeromonas and earlier studies on 16S rRNA, gyrB, rpoD and universal target region of cpn60 gene sequences of the members of genus Aeromonas, recommendations were made to transfer Aeromonas sharmana to a new genus. During the present study, phylogenetic analysis with 16S rRNA, cpn60 and dnaJ gene sequences using Neighbour joining program of the MEGA6 software package showed that strain AR1T formed a separate clade with Aeromonas sharmana GPTSA-6T among the members of the family Aeromonadaceae. Hence, based on the comparative polyphasic data obtained during the present study and also on the previous recommendations, a novel genus Pseudaeromonas gen. nov. within the family Aeromonadaceae is proposed to accommodate Pseudaeromonas sharmana gen. nov. comb. nov. with strain GPTSA-6T (=DSM 17445T =MTCC 7090T =CIP 109378T =CCUG 54939T) as the type species of the genus.
Isolation and Characterization of Novel Paucibacter Species

Ji-hye Han, Kiwoon Baek, and Mi-hwa Lee*
Bacterial Resources Research Division, Freshwater Bacterial Resources Research Bureau, Nakdonggang National Institute of Biological Resources

Four strains belonging to the genus Paucibacter were isolated from sediment of Nakdong River including Neodeol spring (Head of Nakdong River, Taebaek) and Changnyeong Hanam weir. The genus Paucibacter is comprised only one species, P. toxinivorans that degrades cyclic cyanobacterial hepatotoxins microcystins and nodularin. On the basis of the 16S rRNA comprised only one species, Paucibacter toxinivorans 2C20 with 97.75–98.38% similarities and they were very similar to each other (98.48–99.97%). The properties of major cellular fatty acids, quinone and polar lipids of four isolates were within the general range for the genus Paucibacter, however many biochemical characteristics especially, utilization carbon source and whole cell protein profiles using matrix-assisted laser desorption ionization time-of-flight analysis distinguished these isolates from type species. Thus, three strains represent novel species of the genus Paucibacter.

Isolation and Characterization of Novel Spongiibacterium species

Ji Hee Lee, Joo Won Kang, Mi Sun Kim, Seon Choi, Hee Geon Yang, Wan Gyu Kim, and Chi Nam Seong*
Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

A Gram-stain-negative, rod-shaped, aerobic bacterium, designated strain 119BY6-57 was isolated from marine sponge collected from Jeju Island. Strain 119BY6-57 grew between 10 and 37°C, with an optimum of 20 and 30°C. The pH range for growth was between 6.0 and 10.0, with an optimum of pH 7.0. The range of NaCl concentration for growth was between 0.0 and 9.0% (w/v), with an optimum of 1.0 and 2.0%. Catalase- and oxidase-positive. 16S rRNA gene sequence analysis showed that strain 119BY6-57 belonged to genus Lysobacter, the closest member being Lysobacter daejeonensis GH1-9, with a gene sequence similarity of 97.25%. The major fatty acids were iso-C_{15:0} (31.8%), iso-C_{16:0} (16.4%), summed feature 9 (C_{12:0} 3-OH/C_{14:0} 10-methyl) (13.1%). The major isoprenoid quinone was Q-8. The major polar lipid present was phosphatidylethanolamine. The G+C content of the genomic DNA was 69.9 mol%. On the basis of the data presented in this study, strain 119BY6-57 is considered to represent a novel species of the genus Lysobacter.

Hymenobacter aquatilis sp. nov., Isolated from a Mesotrophic Artificial Lake

Heeyoung Kang, Inseong Cha, Haneul Kim, and Kiseong Joh*
Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

A Gram-stain-negative and non-motile bacterial strain that formed straight rods and reddish colonies, designated HMF3095, was isolated from freshwater of a mesotrophic artificial lake in Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain HMF3095 belonged to the genus Hymenobacter and was most closely related to Hymenobacter seoulensis 16F7G (96.7% sequence similarity), Hymenobacter latericoloratus YIM 77920 (96.3%) and Hymenobacter luteus YIM 77921 (96.3%). The major fatty acids were iso-C_{15:0} and/or anteiso-C_{17:0} (31.8%), iso-C_{15:0} and/or C_{16:1}ω6c, summed feature 4 (iso-C_{15:0} and/or anteiso-C_{15:0}) (13.1%) and anteiso-C_{15:0}. The major isoprenoid quinone was menaquinone 7 (MK-7). DNA G+C content was 58.9 mol%. On the basis of the evidence presented in this study, strain HMF3095 represents a novel species of the genus Hymenobacter, for which the name Hymenobacter aquatilis sp. nov. is proposed. The type strain of the species is strain HMF3095 (=KCTC 52398 =NBRC 112669).

Spongiibacterium aquimarinus sp. nov., Isolated from Seaweed Ecklonia cava

Ji Lee, Joo Won Kang, Mi Sun Kim, Seon Choi, Hee Geon Yang, Wan Gyu Kim, and Chi Nam Seong*
Department of Biology, Sunchon National University

A Gram-stain-negative, rod shaped, non-motile and yellow pigmented bacterium, designated strain A018, was isolated from a seaweed Ecklonia cava obtained from the South Sea (34°02′00″N, 127°20′00″E), Republic of Korea. Spongiibacterium pacificum sw169 was the nearest neighbor of strain A018 with 96.5% 16S rRNA gene sequence similarity. Growth occurs at 10–35°C (optimum, 25–30°C), at pH 7–9 (optimum, pH 7–8) and with 2–5% (w/v) sea salts (optimum, 3%). Flexirubin-type pigments are absent. Catalase-positive and oxidase-negative. The major quinone was menaquinone 6 (MK-6). The DNA G+C content of the strain was 38.5 mol%. On the basis of phenotypic-, chemotaxonomic data and phylogenetic inference, strain A018 should be classified into the genus Spongiibacterium, as a member of a novel species, for which the name Spongiibacterium aquimarinus sp. nov. is proposed. The type strain is A018 (=KCTC 52335T).

[This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea. It was also supported by the CK (university for Creative Korea)-I]
**A022**

*Shewanella saliphilus* sp. nov., *Shewanella ulleungensis* sp. nov. and *Shewanella litoralis* sp. nov., Isolated from Coastal Seawater

Bo-Ram Yun1, Min-Kyeong Kim1, Sunjoo Park2, and Seung Bum Kim1,*

1Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, 2MicroID Co. Ltd.

Three strains designated MMS16-UL2501, MMS16-UL2531 and MMS16-UL4821 were isolated from seawater near Ulleung Island, Korea. The isolates were Gram-negative, non-spor-forming, rod-shaped, and motile by flagellum. All isolates grew at 4-30°C (optimum, 25°C) and at pH 6-10 (optimum, pH 7). Strains MMS16-UL2501, MMS16-UL2531, and MMS16-UL4821 grew optimally in the presence of 4.5, 2.5, and 2.5% NaCl, respectively. Phylogenetic trees based on 16S rRNA gene sequences revealed that all strains clustered with *Shewanella algaico* St-61. However, the 16S rRNA gene sequence similarity between the three isolates and *S. algaico* St-61 was in the range of 98.1-99.2%, and that among the isolates was 98.5-99.0%. The main quinones for all strains were Q-7, Q-8, MK-7, and MMK-7, and the composition varied among isolates. The DNA G+C contents of the strains also varied between 42.1 and 43.7 mol%. Phenotypic properties distinguished the strains from one another as well as from *S. algaico*. Based on the polyphasic analysis, each strain is considered to represent a novel species of *Shewanella*, for which the names *Shewanella saliphilus* sp. nov. (type strain, MMS16-UL2501), *Shewanella ulleungensis* sp. nov. (type strain, MMS16-UL2531) and *Shewanella litoralis* sp. nov. (type strain, MMS16-UL4821) are proposed.

**A023**

**Draft Genome Sequence of Bacteriophage BK30P, Lytic Phage that Infects *Macromonas* sp.**

Kiwoon Baek1,2, Ji-Hye Han1, and Mi-Hwa Lee1,*

1Bacterial Resources Research Division, Freshwater Bioneresources Research Bureau, Nakdonggang National Institute of Biological Resources, 2Department of Biological Sciences, Inha University

Bacteriophage BK30P is a lytic bacteriophage that infects the genus *Macromonas* sp. strain BK30, a Freshwater bacterium affiliated with Burkholderiales. Both the bacteriophage and the host bacterial strain were isolated from surface freshwater samples collected off the Nakdong river of Korea. The phage particle has an icosahedral capsid with a diameter of ~47 nm and a long tail of ~75 nm in length; these characteristics constitute the distinctive morphology of the myoviridae family. The complete genome sequence of phage BK30P is 43,064 bp long with 58.6% G+C content. This complete genome sequence is the first report of a lytic phage that infects *Macromonas*, for which the name “*Macromonasphage*” is proposed.

**A024**

*Lacihabitans jumunjinensis* sp. nov., Isolated from a Lagoon

Heeyoung Kang1, Haneul Kim1, JaeHo Song2, Jang-Heon Cho1, Kiseong Joh1, and Yochan Joung1,*

1Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, 2Department of Biological Science, Inha University

A non-motile, orange-pigmented bacterium, designated strain HME71034, was isolated from lagoon water in Republic of Korea. A phylogenetic tree based on 16S rRNA gene sequences showed that strain HME71034 formed a lineage within the genus *Lacihabitans* and family Cytophagaceae. The strain HME71034 was closely related to *Lacihabitans soyangensis* (95.7% sequence similarity). The major fatty acids of strain HME71034 were iso-C15:0, tiglamide, and summed feature 3 (comprising C16:1ω7c and/or C16:1ω6c). The major respiratory quinone was MK-7. The major polyamine was spermidine. The major polar lipids were phosphatidyethanolamine (PE), two unidentified aminolipids (AL), one unidentified aminophospholipid (APL) and three unidentified polar lipids (PL). The DNA G+C content of strain HME71034 was 40.6 mol%. On the basis of the evidence presented in this study, strain HME71034 represents a novel species within the genus *Lacihabitans*, for which the name *Lacihabitans jumunjinensis* sp. nov. is proposed. The type strain is HME71034 (=KCTC 23619 =CECT 7956).

**A025**

*Nisaea acidiphila* sp. nov., Isolated from a Tropical Marine Algal Debris

Ji-Hye Oh, Sung-Hyun Yang, Yeonju Lee, and Kae Kyoung Kwon*

Marine Biotechnology Research Center, Korea Institute of Ocean Science & Technology

A Gram-negative, aerobic, rod-shaped (1.40-0.46 μm × 0.53±0.17 μm) and motile marine bacterium, designated as MEBiC11861, was isolated from a marine algal debris collected at Kosrae, Federation State of Micronesia (162°57’23.1”E, 5°21’13.0”N). The 16S rRNA gene sequence analysis revealed that strain MEBiC11861 showed high similarity with members of the genus *Nisaea* (97.8-98.0%). Growth was observed at 10-42°C (optimum 26-29°C), at pH 4.0-8.5 (optimum pH 5.0) and with 0-10% (optimum 0.5%) NaCl. The predominant cellular fatty acids are C16:0 (5.6%), C18:0 (29.0%), C12:0 3-OH (4.3%), summed feature 3 (C12:0 a9c7c and/or C13:0 a9c6c; 9.9%), summed feature 8 (C15:0 a7c and/or C16:0 a6c; 31.2%), and C16:0 cyclo a8c (10.6%). The DNA G+C contents is 65.6 mol%. The major respiratory quinone is Q-10. Several phenotypic characteristics such as utilization of glutonate, malate, adiate, arabinose etc., DNA G+C ratio, composition of cellular fatty acids, and growth range of pH and salinity differentiate strain MEBiC11861 from members of the genus *Nisaea*. On the basis of this polyphasic taxonomic data, strain MEBiC11861 should be classified as a novel species in the genus *Nisaea* and it is proposed as *Nisaea acidiphila* sp. nov. The type strain is MEBiC11861 (=KCCM 43046 =ICIM 30369). Emended descriptions of the genus *Nisaea* Unios et al. 2008 is also given. [Supported by Marine Biotechnology Program (grant number; 20140513) funded by the MOF, Korea]
**A026**

Marinobacterium aestuarii sp. nov., a Marine Bacterium Isolated from Estuary Sediment

Jaejoon Jung1, Seung Seob Bae2, Yoon Yong Yang3, and Kyunghwa Baek4*  
1Department of Applied Biotechnology, MABIK, 2National Marine Bio-resources and Information Center, MABIK

A Gram-staining-negative, aerobic, motile, nonflagellated rod-shaped bacterium, designated ST58-10T, was isolated from an estuarine sediment in Korea. Growth of strain ST58-10T was observed at 4–35°C (optimum, 20-25°C), pH 6.0–9.0 (optimum, pH 7.0–8.0) and 0.7–7% NaCl (optimum, 2–3%). Phylogenetic analyses based on 16S rRNA gene sequences showed that strain ST58-10T formed a phyletic lineage within the genus Marinobacterium of the family Oceanospirillaceae. Strain ST58-10T was most closely related to Marinobacterium profundum PAMC 27536T (99.5%) and Marinobacterium rhizophilum CL-Y19T (98.3%), to other members of the genus Marinobacterium (94.5–91.5%). However, the mean DNA-DNA hybridization value estimated by genome-to-genome distance calculation was 50.9 ± 2.8 with M. profundum PAMC 27536T and 50.6 ± 7.4% with M. rhizophilum DSM18823T, respectively. Major fatty acids of strain ST58-10T were summed feature 3 (comprising C16:1ω7c/C16:1ω6c and summed feature 8 (18:1ω7c) and C18:0 and contained ubiquinone (Q-8) as the sole isoprenoid quinone. The G+C content of the genomic DNA was 58.78 mol%. On the basis of the phenotypic, chemotaxonomic and molecular properties, strain ST58-10T represents a novel species of the genus Marinobacterium, for which the name Marinobacterium aestuarii sp. nov. is proposed. The type strain is ST58-10T (=KCTC52193T =NBRC112103T).

[This work was supported by National Marine Biodiversity Institute of Korea (2017MO0900).]

**A028**

Parasphingopyxis algicola sp. nov., Isolated from a Marine Red Alga Asparagopsis taxiformis

Sang Eun Jeong and Che Ok Jeon*  
Department of Life Science, Chung-Ang University

An aerobic Gram-staining-negative, orange-pigmented, rod-shaped bacterium, designated ATAX6-5T, was isolated from a marine red alga Asparagopsis taxiformis in South Korea. Cells were catalase- and oxidase-positive reactions. Growth of strain ATAX6-5T was observed at 5–35°C (optimum, 30°C), at pH 6.0–9.0 (optimum, pH 7.0) and in the presence of 0–6.0% (w/v) NaCl (optimum, 2%). Ubiquinone-10 was detected as the sole isoprenoid quinone and C15:0, C17:0 3-OH and C18:0 6-OH were identified as the major cellular fatty acids. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and sphingoglycolipid were the major polar lipids. The G+C content of the genomic DNA was 60.4 mol%. Strain ATAX6-5T was most closely related to Parasphingopyxis lamellibrachiae JAMH10132T with a 96.89% 16S rRNA gene sequence similarity. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain ATAX6-5T formed a phylogenic lineage with Parasphingopyxis lamellibrachiae JAMH10132T within the family Sphingomonadaceae. On the basis of phenotypic, chemotaxonomic and molecular features, strain ATAX6-5T clearly represents a novel species of the genus Parasphingopyxis, for which the name Parasphingopyxis algicola sp. nov. is proposed. The type strain is ATAX6-5T (=KACC 18993T =JCM 31719T).

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**A027**

Upregulated MIR144* Expression Levels in Human Macrophages and Disease Sites from Tuberculosis Patients

Jin Kyung Kim, Hye-Mi Lee, Tae Sung Kim, Yi Sak Kim, and Eun-Yeong Jo*  
Department of Microbiology and Medical Science, Chungnam National University School of Medicine

MicroRNAs (miRNAs) can regulate posttranscriptionally target gene expression. Tuberculosis (TB) is an infectious disease caused by the bacterium Mycobacterium tuberculosis (Mtbb). However, the precise function of miRNAs is unclear in human TB. To evaluate the miRNA expression profiles of peripheral blood mononuclear cells (PBMCs) from patients with pulmonary TB, we analyzed 2 miRNA microarray datasets. Fourteen miRNAs in GSE 29190 were upregulated in active pulmonary TB patients with pulmonary TB, we analyzed 2 miRNA microarray datasets. Fourteen miRNAs in GSE 29190 were upregulated in active pulmonary TB patients compared with healthy controls (HCs). The heatmap revealed that MIR144* showed the greatest magnitude of upregulation among the 10 miRNAs. We then compared the expression of MIR144* in PBMCs from active pulmonary TB patients and HCs. The expression levels of MIR144* were significantly higher in PBMCs from active pulmonary TB patients than in HCs. In addition, MIR144* expression was upregulated in samples collected from disease sites in pulmonary and extrapulmonary TB patients. We further found that MIR144* expression was upregulated in human monocyte-derived macrophages (MDMs) after infection with Mtbb in a multiplicity of infection (MOI)-dependent manner. These data indicate that MIR144* expression is robustly increased in human MDMS following Mtbb infection, and that MIR144* levels are upregulated in PBMCs/tissues from TB patients compared with HCs.

**A029**

Pan-genome and Metatranscriptome Analyses Provide Insights into the Genomic and Metabolic Features of Leuconostoc mesenteroides in Kimchi Fermentation

Byung Hee Chun, Hye Hee Jeon, Kyung Hyun Kim, Hyesu Jung, and Che Ok Jeon*  
Department of Life Science, Chung-Ang University

To investigate the comprehensive characteristics of Leuconostoc (Leu.) mesenteroides, we performed the pan-genome analysis using the downloaded in GenBank database and sequenced whole genomes of eighteen Leu. mesenteroides strains — average nucleotide identity (ANI) and in silico DNA-DNA hybridizations (DDH) values also supported that all they belong to Leu. mesenteroides. A phylogenetic tree was constructed using all core genes to investigate their phylogenetic relationships, which were a little different from the phylogenetic relationships based on 16S rRNA gene for the analysis of functional capabilities of Leu. mesenteroides, we performed relatedness based on molecular phenotypes and clusters of orthologous groups (COG) analysis. and all functional genes of eighteen genomes were cumulatively mapped onto the KEGG pathways to investigate metabolic capability. As the results, genes associated with carbohydrate metabolism and phosphotransferase system (PTS) were abundant, suggesting that Leu. mesenteroides was adapted and evolved under environments with various carbon sources. Finally, potential pathways for the sugar metabolism of Leu. mesenteroides were reconstructed based on genomic analysis. This is the first study to investigate the metabolic capabilities and diversities of Leu. mesenteroides.

[This work was supported by the Strategic Initiative for Microbiomes in Agriculture and Food, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.]
Flavobacterium chuncheonense features, strain S1-47 was 99.6%. Based on the phenotypic, chemotaxonomic and molecular features, strain IMCC25901 was isolated from freshwater lakes in Korea. Three Gram-negative, non-motile, rod-shaped bacterial strains were identified as Flavobacterium hwacheonense, Flavobacterium chungpyungense and Flavobacterium myungsuense from Lake Paro and strains IMCC26013 and IMCC26026 were from Lake Soyang. Phylogenetic analysis based on 16S rRNA gene sequences showed that three strains belonged to the genus Flavobacterium, with a high bootstrap value and their 16S rRNA gene sequence similarity was 99.6%. Based on the phenotypic, chemotaxonomic and molecular features, strain S1-47 clearly represents a novel species of the genus Alibirhodobacter, for which the name Alibirhodobacter aequarii sp. nov. is proposed. The type strain is S1-47 (=KACC 18884^T =JCM 31536). An emended description of the genus Alibirhodobacter is also proposed.

Flavobacterium hwacheonense sp. nov., Flavobacterium chungpyungense sp. nov., and Flavobacterium myungsuense sp. nov. were isolated from freshwater lakes.

A Gram-negative, non-motile, rod-shaped bacterial strain was isolated from fresh water lakes in Korea. Strain IMCC25901 was isolated from Lake Paro and strains IMCC26013 and IMCC26026 were from Lake Soyang. Phylogenetic analysis based on 16S rRNA gene sequences showed that three strains belonged to the genus Flavobacterium and strains IMCC25901, IMCC26013, and IMCC26026 were most closely related to Flavobacterium yonnginense (96.7% sequence similarity), Flavobacterium psychrophilum (96.5%) and Flavobacterium myungsuense (97.7%), respectively. Optimal growth conditions of these strains were observed at 17°C, pH 7.5 and without NaCl. DNA G+C contents of three strains ranged from 33.7 to 37.8 mol%. DNA-DNA relatedness of strain IMCC26026 with F. myungsuense was 56.4%, showing a novel species status of strain IMCC26026. Major fatty acids of three strains were iso-C_{15:0} 3-OH, G, iso-C_{16:1}ω7c, iso-C_{17:0}ω6c and/or C_{16:1}ω7c. Respiratory quinone detected in the strains was MK-6. The G+C content of the genomic DNA was 33.7%. Major respiratory quinone was MK-6. On the basis of phenotypic and genotypic characteristics, strain IMCC26026 represented a novel species in the genus Flavobacterium, for which the name Flavobacterium hwacheonense sp. nov. is proposed.

Leucobacter ruminantium sp. nov., Isolated from the Bovine Rumen

A Gram-negative, non-motile, rod-shaped, yellow-pigmented marine bacterium, designated MEBiC07310^T was isolated from tidal flat sediments in Taean province, South Korea. Phylogenetic analysis based on the 16S rRNA gene sequences indicated that strain MEBiC07310^T was affiliated with members of genus Flavobacterium belong to the Bacteriodetes phylum and showed that the strain was most closely related to Flavobacterium haurii (97.6%) and followed by Flavobacterium indicum(GPTSA 1009^T (95%) and Flavobacterium cucumis (92.5%). Growth was observed at 17–45°C (optimum 32°C), at pH 5–48 (optimum pH 7.0), and with 0–43% NaCl (optimum 1%). The major fatty acids of strain MEBiC07310^T were iso-C_{15:0} (25%), iso-C_{16:0} 3-OH (8.4%), iso-C_{15:0} 3-OH (18.6%), summed feature 1 (13.2%, iso-C_{15:1}ω7c and/or C_{13:0} 3-OH) and summed feature 3 (16.9%, C_{16:1}ω7c and/or C_{16:1}ω6c). The G+C content of genomic DNA was 33.7% and major respiratory quinone was MK-6. On the basis of phenotypic and genotypic characteristics, strain MEBiC07310^T represents a novel species in the genus Flavobacterium, for which the name Flavobacterium sediminis sp. nov. is proposed.

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Strain H0056, a Novel Member of the Genus Flavobacterium

ChaeYun Baek1, Su-Kyoung Shin2, Gun-Soo Park2,3, and Hana Yi1,4*

1Department of Public Health Sciences, Graduate School, Korea University, 2Division of Functional Food Research, Korea Food Research Institute, 3Convergent Research Center for Emerging Virus Infection, Korea Research Institute of Chemical Technology, 4School of Biosystem and Biomedical Science, Korea University

A Gram-staining-negative, aerobic, non-motile, rod-shaped, and yellow-pigmented bacterial strain, designated strain H0056, was isolated during the study of indigenous bacterial diversity in Korea. The isolate grew on R2A, TSA, NA, and MA. Best growth was observed at pH 7.5, at 30°C, and in the absence of NaCl. In the 16S rRNA gene tree, the isolate formed a distinct branch within the genus Flavobacterium, a member of the family Flavobacteriaceae. The highest sequence similarity was observed with F. arsenoxidans (97.6%), and followed by F. ginsengioli (97.5%) and F. defluvi (97.4%). The low sequence similarity (<98.7%) and tree topology demonstrated the taxonomic independence of the strain at the species-level. The phenotypic properties including Tweenase and oxidase activity also distinguished the new isolate from its close relative species. Thus, based on the genomic and phenotypic data, it is fair to say that the isolate is a novel species candidate of the genus Flavobacterium. The polyphasic study including whole genome sequencing is still underway. [This work 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 and by the National Research Council of Science & Technology (NST) grant by the Korean government (MSIP) [No. CRC-16-01-KRICT].]

A Novel Species Candidate belonging to the Genus Muclaginibacter

Soohyun Maeng1, Su-Kyoung Shin1, Jin-Soo Maeng2,3, and Hana Yi1,2,3,4

1Department of Public Health Sciences, Graduate School, Korea University, 2Division of Functional Food Research, Korea Food Research Institute, 3Convergent Research Center for Emerging Virus Infection, Korea Research Institute of Chemical Technology, 4School of Biosystem and Biomedical Science, Korea University

A Gram-reaction-negative, aerobic, non-motile, rod-shaped bacterium, designated H0046T, was isolated and subjected to be a taxonomic investigation. The phylogenetic analysis of 16S rRNA gene sequence placed the isolate within the genus Muclaginibacter, a member of the family Sphingobacteriaceae. The highest sequence similarity was observed with Muclaginibacter oryzae (98.0%) showing the independence of the new isolate from its close relative species. Thus, based on the genomic and phenotypic data, it is fair to say that the isolate is a novel species candidate of the genus Flavobacterium. The polyphasic study including whole genome sequencing is still underway. [This work 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 and by the National Research Council of Science & Technology (NST) grant by the Korean government (MSIP) [No. CRC-16-01-KRICT].]

A Proposal of Leuconostoc mesenteroides subsp. jonggjibkimchii subsp. nov. and Reclassification of Leuconostoc mesenteroides subsp. suionicum (Gu et al., 2012) as Leuconostoc suionicum sp. nov. Based on Complete Genome Sequences

Hye Hee Jeon, Kyung Hyun Kim, Byung Hee Chun, Byung Hee Ryu, Nam Soo Han, and Che Ok Jeon*

Department of Life Science, Chung-Ang University

The type strains of Leuconostoc (Leu.) mesenteroides including four validly described subspecies, Leu. mesenteroides subsp. mesenteroides, Leu. mesenteroides subsp. cremoris, Leu. mesenteroides subsp. dextranicum and Leu. mesenteroides subsp. suionicum, and strain DRC1506T, used as a starter for commercial kimchi production, were phylogenetically analyzed based on their complete genome sequences. Although the type strains of the five Leu. mesenteroides subspecies shared very high 16S rRNA gene sequence similarities (>99.72%), the analysis of ANI, in silico DDH and core genome-based relatedness suggested that they could form five different phylogenetic lineages. The type strains of five Leu. mesenteroides subspecies shared higher ANI and in silico DDH values than the thresholds (95-96% and 70%, respectively) generally accepted for different species delineation, whereas the type strain of Leu. mesenteroides subsp. suionicum (strain DSM 20241T) shared lower ANI (94.1%) and in silico DDH values (57%) with other four Leu. mesenteroides lineage strains. Here, we report that strain DRC1506T represent a novel subspecies within the species Leuconostoc mesenteroides, for which the name Leu. mesenteroides subsp. jonggjibkimchii subsp. nov. is proposed. The type strain is DRC1506T (=KCCM 43249T =JCM 31787T). In addition, Leu. mesenteroides subsp. suionicum is also reclassified as Leu. suionicum, sp. nov., comb. nov. (type strain DSM 20241T =ATCC 9135T =KIBB 8159T =CMB 6992T).

A Novel Tenacibaculum sp. Isolated from a Squid

Su-Kyoung Shin1 and Hana Yi1,2,4*

1Department of Public Health Sciences, Graduate School, Korea University, 2School of Biosystem and Biomedical Science, Korea University

A novel Gram-reaction-negative, aerobic, rod-shaped bacterium, designated strain LPB0136T, was isolated from a squid collected from the East Sea. Cell growth occurred aerobically at 4–25°C, at pH 5–9 and in the presence of 2–5% NaCl. The complete genome sequence determined in this study revealed that strain LPB0136 possessed a circular chromosome with a total length of 3,019,213 bp. The genome had a 30.7 mol% G+C content and contained 2,669 protein-coding genes and 48 RNA genes. Phylogenetic analysis based on its 16S rRNA gene sequence indicated strain LPB0136T belongs to the genus Tenacibaculum and is most closely related to T. aestuarii SMK-4 (95.9% 16S rRNA gene sequence similarity) and T. caeniipilagii-HI26M (95.9%). The respiratory quinone was menaquinone-6 and major fatty acids were iso-C15:0 3-OH, iso-C15:0 3-OH, iso-C15:0 3-OH, iso-C15:0 3-OH, iso-C15:0 3-OH, and C17:1 α9c. The size of genome, chemotaxonomic features, and physiological characteristics supported the assignment of strain LPB0136T in the genus Tenacibaculum. However, the low 16S rRNA gene sequence similarity and a number of enzymatic properties distinguished the isolate from other closely related members of the genus Tenacibaculum. On the basis of polyphasic taxonomic data, strain LPB0136T should be proposed as a novel species of the genus Tenacibaculum. [This work 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.]
Isolation of Two Novel Marine Bacteria belonging to the 4-Org1-14 and OCS116 Clades of Alphaproteobacteria and Their Genomic Characterization

Yeonjung Lim, Ilnam Kang, and Jang-Cheon Cho*

Department of Biological Sciences, Inha University

In this study, we have isolated and characterized two marine bacterial strains affiliated with the 4-Org1-14 and OCS116 clades, two abundant marine alphaproteobacterial groups, for which culture-dependent studies have been performed rarely. Strain IMCC3096 was non-motile, ivory-colored, and rod-shaped aerobic bacterium. Cellular growth occurred at 20–30°C, pH 6.0–7.0, and with 0.5–4.0% (w/v) NaCl. Phylogenetic analysis indicated that IMCC3096 belonged to the 4-Org1-14 clade, with <92% similarities to type strains of closely related phylogenetic groups. Strain IMCC20636 was non-motile, gray-colored, rod-shaped, and aerobic bacterium. Optimal growth was observed at 15–30°C, pH 7.0–7.5, and with 1.0–3.0% (w/v) NaCl. Phylogenetic analysis indicated that the strain belonged to the OCS116 clade of the order Rhizobiales, with <94% similarities to type strains of closely related families. The complete genomes of strains IMCC3096 and IMCC20636, determined by PacBio sequencing, were 4.29 Mb and 4.79 Mb in size with G+C contents of 64.6% and 53.9%, respectively. Since the genome sequences of the two strains are regarded as the sole genomic resource available currently for the 4-Org1-14 and OCS116 clades, further genomic and physiological analysis will contribute to understanding taxonomic and functional diversity of marine Alphaproteobacteria.

[This study was supported by a grant from the Marine Biotechnology Development, College of Pharmacy, Wonkwang University, Korea Polar Research Institute, KORDI and the Korea Polar Research Institute, KORDI.]

Genome Characterization and Polyphasic Taxonomy of Strain GR16-43 of Betaproteobacteria, Isolated from the Freshwater by Using Dilution-to-extinction

Ahyoung Choi1,2, Kiwoon Baek1,2, Eujin Chung1,2, and Gang-Guk Choi1,2*

1Nakdonggang National Institute of Biological Resources, 2Culture Techniques Research Division, Bacterial Resources Research Division

Culturing and characterization of freshwater bacteria is crucial to understand physiology and the ecological roles of oligotrophic freshwater microorganisms. Although many isolated belong to freshwater major phylogenetic groups have been successfully cultivated using the dilution-to-extinction method, there still remain many freshwater bacterial groups that have no cultured representatives. Depth, pH, carbon substrate preferences, and seasonal factors are all known to differentiate closely related organisms within these lineages. Strains GR16-43, belonging to the Betaproteobacteria, was isolated from a surface freshwater sample in the Geomnyong pond, by dilution-to-extinction culturing. In this report, we present the phylogenetic analyses, phenotypic characterization, and genome property of strain GR16-43, the first cultivated isolated of the Betaproteobacteria. On the basis of polyphasic analyses, strain GR16-43 is regarded to be a novel order in the Betaproteobacteria for which the name Nimbirrimonas geomnyongensis.

Characterization of Flavobacterium sp. Strain I3-3 Isolated from River Water

Kishor Sureshbab Patil and Jong-Chan Chae*

Division of Biotechnology, Chonbuk National University

A yellowish flexirubin pigment producing strain I3-3T isolated from river water in South Korea was studied to determine its taxonomic position. Cells of the isolate were rod shaped, Gram stain negative, aerobic, non-motile, and ampicillin resistant. The strain reduced nitrite to nitrate and showed catalase and oxidase activities. Optimum growth was observed at 26–30°C, pH 6.0–8.0 and upto 1.5% (w/v) NaCl, respectively. On the basis of phenotypic and phylogenetic distinctiveness, strain I3-3T is considered to represent novel species in the genus Flavobacterium and comparison of the 16S rRNA gene sequence with the sequences of the type strains of the most closely related species showed highest sequence similarities to Flavobacterium nitrogenifigens (96.95 %) and Flavobacterium compostaboris (97.93%). Further phenotypic and genotypic data will help us to propose the strain I3-3T as novel species of the genus Flavobacterium.

Cold Adaptation and Diversity of Bacteria Isolates from Chukchi Sea

Jae-Young Son1, Yu Ri Choe1, Yu-Jin Son1, Seo-Lim Hwang1, Hyun Cheol Oh1, Jong Ha Im2, and Jae Hak Sohn1,2*

1Major in Food Biotechnology, Division of Bioindustry, College of Medical and Life Sciences, Silla University, 2Institute of Pharmaceutical Research and Development, College of Pharmacy, Wonkwang University, Korea Polar Research Institute, KORDI

We survey the cold adapted characteristic and the diversity of a culturable bacteria isolated from the water column and sediment of Chukchi Sea. Samples collected from Chukchi Sea was inoculated on Marine agar plate by spread plate method and then incubated at 10°C for 20 days. The 296 bacterial strains was isolated by morphological characteristics of grown colonies. For the growth of isolated bacteria in different temperature, bacteria were inoculated in Marine agar plate and then cultured at 5°C, 10°C and 25°C for 20 days. As a result, the 22 isolates were psychrophilic form, and 15 strains were psychrotolerant form. From the phylogenetic analysis based on 16S rRNA gene sequence, psychrophilic bacteria consisted 22 taxa from 8 genera (Bacillus (10), Pseudoalteromonas (3), Paenibacillus sp., Stenotrophomonas (2), Brevibacterium (1), Alteromonas (1), Sediminicola (1), Sulfitobacter (1)) and psychrotolerant bacteria consisted of 15 taxa from 4 genera (Bacillus (10), Pseudoalteromonas (3), Brevibacterium (1), Stenotrophomonas (1)). From these results, the isolated strains cultured at low temperature showed a high diversity of psychrophilic bacteria rather than psychrotolerant bacteria.
**AO42**

**Diversity and Bioprospecting of Cold Adapted Marine Fungi Isolated from the Sediments of Ross Sea**

Jae-Young Son¹, Yu Ri Choe¹, Yu-In Son¹, Seo-Lim Hwang¹, Hyun Cheol Oh², Jonghun Im³, and Jae Hak Sohn¹**¹

¹Major in Food Biotechnology, Division of Bioindustry, College of Medical and Life Sciences, Silla University, ²Institute of Pharmaceutical Research and Development, College of Pharmacy, Wonkwang University, ³Korea Polar Research Institute, KORDI

We surveyed the diversity of marine fungi isolated from Ross Sea and their capability to produce bioactive compounds. Sediment sample were collected using box core sampler in the 6 stations of Ross Sea. Sample diluted by 10 fold dilution method after homogenization, were inoculated on ZoBell 2216e, YPG and GYA agar plate and then incubated at 10°C for 20 days. 52 fungal colonies isolated and preserved in 15% (v/v) glycerol solution. From the results of identification using internal transcribed spacer (ITS) sequences, isolates consisted of 52 taxa from 12 genera (Cladosporium (23), Penicillium (16), Aspergillus (3), Engyodontium (2), Epicoccum (1), Penicillora (1), Pseudococcoarellia (1), Talaromyces (1), Acrocomium (1), Acrodictium (1), Usitilago (1), Pithomyces (1)). Isolates were tested for their ability to grow at low temperatures (5, 10, and 20°C). Most isolates were psychrotolerant fungi. Isolates were cultured using PDA at 20°C for 15 days and extracted with ethyl acetate. From the PTP1B inhibitory assay using fungal extracts, 6 extracts displayed strong inhibitory activity. These results suggest that marine fungi isolated from the sediment of Ross Sea might be a valuable resource for the screening of bioactive compound.

**AO43**

**Microbial Communities from Commercial Salts of Danakil Depression, Ethiopia and Genome Sequencing of Halobrum sp. SAH-A6**

Kyu-Hyeong Hee Park¹, Min Gyeong Woo¹, Sun-Hee Park¹, Ashagrie Gibtani¹, Do Kyung Oh¹, Hye Won Kim¹, Na Kyeong Park¹, Dong-Woo Lee², Han-Seung Lee³, and Sang-Jae Lee¹**¹

¹Major in Food Biotechnology and The Research Center for Extremophiles & Marine Microbiology, Silla University, ²Department of Biotechnology College of Biological and Chemical Engineering Addis Ababa Science and Technology University, Ethiopia, ³School of Applied Biosciences, Kyungpook National University

Microbial communities were investigated with a metagenomic approach in four commercial salts: Ethiopian Aldera salt (EAS), Ethiopian rock salt (ERS), Korea Jangpan salt (KJS), and Korean Topan salt (KTS). These microbial communities contained 48.22 – 61.4% Bacteria, 37.72 – 51.26% Archaea, 0.51- 0.86% Eukarya, and 0.005-0.009% unclassified reads. Among bacteria, the communities in these salts were dominated by the phyla Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes. Of the archaea, 91.58% belonged to the class Halobacteria, whereas the remaining 7.58%, 0.83%, and 0.01% were Nanoarchaea, Methanobacteria, and Thermococci, respectively. The draft genome sequence of strain SAH-A6, isolated from ERS. The genome comprised 3,325,770 bp, with the G + C content of 68.0%. The strain has many genes which are responsible for secondary metabolites biosynthesis, transport and catabolism as compared to other Halobrum archaea members. Abundant genes responsible for numerous transport systems, solute accumulation, and aromatic/sulfur decomposition were detected. The first genomic analysis encourages further research on comparative genomics, and biotechnological applications. The NCBI accession number for this genome is SAMN04278861 and ID: 4278861, and strain deposited with accession number KCTC 43215. This study indicated the occurrence and diversity of halophilic bacteria and archaea in commercial salts that could be important in the gastrointestinal tract after ingestion

**AO44**

**Labrenzia sp. Nov., Isolated from Marine Sponge Callypsogorgia elegans in Jeju**

So-Hyun Park, Ji-Hyun Kim, Hae-Ri Lee, Kyung-Mi Moon, and Moon-Soo Heo**¹

¹Jeju National University

A Gram-staining-negative, aerobic, light brown pigment bacterium, designated strain CE80 was isolated from marine sponge Callypsogorgia elegans in Jeju. Strain CE80 was isolated and grown optimally at 25°C, pH 6.0-7.0 (optimum pH 7.0-8.0). The strain was able to grow at NaCl concentrations of 1.0-5.0% (w/v), with optimum growth at 1.0-3.0% (w/v) NaCl. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain CE80 belonged to the genus Labrenzia and were closely related to Labrenzia suevadai YC6927 (97.01%), Labrenzia aggregata IAM 12614 (95.90%) and Labrenzia alexandrii DFL-11 (95.90%), Labrenzia marina mano18 (95.72%) and Labrenzia alba CECT 5094 (95.34%). The major fatty acids (>2%) of strain CE80 were C18:0 (66.76%), C16:1ω7c (10.26%), C8:0 (7.11%), 11-methyl C16:0 (4.47%), C15:0ω6c (2.63%), C18:3ω6cω9cω12c (2.25%) and unknown 14:0 (2.05%). The major respiratory quinone was Q-10. The Polar lipid were phosphatidylglycerol, diphosphatidyglycerol, phosphatidylethanolamine, phosphatidylcholine, two unknown aminolipids, and three unknown lipids. The DNA G+C content of strain CE80 was 55.86 mol%. On the basis of physiological and biochemical characterization, phylogenetic and chemotaxonomic analysis, it is proposed that isolated a new species, Labrenzia sp. nov., Type strain CE80 (= KCTC 42149 = ICMP30735).

**AO45**

**Description of Arthrobacter silviterrae sp. nov., a Bacterium Isolated from Forest Soil**

Bora Chu, Shin Ae Lee, Jeong Myeong Kim, Soo-Jin Kim, Hayoun Cho, Jae-Hyung Ahn, Soon-Wo Kwon, and Hang-Yeon Weon**¹

¹National Institute of Agricultural Sciences

A novel actinomycete strain, designated KIS14-16*¹, was isolated from a forest soil in Ongjin county, South Korea, and characterized using polyphasic taxonomy. Cells are aerobic, Gram-stain-positive, non-flagellated, short-rod. Colonies are light yellow, convex and round. Comparison of 16S rRNA gene sequences showed that strain KIS14-16 is a member of the genus Arthrobacter, exhibiting highest sequence similarity with Arthrobacter livingstonensis U12* (97.7%), Arthrobacter cryoconiti C6-08* (97.6%), and Arthrobacter stackebrandii CCM 2783* (97.1%). and less than 97.0% sequence homology with all the other validly named taxa. DNA-DNA relatedness and phenotypic data readily distinguished strain KIS14-16 from phylogenetically related type strains. The peptidoglycan type of strain KIS14-16 was A3α, with an interpeptide bridge comprising L-Thr, Gly, and L-Ala. Strain KIS14-16 contained a large amount ofMK-9(H2) and the relatively small amounts of MK-10(H2) and MK-8(H4). The main polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidyl ethanolamine and one unidentified glycolipid. On the basis of these phenotypic, chemotaxonomic and phylogenetic data, strain KIS14-16*¹ should be designated as a representative of a novel species of the genus Arthrobacter, for which the name Arthrobacter silviterrae sp. nov. is proposed. The type strain is KIS14-16*¹ (= KACC 17303 = DSM 27180 = NBRC 109660). [Supported by grant from RDA]
A novel Gram-negative bacterial strain, designated T16R-256T, was isolated from the rhizosphere soil of tomato plants grown in greenhouse in Republic of Korea, and characterized using polyphasic taxonomy. Cells are aerobic, non-flagellated, and rod. Colonies are light yellow, convex (optimally at 28°C), and pH range of 7.0–9.0 (optimally at 7.0–8.0), and in 4% NaCl (w/v). Comparison of 16S rRNA gene sequences showed that strain T16R-256T is a member of the genus Parapedobacter, exhibiting highest sequence similarity with Parapedobacter pyrenivorans P-4T (94.2%), Parapedobacter indicus RK1T (93.7%), Parapedobacter koreensis Jip14T (93.7%), Parapedobacter luteus DSM 22899T (93.6%), Parapedobacter soli DCY14T (93.4%). The main polar lipids were phosphatidylethanolamine, sphingolipid, one aminophospholipid, two aminolipids and three lipids. The major fatty acids (>10% of the total fatty acids) were iso-C15:0, iso-C17:0 3-OH, and iso-C16:0 2-OH/C16:1 a9c. Strain T16R-256T contained MK-7 as predominant respiratory quinone. The genome DNA G+C content of the type strain is 55.5 mol%. On the basis of these phenotypic, chemotaxonomic and phylogenetic data, strain T16R-256T should be designated as a novel species of the genus Parapedobacter, for which the name Parapedobacter lycopersici sp. nov. is proposed. The type strain is T16R-256T (= KACC 18788T = JCM 31602T).

This research was supported by RDA.
Identification and Characterization of Yeasts Isolated from Korean Nuruk

Kyung-youn Hong, Seung-Beom Hong, Soon-Wo Kwon, Soo-Jin Kim, and Jeong-Seon Kim*

Agricultural Microbiology Division, National Institute of Agricultural Science, Rural Development Administration

In Korea, we have used Nuruk as ‘traditional fermentation starter’ when alcohol beverages were produced with material containing starch for starch saccharification and alcohol fermentation. Naturally fermented Nuruk include various microorganisms because Nuruk making condition is suitable for microbial culture. We isolated yeasts and analyzed diversity and fermentation characteristics of yeasts from Nuruk. Eight Nuruks were collected and the yeasts were isolated from six of Nuruks based on morphology of colony and spore. Yeasts were identified using ITS rDNA sequences and they were compared with type strains. The results of identification have shown that 8 species were distributed in Nuruk, including Wickerhamomyces anomalus and Saccharomyces fibuligera etc. In most Nuruk, Saccharomyces fibuligera was present and the species had amylase and proteolytic activity. Torulaspora delbrueckii showed the highest amylase activity. The yeasts had excellent fermentation characteristics were deposited in KACC for fermentation studies. We should continually research for isolation of various fermentation yeasts. [Supported by grants from National Institute of Agricultural Science, Rural Development Administration [Project No. PJ011248].]

Flavobacterium foetidum sp. nov., Isolated from Ginseng Soil

Ji-Hye Bu and Chang-Jun Cha*

Department of Systems Biotechnology, Chung-Ang University

A yellow-pigmented, rod-shaped, Gram-staining-negative, aerobic and devoid of flagella, but showed gliding motility bacteria strain, designated CJ42, was isolated from the ginseng soil in Anseong, South Korea. Strain CJ42 grew optimally at 30°C and pH 7.0 and in the absence of NaCl on tryptic soy agar. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain CJ42 belonged to the genus Flavobacterium within the family Flavobacteriaceae and was most closely related to Flavobacterium phragmitis DSM 23314 (96.7% similarity). Flexirubin-type pigments were present. The major isoprenoid quinone was menaquinone 6 (MK-6). The predominant polar lipids were phosphatidylethanolamine, an unidentified aminoglycolipid and two unidentified glycolipids. The G+C content of the genomic DNA was 30.7 mol%. On the basis polyphasic taxonomic approach, strain CJ42 represents a novel species in the genus Flavobacterium, for which the name Flavobacterium foetidum sp. nov. is proposed. The type strain is CJ42T.

Paenibacillus translucens sp. nov., Isolated from Tidal Flat Sediment

Yong-Seok Kim and Chang-Jun Cha*

Department of Systems Biotechnology, Chung-Ang University

A Gram-staining-variable, aerobic, rod-shaped, motile and spore-forming bacterial strain, designated CJ11T, was isolated from the tidal flat sediment in Ganghwa-do, South Korea. Strain CJ11T grew optimally on R2A at 30°C and pH 7.0. Sequencing results of the 16S rRNA gene revealed that strain CJ11T possesses two copies of the 16S rRNA gene varying at 5 positions. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain CJ11T belonged to the genus Paenibacillus, within the family Paenibacillaceae and was most closely related to Paenibacillus tarimensis KACC 14087T (96.20–96.28% similarity). The DNA G+C contents of the genomic DNA was 51.0 mol%. The major isoprenoid quinone was menaquinone 7 (MK-7). On the basis of the polyphasic taxonomic study, strain CJ11T represents a novel species in the genus Paenibacillus, for which the name Paenibacillus translucens sp. nov. is proposed. The type strain is CJ11T.

Ketobacter alkanivorans gen. nov., sp. nov., an n-Alkane-degrading Bacterium Isolated from Yellow Sea

Seol-Hee Kim and Sung-Kuen Rhee*

Department of Microbiology, Chungbuk National University

Strain G15T was isolated from a surface seawater collected from Garorim Bay. The isolated strain was aerobic, gram-negative, rod shape, motile with a polar flagellum and negative for catalase and oxidase. The optimum pH, salinity and temperature for the growth were determined to be pH 7.5–8.0, NaCl 3% (w/v) and 25 °C, while the range was pH 6.0–9.0, NaCl 1–7% (w/v), 18–38 °C. Growth was observed with acetate and hexadecane. Phylogenetic analysis based on the 16S rRNA gene sequences showed that strain G15T was affiliated to the genus Alcanivorax and was most closely related to Alcanivorax dieselolei B-5T (92.0% similarity) and Alcanivorax marinus R8-12T (91.7%). The major cellular fatty acids in strain G15T were C16:0 α6/C18:1 α7c (28.73%), C18:1 α6/C18:1 α7c (26.26%) and C16:1ω7c (11.98%) and the profile was distinct from those of the closely related species. Major respiratory quinone of strain G15T was Q-8. The main polar lipids were phosphatidylethanolamine and phosphatidylglycerol. The G+C content of the genomic DNA of strains G15T was determined to be 51.2 mol%. Based on phenotypic, chemotaxonomic, and phylogenetic studies, strain G15T was considered to represent a novel species of a novel genus of the family Alcanivoracaceae, for which we propose the name Ketobacter alkanivorans gen. nov., sp. nov., and the type strain is G15T (= KCTC 52659T = JCM 31835T). [Supported by the Ministry of Oceans and Fisheries, Korean.]

Flavobacterium phragmitis DSM 23314

DSM 23314

A050

A052

A051

A053
**A Novel Method for Screening of Fast-growing Methanotrophs Using the Chemostat Principle**

Jisun Kim and Sukhwan Yoon*

Korea Advanced Institute of Science and Technology

Despite the high potential of methanotrophs as biocatalysts, their slow growth rates have barred prompt genetic modifications and biomass production. In this study, the chemostat principle was adopted to isolate fast-growing methanotrophs. The soil from Gapcheon Stream, Daejeon was collected and enriched in batch culture in NMS medium before inoculation into a continuously stirred tank reactor (CSTR) with 20% CH₄ (in air) in the headspace and NMS medium in the aqueous phase. After initial fed-batch incubation in the CSTR, the dilution rate was increased from 0.1 h⁻¹ to 0.35 h⁻¹ with an increment of 0.05 h⁻¹. The sample collected at the highest dilution rate was used for isolation. The shift in microbial population and enrichment of fast-growing methanotrophs were monitored with 16S rRNA gene amplicon sequencing targeting the V6-V8 region. Diverse methanotrophs constituted <0.09% of the microbial population in the soil sample were enriched in initial batch incubation. After start of chemostat operation, Methylobacillum spp. was remarkably enriched and only one methanotroph OTU remained in the reactor, which grouped with Methylobacillum sp. LW13. The isolated strain exhibited exponential growth rate larger than 0.3 h⁻¹, the highest growth rate ever reported. The novel isolation method successfully screened for fast-growing methanotrophs and significantly shortened the time for isolation.

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**Microbial Community in Produced Water Samples**

Daehyun D. Kim², Courtney R. A. Toth², Coryne O’Farrell², Oscar Montoya³, Lisa M. Gieg¹, and Tae-hyuk Kwon¹, and Sukhwan Yoon*++

¹Department of Civil and Environmental Engineering, Korea Advanced Institute of Science and Technology (KAIST), ²Department of Biological Sciences, University of Calgary, Calgary, Alberta, T2N 1N4, Canada

Microbial enhanced oil recovery (MEOR) has recently garnered interest as a low-cost and environmentally-friendly alternative to thermal or chemical oil recovery techniques. The main targets of MEOR have been oil reservoirs with relatively low temperature (30-50°C). As preliminary investigation for a research project to develop MEOR technique for higher temperature (~80°C) oil reservoirs, we have investigated the indigenous microbial community structure of five different mid-temperature wells in Houston, TX, US (70-80°C) and Crossfield, AB, Canada (75°C). Produced water samples from Houston (4 spatially separated wells) and Crossfield (1 well, 1 oil-water separator) were analyzed for their community composition. Miseq sequencing of PCR-amplified V6-V8 region of 16S rRNA gene yielded 48,502-106,985 reads, which were processed with QIIME. Despite the geographical distance and difference in the chemical compositions, the OTU compositions of the two sites exhibited high similarity, as indicated by their close association in the NMDS plot. The major groups of recovered methanotrophs were Methylomonas (13.1%), and Pseudomonadaceae (16.8%), which have been recognized to associate with marine invertebrates comprised a greater proportion of the operational taxonomic units (OTUs) shared in all samples. Community diversity according to the shannon index was highest in S. gracillima in Wakayama, Japan and the index of dominance showed the highest in S. gracillima in Kenting, Taiwan. Soft coral, S. gracillima were enriched in OTUs from the families Haliclonaeeae, Mycoplasmataceae, Alteromonadaceae, Anaplasmataceae, and Rhodobacteraceae. The data presented here provide a broader characterization of the bacterial community in S. gracillima that habit in different environment through the Kuroshio Current.

[This study was supported by KIOST (PE99541).]

**Comparing Bacterial Communities Associated with Soft Coral Scleronephthya gracillimum**

Seonwook Woo

Korea Institute of Ocean Science and Technology

Environmental impacts can induce the change of relationship between coral and its symbiotic microbial community. Furthermore the microbial community change could lead the coral diseases and the opportunistic infections in corals exposed to the increased temperature. In this study we collected a Kuroshio current linked soft coral, Scleronephthya gracillimum from a variety of latitude. Coral samples were collected from six geographically distinct areas in Taiwan, Japan, and Korea to determine if there were biogeographic differences between the latitude. The results showed total 88 species of bacteria were found in S. gracillima and they were classified to 72 genus, 41 family, 32 order, 16 class and 13 phylum. The genus Endozoicomonas, a gammaproteobacteria that is frequently found to associate with marine invertebrates comprised a greater proportion of the operational taxonomic units (OTUs) shared in all samples. Community diversity according to the shannon index was highest in S. gracillima in Wakayama, Japan and the index of dominance showed the highest in S. gracillima in Kenting, Taiwan. Soft coral, S. gracillima were enriched in OTUs from the families Haliclonaeeae, Mycoplasmataceae, Alteromonadaceae, Anaplasmataceae, and Rhodobacteraceae. The data presented here provide a broader characterization of the bacterial community in S. gracillima that habit in different environment through the Kuroshio Current.

[Supported by NRF (Award2016004832) and MOTIE as “U-City Master and Doctor Course Grant Program.”]

**Strain OB3b Interferes with N₂O Reduction in Pseudomonas stutzeri Strain DCP-Ps1**

Jin Chang¹, Doyoung Park¹, Wenyu Gu², Jeremy D. Semrau², Alan A. DiSpirito², and Sukhwan Yoon*++

¹Korea Advanced Institute of Science and Technology (KAIST), ²University of Michigan, Iowa State University

Methanobactin (Mb) is a Cu chelator secreted by methanotrophs for scavenging of Cu from Cu-deficient environment. Cu scavenging may result in deprivation of Cu from the environment and thus, may have negative impact on the biochemical reactions that require Cu for their activities. Mb-OB3b accumulated 435.7 - 453.3 µmoles of N₂O reduction activity in the absence of Mb or strain OB3b cells, transient N₂O evolution from strain DCP-Ps1 was monitored upon incubation in a minimal salts medium amended with 450 µmoles NO₃⁻. In the headspace and NMS medium in the aqueous phase. After initial fed-batch incubation in the CSTR, the dilution rate was increased from 0.1 h⁻¹ to 0.35 h⁻¹ with an increment of 0.05 h⁻¹. The sample collected at the highest dilution rate was used for isolation. The shift in microbial population and enrichment of fast-growing methanotrophs were monitored with 16S rRNA gene amplicon sequencing targeting the V6-V8 region. Diverse methanotrophs constituted <0.09% of the microbial population in the soil sample were enriched in initial batch incubation. After start of chemostat operation, Methylobacillum spp. was remarkably enriched and only one methanotroph OTU remained in the reactor, which grouped with Methylobacillum sp. LW13. The isolated strain exhibited exponential growth rate larger than 0.3 h⁻¹, the highest growth rate ever reported. The novel isolation method successfully screened for fast-growing methanotrophs and significantly shortened the time for isolation.

[Supported by NRF (Award2016004832) and MOTIE as “U-City Master and Doctor Course Grant Program.”]
Physiological and Genomic Features of a Novel Acidophilic Arsenic-oxidizing Bacterial Strain AS8 belonging to the Genus Herminiimonas

Ye-Eun Kim and Soo-Je Park*

Department of Biology, Jeju National University

The toxic metalloid arsenic is widely distributed in both natural and anthropogenic environments in two inorganic arsenic forms, arsenite [As(III)] and arsenate [As(V)]. Strain AS8 belonging to the genus Herminiimonas was isolated from a heavy-metal contaminated soil in Daegu and represents a novel species which has a capability for arsenic oxidation. Cells of strain AS8 were rod and stained Gram-stain-negative, and formed small beige-pigmented colonies. It grew heterotrophically in the range of 20–40°C (optimun 25–30°C), pH 3.0–9.0 (optimun 5.0–6.0), and 0–1% NaCl (w/v, optimun 0.2–0.4%). Under chemolithotrophic conditions, the strain utilized limited organic acids and amino acids as the carbon and/or nitrogen sources but not the electron sources. Unexpectedly, most carbohydrates did not support growth as a sole carbon sources. These results were supported by genome sequencing, as very few ABC transporters capable of oligo/nosaccharide uptake were identified in the AS8 genome. However, the genome did harbor gene sequences required for colonization, flagella biosynthesis, urea degradation, and heavy-metal and antibiotic resistance. It can also grow as a sulfur oxidizer in a complex medium (i.e. trypticase soy agar). Based on these polyphasic and genomic analyses, we propose the AS8 strain be named Herminiimonas arsenitoxidans sp. nov.

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Molecular Analysis of Soil Bacterial Community Structures for Environmental Risk Assessment with Varieties of Genetically Modified Soybean and Hot Pepper

Jungpyo Yun, Dong-Uk Kim, Hysoon Lee, Suyeon Lee, and Jong-Ok Ka*

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

The impacts of transgenic plants on soil microbial community structures were assessed by using both cultivation and molecular methods. Two varieties of crops, soybean and hot pepper, were used in this study. The field plot consisted of four subplots planted with genetically modified (GM) soybean (Glycine max L. Merr, introduction of osmotic pressure inducible gene [AtSIZ-6]), non-GM soybean (Glycine max L. Merr), GM hot pepper (Capsicum annuum L., introduction of herbicide resistance gene [bar]), and non-GM hot pepper (Capsicum annuum L.). The microbial populations of bacteria, actinomycetes, and fungi measured by cultivation methods were quite similar among the four subplots. However, the population of Rhizobium, symbiotic nitrogen-fixing bacteria in legume plants, was much larger in soybean soils than in hot pepper soils. Analysis with denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA genes showed that there were no significant differences in bacterial communities between GM and non-GM, but there were substantial differences between soybean and hot pepper. The level of the introduced gene (AtSIZ-6, bar) into GM crops was greatly increased in soil when the crops were actively growing in the experimental field, but thereafter its level was gradually decreased to the initial level.

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Sexual Dimorphisms in the Gut Microbial Community of 16 Mottled Skates, Raja pulchra

Eun Bae Kim1,2,3, Jihyun Won1, Gwi-Deuk Jin1, Jongbin Park1, Inhwan You2, and Eun Bae Kim1,2,3*

1Department of Animal Life Science, College of Animal Life Sciences, Kangwon National University, 2Division of Applied Animal Science, College of Animal Life Sciences, Kangwon National University, 3Institute of Animal Resources, College of Animal Life Sciences, Kangwon National University

Raja pulchra, Cham-hong-eo in Korean, is a kind of wild mottled skates. Previous studies showed sexual dimorphisms such as body weight and body size. In this study, we analyzed the gut microbiota of 16 skates (8 males and 8 females). Their body weights ranged from 7.7–11 kg (females, 9.4±1.1 kg) or 4.25–5.5 kg (males, 4.9±0.4 kg). The gut samples were collected from the large intestine after sacrificed. To analyze microbial profiles, we extracted gDNA of the gut content and amplified 16S rRNA V4 region by using barcoded primers. The amplicons were sequenced using the Illumina MiSeq Sequencer. Firmicutes and Bacteroidetes were more abundant in the female skates. Photobacterium, Lactobacillus, Prevotella comprised the most of the gut microbiota. The Photobacterium genus are well-known as aquatic bacteria that are frequently found in fishes. We identified sexual dimorphisms in the gut microbiota of the mottled skates. Future research is required to clarify any association between gut microbiota and skate physiology such as body weight.

[Supported by Marine Biotechnology Program (PJ200620) from Ministry of Oceans and Fisheries and by the BK21 Plus.]

Surveillance Studies of Triclosan-resistant Genes in Major Pathogenic Microorganisms Using Genome-wide Analysis

Raees Khan, Nazish Roy, Kihyuck Choi, and Seon-Woo Lee*

Applied Bioscience of Dong-A University

The use of triclosan [TCS] outnumbers the knowledge about distribution of TCS resistance determinants in a majority of pathogenic bacteria. We aimed to evaluate the prevalence of TCS resistance determinants in major pathogenic bacteria and to assess the selective enrichment of potentially pathogenic genera in TCS contaminated environments. Genome-wide in silico analysis against a TCS-resistant gene [TRG] database and microbiome analysis of TCS contaminated soil samples were performed. Results revealed abundant potential TCS resistance determinants among majority of bacteria, including various enoyl-acyl carrier protein reductase [ENR] homologues, AcrB efflux pumps, and ENR substitutions. We found that most of the human and soil-borne plant pathogens contained TCS resistance determinants homologues. Most of these organisms carried completely TCS-tolerant ENR homologues. FabI ENR-the only known effective target for TCS, was found either co-localized with other TCS resistance determinants or had substitutions associated with TCS resistance. Furthermore, from microbiome analysis enrichment of pathogenic genera with intrinsic TCS resistance determinants was observed. In summary, TCS may not be as effective against the majority of bacterial pathogens and its excessive use may selectively enrich for not only TCS-resistant bacterial pathogens, but possibly for additional resistance to multiple antibiotics.

[Supported by grant from Rural Development Administration of Republic of Korea]
Compartmentalizing the Role of Rhizosphere Microbiome and Endophytic Microbiome against Bacterial Wilt Incidence on Tomato Plants

Nazish Roy, Jinhee Choi, Pyeong An Lee, Raees Khan, Kihyuck Choi, and Seon-Woo Lee*
Applied Bioscience of Dong-A University

The soil and endophytic microorganisms harbored by plants are crucial in establishing resistance against plant pathogens. Previously, we developed an analysis system for plant-microbiome interaction (ASPMI) and examined the assembly of rhizosphere microbiome and its role in bacterial wilt incidence caused by Ralstonia solanacearum. The rhizosphere community structure and disease incidence of Hawaii 7996 differed greatly with each different type of soil microbiome treatments suggesting disease suppression as a function of rhizosphere microbiome. Bacterial wilt incidence in tomato cultivar Hawaii 7996 was variable under 4 different root microbiome environments. Microbiome analysis by 16S rRNA gene amplicon sequencing revealed that rhizosphere microbial community were remarkably different in tomato treated with 4 different microbiome. Therefore, we hypothesize that the rhizosphere microbiome plays an important role in establishing disease resistance as compared to the endophytic microbiome. We are currently investigating the exclusive role of endophytic microbiome in establishing disease resistance by compartmentalizing the rhizosphere microbiome. We further plan to investigate the difference in the endophytic community structure developed when treated with different soil types and their respective effects on disease incidence. [Supported by grant from Rural Development Administration of Republic of Korea]

Genome Analysis of Flavobacteriales Bacterium Strain UJ101

Junghee Kim¹, Kae Kyoung Kwon², and Hyun-Myung Oh*¹
¹Pukyong National University, *Korea Institute of Ocean Science & Technology

Flavobacteriales bacterium strain UJ101 was isolated from a xanthid crab shell collected from East Sea of Korea. Here we report the complete genome sequence of strain UJ101 for the study of metabolic interaction between UJ101 and its host organism. Single molecule real-time technology (PacBio RSII) was used for the single circular chromosome that is 3,074,209 base pairs in length and the GC content was 30.74%. The genome of UJ101 contains 2,698 ORFs with 46 tRNAs and 9 rRNAs genes. According to the annotated list of genes Embden–Meyerhof and pentose phosphate pathway is well conserved, but key enzymes of Entner–Doudoroff pathway were impaired. TCA and glyoxylate cycle were conserved while carbon fixation and one carbon metabolism were mostly lacking except formaldehyde dehydrogenase. UJ101 encodes degradation enzyme including 8 glycosyl transferases, 3 amylases, and 8 peptidases. Biosynthetic enzymes for lysine, tryptophan, phenylalanine, and tyrosine were also impaired. Alcohol and/or organic acid fermentation could not be expected. Genomes from Flavobacteriales and related groups were chosen for comparative genomic analysis. Strain UJ101 was compared with bacterial genomes isolated from other marine animals (3 strains from invertebrate and 5 strains from fishes). Other related genomes from the same genera were included although they were isolated from seawater and marine sediments.

Cloning of Cytosine N4-methyltransferases from Marine Alphabacteria

Junghee Kim¹, Kae Kyoung Kwon², and Hyun-Myung Oh*¹
¹Pukyong National University, *Korea Institute of Ocean Science & Technology

DNA methylation is involved in a diversity of 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 5-adenosyl methionine as a co-substrate. Using single molecule real-time sequencing method (SMRT), methylation patterns of Celeribacter marinus IMCC12053 and Novosphingobium pentaromaticivorans US6-1 were compared using Gibbs motif sampler program. Both strains have been observed to change adenosine of 5'-GANTC-3' as N6-methyladenosine, and N4-cytosine of 5'-CpG-3' (IMCC12053) and 5'-GpC-3' (US6-1) as N4-methylcytosine. Using phylogenetic analysis exocyclic DNA methyltransferases from both of the species were chosen for cloning. In this study cloned exocyclic exocyclic DNA methylases are presented, and the potential use of novel type of CpG and GpC methylases in molecular biology and epigenetics.
**B013**

Metabolic and Stress Responses of *Acinetobacter oleivorans* DR1 during Long-chain Alkane Degradation

Chulwoo Park1, Bora Shin1, Jaejoon Jung1, and Woosun Park1,2

1Laboratory of Molecular Environmental Microbiology, Department of Environmental Science and Ecological Engineering, Korea University, 2National Marine Biodiversity Institute of Korea

*Acinetobacter oleivorans* DR1 can utilize C17–C30 alkanes as a sole carbon source, but not short-chain alkanes (C6, C12). Transcriptomic and qRT-PCR analyses under triacontane (C30) condition suggested that genes participating in the synthesis of trehalose, poly 3-hydroxybutyrate (PHB), siderophore, and unsaturated fatty acid were highly upregulated. Induced in the synthesis of trehalose, poly 3-hydroxybutyrate (PHB), siderophore, and mutant analyses of AHs showed that *aceA* and *pta* genes suggested unusual ATP synthesis during C30 degradation. Growth assay of *aceA* knock out mutant indicates that glyoxylate shunt pathway is essential metabolism under C30 condition. Further expression and mutant analyses of AHs showed that *alkB1* and *alkB2* are major AH-encoding genes under C17–C30 alkanes, but inducible *almA* genes on LC alkanes help to degrade C30. Phylogenetic analyses and large incongruity between phylogenies of 16S rRNA and each AH gene represented that *Acinetobacter* is novel species to have multiple alkane hydroxylation system and uniqueness of three different AHs possessing bacteria.

[This work was supported by grants from National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. NRF-2014 R1A2A2A05007010).]

**B014**

Fragile Skin Microbiome in Megacity under Influence of Predominantly Niche Based Assembly Process

Hye-Jin Kim1, Jin Ju Kim2, Taeyeun Kim1, Hanbyul Kim1, Susun An1, and Woo Jun Sul2

1Department of Systems Biotechnology, Chung-Ang University, 2Safety Research Institute, Amorepacific R&D Center

The skin is the largest organ of the human body and functions as the first line of defense against injury and infection by providing a physical barrier and immune systems protections. Skin microbiome, composed of a wide range of bacteria, fungi, and viruses, also provides a critical role of host defense. The diversity, composition, and stability of skin microbiome are range of bacteria, fungi, and viruses, also provides a critical role of host defense. The diversity, composition, and stability of skin microbiome are also a critical role of host defense. The diversity, composition, and stability of skin microbiome are also a critical role of host defense. The diversity, composition, and stability of skin microbiome are also a critical role of host defense. The diversity, composition, and stability of skin microbiome are also a critical role of host defense. The diversity, composition, and stability of skin microbiome are also a critical role of host defense. The diversity, composition, and stability of skin microbiome are also a critical role of host defense.

[This research was supported by Basic Science Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education and Amore-Pacific Co. R&D Center.]

**B015**

Occurrence of Season-specific Potentially Pathogenic *Vibrio* spp. on the Southern Coast of South Korea

Doris Yoong Wen Di1, Anna Lee2, Jeonghwan Jang1, Dukki Han1, and Hor-Gil Hur1

1School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology, 2Centers for Disease Control and Prevention, 3BioTechnology Institute, University of Minnesota, Saint Paul, Minnesota, USA

*Vibrio* species are widely distributed in warm estuarine and coastal environments, and they can infect humans through the consumption of raw and mishandled contaminated seafood. In this study, we aimed to isolate and observe the distribution of enteropathogenic *Vibrio* spp. from environments of the southern coast of South Korea over a season cycle. A total of 10,983 *Vibrio* spp. were obtained from tidal water and mud samples from five sampling sites along the southern west coast of South Korea. We found that *Vibrio alginolyticus* (n = 6,262) and *Vibrio para-haemolyticus* (n = 1,757) were ubiquitous in both tidal water and mud year round, whereas *Vibrio cholera* (n = 24) and *Vibrio vulnificus* (n = 130) were seasonally specific to summer. While all *V. cholerae* isolates were nontoxicogenic (non-O1 and non-O139), more than 88% of *V. vulnificus* isolates possessed the virulence factors elastolytic protease (vwp). Interestingly, *V. para-haemolyticus*, which was omnipresent in all seasons, contained the virulence factors thermostable direct hemolysin (tdh) and TDH-related hemolysin (trh) in higher amounts in June ([29 trh] and September ([14 tdh; 36 trh]; 12 tdh and trh) than in December (4 trh) and February (3 tdh), and virulence factors were absent from isolates detected in April. To understand the phenomena where virulence factors were only detected in warm season but absent in cold season although the locations are static, long-term monitoring and particular seasonal study are necessary.
Monitoring of Various Antibiotic Resistance in Three Rivers of South Korea
Hanseob Shin, Doris Yoong Wen Di, Dukki Han, Seonyun Moon, and Hor-Gil Hur
Gwangju Institute of Science and Technology

An amount of antibiotics have been used in South Korea for various treatment of disease compared to other OECD countries. The over-use of antibiotics could potentially result in spread of antibiotic resistance genes (ARG) into the environment. In this study, ARG and ARB were surveyed from three rivers of South Korea to assess fate of antibiotic resistance in the aquatic environment. Nine water samples were collected from upstream (n=3) and downstream (n=3) at discharging point of a wastewater treatment plant (WWTP) in Han (H), Yeongsan (YS) and Nakdong (ND) rivers as well as effluent waters (n=3) from the WWTPs. Total 900 resistant strains (n=10) were isolated from the water samples. The isolates were identified by 16s rDNA sequencing. To monitor ARG in the collected samples, qPCR-based SmartChip technique was applied in the study. The result of genus identification shows that Acinetobacter spp. were dominant (52-90%) in all the samples, except YS upstream which mainly has 29% of Chryseobacterium spp.. ND effluent (84%), upstream (90%), and downstream (73%) possess the highest Acinetobacter spp. among the samples. The total CFU of ARB was found to be higher in the downstream (3.42 × 10^6) than the upstream (4.89 × 10^5) and the lowest in the effluent (2.92 × 10^5). SmartChip analysis showed that ARG for aminoglycosides are highest in the effluent (25.6-32.9%), upstream (23.5-30.5%), and downstream (19.8-29.9%).

[Supported by “the Environmental Health Action Program (RE201603079)” funded by the Korea Ministry of Environment (MOE)]

Microbial Community Structures of Activated Sludge in Anaerobic/Anoxic/Oxic Reactors Operating at Full-scale Wastewater Treatment Plants Revealed by 454 Pyrosequencing
Sunja Cho1 and Youngok Lee2*
1Pusan National University, 2Daegu University

To understand the differences of bacterial- and archaeal diversity depending on both basins and kind of wastewater treated by anaerobic/anoxic/oxic (A2O) process, 10 sludges were harvested from three different municipal WWTPs and analyzed as follows: Metagenomic DNAs from sludges were extracted and amplified by PCR with 27F/S18R primers and pyrosequenced using Roche 454 GS FLX Titanium. As results, total 1,261 OTUs and 75 OTUs were obtained for Bacteria and Archaea respectively. The major group in Archaea was affiliated with Euryarabaeota (over 97%), whereas the most frequently occurred bacterial group on the phylum level belonged to Proteobacteria, which varied from 41.5% to 54.2% (average 46%) obtained from 10 different sludges, and followed by Bacteroidetes, Actinobacteria, Chloroflexi and Firmicutes in the order except one among the 10 samples regardless of operational condition and kind of treated wastewater. Therefore, it could be concluded that the bacterial- and archaeal composition in sludge obtained from different basins of several WWTPs operated by A2O process were almost similar to one another.

Characterization of a Novel Plant Growth Promoting Dormant Bacterium from Tomato Rhizosphere
Roniya Thapa Magar, Min Kyeong Jung, Hyun Gi Kong, Seung Yeu Lee, and Seon-Woo Lee*
Applied Bioscience of Dong-A University

Plant growth promoting rhizobacteria (PGPR) and their interactions with plants have been studied as an alternative application in environmental-friendly agriculture. To isolate and characterize novel bacterial strains that could possibly benefit plant health and growth, soil suspensions from tomato rhizosphere were spread onto various bacterial growth media. We isolated tiny slow-growing bacterial colonies (dormant) which were able to grow only in the presence of selected another bacteria (helper) for further research. These dormant and helper bacteria were identified as Bacillus circulans and Pseudomonas putida respectively, based on 16s rRNA gene sequence. To analyze the PGP effect on tomato (Zuken), we treated the dormant and helper bacteria into tomato plants using either individual culture or mixture of bacterial suspension. This result revealed that the treatment of only dormant displayed a significant PGP effect with increasing fresh weight on Zuken cultivar whereas the other treatments (control, mixture suspension and only helper) did not show PGP activity. For identification of the genes involved in helper-dormant interaction, transposon inserted mutant strains were generated using helper strain. Among the mutant strains, one of mutants, 2165 strain enhancing the growth of the dormant bacteria was selected for future genetic characterization.

[Supported by grant from Rural Development Administration of Republic Korea]
Metagenomic Analysis on the Influence of Ocean Acidification on Microbial Communities in a Mesocosm Experiment

Min-Jung Kwak[1], Byung Kwon Kim[1,2], Ki-Tae Park[1], Eun Jin Yang[1], Soon-Kyeong Kwon[1], Kyungsoon Shin[3], Kitack Lee[3], and Ji Hyun F. Kim[1,4]†


Since the Industrial Revolution, human activities have resulted in dramatically rising atmospheric concentration of carbon dioxide, which in turn may cause the climate change among others. Especially, CO2 in the atmosphere is absorbed by the ocean to change the oceanic chemistry. These global environmental changes may influence marine organisms in many ways including nutrient flow and primary production. To investigate the influence of ocean acidification on marine microbial ecology, we looked into the dynamics of microorganism and their gene contents by analyzing the metagenomes in a mesocosm experiment. Results showed that, under the high CO2 condition, the abundances of diatom and genes for primary production increased, whereas increase at high CO2 was not observed for some phototrophic taxa that are present at low CO2. Dynamics of the genes associated with nitrogen cycle and dimethylsulphide production indicated that the concentration of NH4+ increase and that of dimethylsulphide decreases in the acidified seawater. These processes finally will promote the seawater acidification more and more. In conclusion, our analysis results demonstrated that acidified seawater make the ocean more acidic and this finally will decrease of microbial diversity in the future.

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Ameliorating Role of *Leifsonia soli* SE134 to Inhibit Cu Toxicity in Tomato

Sang-Mo Kang, Muhammad Waqas, Ko-Eun Lee, Arjun Adhikari, and In-Jung Lee*†

School of Applied Biosciences, Kyungpook National University

Rhizosphere bacteria isolated from the root zone have already been reported as key factor associated for healthy plant growth to sustain agriculture. The present study focused on the effect of PGPR *Leifsonia soli* SE134 on tomato grown beyond the threshold level of Cu toxicity. The isolate was found to exhibit Cu resistant and plant growth promotion characteristics. Deleterious effect of Cu toxicity was elucidated on plant growth parameters like shoot length root length, stem diameter, shoot dry weight, root dry weight and chlorophyll content of tomato plants. However, the inoculated plant with the isolate enhanced plant growth promoting attributes, stimulated the polyphenol and flavonoids content, modulated the amino acid content(glutamic acid, threonine, phenylalanine, glycine, arginine, and proline), and reduced super oxide dismutase activity. The isolate also minimized the Cu and increased P and Fe content in plant tissues. These results demonstrated that the Phytohormones producing PGPR plays a significant role to inhibit heavy metal stress and to enhance plant growth promotion.

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Comparative Metagenomic Analysis of Wheat-based Nuruk

Ju Yeon Song[1], Ki Young Yoon[1], Jeong-Ah Seo[2], Min-Jung Kwak[2], and Ji Hyun F. Kim[1,4]**

[1]Department of Systems Biology and Division of Life Sciences, Yonsei University, [2]School of Biomedical Science, Soongsil University, [3]Strategic Initiative for Microbiomes in Agriculture and Food, Yonsei University

*Nuruk* is a natural fermentation starter used to produce Korean traditional alcoholic beverages. Composition of micro-organisms is a key factor that influences the quality of alcoholic beverages. It is presumed that the flavor of traditional alcoholic beverages depends on the kinds of raw materials for *nuruk* production. Since a variety of flavors in alcoholic beverages might result from different microbial compositions, we compared the microbial communities in different *nuruk* samples through microbiome approaches involving the 16S ribosomal RNA gene (for prokaryotes), internal transcribed spacers between rRNA genes (for eukaryotes), and the whole metagenome. *Nuruk* samples made from different wheat species were collected in different fermentation steps. To exclude the undesirable wheat genomic DNA, a purification method for preparation of the metagenomic DNA was established and then the extracted DNA was subject to metagenomic sequence analysis. Microbial communities were monitored by targeted amplicon sequencing, and the functional diversity of *nuruk* was analyzed by whole metagenome shotgun sequencing. We could observe microbial dynamics in *nuruk* by fermentation time and obvious difference of the microbial structures by raw materials.

[This work was supported by the Strategic Initiative for Microbiomes in Agriculture and Food, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea [914006-04-3-HD020]].
Extremophiles are microorganisms that thrive in physically or geochemically extreme conditions in terms of high temperature, high NaCl concentration, or anaerobic respiration. The extremophiles have been known to be fastidious about the cultivation and isolation, as compared with mesophilic, non-halophilic, neutral, and aerobic bacteria. Here we analyzed genome sequences of three extremophiles isolated from different extreme conditions. The hyperthermophilic archaeon *Vulcanisaeta thermophila* CBA1501 had been isolated from solfataric soil. The genome of strain CBA1501 had 211 POGs as a singleton, including hydrogen sulfite reductase, and amidase. The extremely halophilic archaeon *Halobacterium noricense* CBA1132 had been isolated from solar salt. Strain CBA1132 had nine putative CRISP-R genes encoding metal resistance determinants. In addition, the potential opportunistic pathogen *Enterococcus faecalis* CBA7120 had been isolated from the human feces. Strain CBA7120 contained 374 pan-genome orthologous groups. The genes related to multidrug resistance efflux pumps were also annotated. The genomic analyses of the extremophiles based on culturomics will provide insights into the metabolism of extremophiles and biotechnological applications of novel extremophilic enzymes.

Seasonal Differences of Bacterial Communities Associated with the Marine Sponge, *Discodermia calyx* Based on DGGE

Ga-Eun Lee and Jin-Sook Park*

Department of Biological Sciences and Biotechnology, Hannam University

The community structure of bacteria associated with the marine sponge, *Discodermia calyx*, collected from Jeju Island in winter (January) of 2013 and summer (August) of 2013, were compared by the PCR-DGGE method. After isolation of the total genomic DNAs from the sponges, the V3 regions of the 16S rRNA genes were amplified and subjected to DGGE profiling. The two sponges displayed different DGGE band patterns. The 16S rRNA gene sequences derived from the DGGE bands showed 75-100% similarities to the known bacterial species in the public database. The bacterial communities from the sponge, captured on winter, contained 4 phyla, 6 classes: *Proteobacteria* (Alpha-, Gamma-, Delta-), *Chloroflexi*, *Actinobacteria* and *Deinococcus-Thermus*. There were 5 phyla, 7 classes observed from the bacterial communities associated with the sponge, captured on summer: *Proteobacteria* (Alpha-, Gamma-, Delta-), *Chloroflexi*, *Actinobacteria*, *Acidobacteria* and *Nitrospirae*. On the sponge, *D. calyx*, more diverse bacterial communities were shown on summer than on winter, and even from the same sponge, there were seasonal differences.

Complete Genome Sequences of *Moraxella osloensis* Strains Isolated from Human Skin

Munkhtsartsral Ganzorig1, Shivalika Pokhriyal2, Sachin Kumar Badaya1, Jae Yun Lim1, Ingyu Hwang2, and Kyong Lee*

1Changwon National University, 2Seoul National University

*Moraxella osloensis*, a Gram-negative bacterium that is saprophytic on skin and mucosa, infrequently causes infections. From human skin we isolated *M. osloensis* strains (YHS-M, TT16, KSH) that utilize Triton X-100 as a sole source of carbon and energy. Triton X-100 has a hydrophilic polyethylene oxide chain (average 9.5 EO units) and an aromatic hydrocarbon lipophilic group. It is a nonionic surfactant of alkylphenol polyethoxylate (APEOn) that has been used worldwide. The fate and biodegradability of APEOn in the environment have received much attention over the last decade. There is much interest in the environmental fate of the APEOn surfactant metabolites, because they can mimic natural hormones and thus have the potential to act as endocrine disrupters in humans. The primary degradation of APEOn by bacterial species generally proceeds through gradual shortening of the ethoxylate chain. The isolated *Moraxella osloensis* strains showed enhanced growth in order of TT16 >YHS-M > KSH with Triton X-100. The YHS genome comprises a 2.57-Mbp chromosome and three plasmids. TT16 and KSH have similar sizes of chromosome but with four plasmids, respectively. Compared to the previous genome sequences of *M. osloensis* strains originating from other environments, intense gene reshuffles in plasmids are observed. [Supported by grants from KRF.]

Drastic Changes of Human Skin Microbiome during Trips to Antarctic Research Stations

Kyu-Chan Lee1, Jeongjun Heo2, Junho Cho1,2, Hye-Jun Kim1, Jinju Kim1, Ok-Sun Kim2, and Woo Jun Sul*

1Systems Biotechnology, Chung-Ang University, 2Division of Life Sciences, Korea Polar Research Institute

Environmental and host factors (sebum, moisture, temperature, and individuality) contribute to characterization of skin microbiome and cause diverse skin microbial communities. Geographical location also contributes to the composition of the skin microbiome. We tracked alteration of skin bacterial communities during two different visits to Antarctica. To analyze dynamic variation of human skin microbiome, we tracked bacterial communities of 2 adults depending on location, sunscreen uses, and outdoor activity. One subject air-travelled to Christchurch (New Zealand) from Seoul (S. Korea) and moved to Jang Bogo Antarctic Research Station (Terra Nova Bay) by a research vessel. The other subject flew to King Sejong Station (Barton Peninsula King George Island) through Sydney (Australia) and Punta Arenas (Chile). They returned to Seoul via the same route. During their Antarctic trips, they collected their own cheek bacterial samples with swabs once per 2 days for 4 months and 2 months, respectively. Phylogenetic marker gene was used for skin bacterial community analyses via Quantitative Insights Into Microbial Ecology and Linear Discriminant Analysis Effect Size pipelines. We found that skin bacterial communities were altered by external exposures (location, and outdoor activity). The variation of bacterial communities was also affected by sunblock uses. These results indicate that extreme Antarctic environment and external factors may cause dynamic changes of human skin microbiome.
Evaluation of Suitability and Safety in Commercial Probiotics for Animal Using Barcoded Pyrosequencing and Multiplex-PCR

Jong Woo Hyeon, Ah Ryeong Son, Tingye Feng, Badei Jia, and Che Ok Jeon*
Department of Life Science, Chung-Ang University

Recently, because of a wide use of antibiotics for animal feed additives, there is growing concern about widespread of antibiotic resistance genes. So, a variety of commercial probiotic products have been developed as alternatives of antibiotics. However, few studies have been conducted to investigate the real microbial composition and contamination by pathogenic bacteria in commercial probiotic products. Bacterial community compositions were investigated in fifty commercial probiotics from twenty-six brands from Korea using barcoded pyrosequencing approach. Pyrosequencing results were processed and classified using RDpipeline and potential pathogenic bacteria were found by Blastn based on PHI (Pathogen Host Interaction) database. Pyrosequencing results indicated that bacterial compositions of some probiotic products were similar with labeled information. However, potentially pathogenic sequences classified as Pseudomonas aeruginosa, Burkholderia cenocepacia, and Escherichia coli at a 97% sequence similarity criterion were approximately 2% of the total sequencing reads. Primer sets targeting virulence genes of known pathogenic bacteria were designed. The virulence genes from commercial probiotics were multiplex-PCR amplified using these primer sets.

This study was supported by the ‘Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01090604)’ of Rural Development Administration, Republic of Korea.

Use of Tetragenococcus halophilus Strain MJ14 as a Starter Culture in Saeu-jeot Fermentation

Se Hee Lee1, Kyung Hyun Kim2, Min Young Jung1, and Che Ok Jeon*1
1Microbiology and Functionality Research Group, World Institute of Kimchi, 2Department of Life Science, Chung-Ang University

To investigate the effects of Tetragenococcus halophilus MJ14, known as a non-biogenic producer, on saeu-jeot fermentation, three sets of saeu-jeot samples (non-starter, starter with or without glucose) were prepared and their pH, viable cells, bacterial communities, and metabolites were analyzed. Viable cells in starter samples increased to the highest values at 25 days and then gradually decreased until the end of fermentation. On contrary, the number of viable cells in non-starter samples was very low during the initial stage, but increased rapidly to those in starter samples at 82 days. Bacterial community based on pyrosequencing showed that T. halophilus was predominant from the beginning to the end of fermentation in starter samples, while in non-starter samples Salinibacterium album, Alkalibacillus salliu, T. muriaticus, and Marinobacteriaceae pterolyticae appeared as major populations after 25 days. Metabolite analysis using 1H NMR demonstrated that amino acid profiles were relatively similar in all samples regardless of the use of starter culture and/or not glucose and lactate was just produced in starter samples with glucose. The generation of trimethyl amine and dimethyl amine from trimethylamine N-oxide was clearly smaller in starter samples. However, cadaverine production in starter samples was higher than that in non-starter samples during the early fermentation period, but cadaverine production became higher in non-starter samples than that in starter samples.

A Taxonomic Analysis Based on Functionally Equivalent Proteins

Dong Su Yu, Eunsu Park, and Jukyeong Eo*
National Institute of Ecology

When taxonomically analyzing some bacterial species, traditional methods using 16S rRNA have often shown a relatively low confidence because 16S rRNA cannot exactly represent functional features of the species even though it is stable in phylogeny. Nevertheless, many researchers still have used similarity based on 16S rRNA in early phase of species identification because of the simple study. Since high-tech methods such as NGS and 3rd generation sequencing were developed to sequence genomes, species identification based on protein function has been more useful than by 16S rRNA. In taxonomic analysis using proteins, selecting functionally equivalent proteins is highly important because of the high accuracy. We developed TaxonFEP system that identifies taxonomically species based on FEP-B algorithm that can predict functionally equivalent proteins. By FEP-B algorithm, TaxonFEP can predict functionally equivalent proteins against UniProt Knowledgebase and identify species by equivalent protein function.

This work was supported by ‘Assessment of Climate Change Risks and Adaptation Strategy Research for Ecosystem in Korea’ under Climate Change and ‘Study on Forest Ecosystem through the Functional Diversity of the Endophyte’ of National Institute of Ecology (NIE).

Interplay between Tomato’s Disease Resistance and Its Rhizosphere Microbiota

Min-Jung Kwak1, Jidam Lee2, Hyun Gi Gong2, Soo Yeon Choi2, Kihyuck Choi2, Pyeong An Lee2, Ju Yeon Song2, Soon-Kyeong Kwon2, Eun Joo Jung1, Seon-Woo Lee2, and Jihyun F. Kim1,2,3,
1Department of Systems Biology and Division of Life Sciences, Yonsei University, 2Department of Applied Biology, Dong-A University, 3Strategic Initiative for Microbiomes in Agriculture and Food (iMAF), Yonsei University

Bacterial wilt is a severe plant disease caused by the soil-borne bacterium Ralstonia solanacearum. We initiated a whole metagenomic analysis of the rhizosphere communities of two tomato cultivars that are resistant or susceptible to bacterial wilt. Hawaii 7996 is resistant to the disease, while Moneymaker is susceptible. Taxonomic analysis of the 16S rRNA reads, which have been extracted from the whole metagenome data using blastn against the SILVA database, revealed that the proportion of Flavobacteria is higher in the rhizosphere of Hawaii 7996 than in the rhizosphere of Moneymaker, whereas the proportion of Betaproteobacteria is higher in Moneymaker than in Hawaii 7996. Through scaffold binning, we were able to reconstruct the genome of a novel uncultured Flavobacteriaceae bacterium from the metagenomic sequences of Hawaii 7996, and we successfully isolated the corresponding bacterium based on its genome information. Our results demonstrate that the rhizosphere community structure and gene repertoire are different between wilt-resistant and -susceptible plants, and suggest that a specific taxon may influence the plant resistance against the wilt pathogen.

[Financial support from the National Research Foundation (NRF-2011-0017670 and NRF-2014M3C9A3300882), the Strategic Initiative for Microbiomes in Agriculture and Food, and BK21 PLUS]
Endophytic yeasts inhabit internal *Mankyua chejuens* and *Dendropanax morbifera* roots which needed to be identified using isolation methods that have been used in yeast biotechnology. A culture-dependent method was necessary for the isolation of many yeast strains associated with *Mankyua chejuens* and *Dendropanax morbifera*. In this study, we spread homogenized *Mankyua chejuens* and *Dendropanax morbifera* roots onto GPY medium containing antibiotics, Triton X-100 and L-sorbose. We isolated each 152 and 81 yeast strains from the roots of *Mankyua chejuens* and *Dendropanax morbifera*. Sequence analysis indicated that the root yeast species represented 140 isolates of *Cyberlindnera*, 11 isolate of the genus *Candida*, and one isolate of the genus *Kluveromyces* in *Mankyua chejuens* and *Vanderwaltozyma* (40 isolates), *Cryptococcus* (40 isolates), and *Kluveromyces* (one isolate) in *Dendropanax morbifera*. Two *Kluveromyces* isolates showed high production of bioethanol. These yeasts may apply valuable approach such as bioethanol producers in the field of biotechnology. Our findings revealed that *Cyberlindnera* represented major dominant genus in *Mankyua chejuens* and *Vanderwaltozyma* and *Cryptococcus* genera in *Dendropanax morbifera* were dominant, in addition, two *Kluveromyces* isolates produced high bioethanol.

**B032**

**Yeasts associated with Roots of Endemic Plants, *Mankyua chejuens* and *Dendropanax morbifera***

Dae-Shin Kim1, Keun Chul Lee2, Jung-Sook Lee2, and Jong-Shik Kim1*  
1World Heritage Office, Jeju; 2Korean Collection for Type Cultures, KRIBB; 3Gyeongbuk Institute for Marine Bio-Industry

Lake Soyang is the largest reservoir in Korea. In a previous study based on 16S rRNA gene amplicon sequencing, the bacterial assemblage of Lake Soyang exhibited seasonal and depth-dependent variation. In this study, to understand the microbial community structure of Lake Soyang more thoroughly, we analyzed shotgun metagenomes for 8 water samples collected from two depths (1 m and 50 m) for four seasons. Metagenome sequencing yielded an average of 16 Gbp (64 million reads; 250-bp paired end) for each sample. Taxonomic profiling by MetaPhlAn showed the prevalence of the acl gene lineage and the LD12 clade and also revealed bacterial community shift following summer stratification and winter mixing, which were congruent with the results from 16S rRNA gene amplicon sequencing. Metagenome assembly by SPAdes produced more than 1.6 million contigs of 1 kb or more. Contig binning by MaxBin resulted in 224 metagenome assembled genomes (MAGs) of ≥ 90% completeness. The sizes and GC contents of the 224 MAGs ranged 1.36–7.85 Mbp and 31.5–70.8%, respectively. Fragment recruitment of the metagenome sequences was performed using the complete genome sequences of the four acl strains (previously isolated from Lake Soyang) and the results showed specific distribution patterns of the acl sub-clades.  

**B033**

**Effect of Carbon and Nitrogen Sources on Kerosene Degradation by *Brevibacterium frigoritolerans* Strain SHD-34**

Seon-Hee Kim and Hyung-Yeol Kahng*  
Department of Environmental Education, Sunchon National University

*Brevibacterium frigoritolerans* SHD-34 was isolated from a crude oil contaminated site in Manlipo dock at Taean seashore, Korea. Strain SHD-34 was first studied for its growth in medium containing diesel, kerosene, or gasoline, suggesting it grew well in kerosene. GC-MS analysis revealed that strain SHD-34 degraded 90.84% of the initial concentration of 1% kerosene at the 7 days incubation time point without any carbon and nitrogen sources. Analysis of the effect on kerosene degradation by additional carbon sources such as glucose, lactose, maltose, citric acid, and D-fructose showed that the degradation rate for kerosene was 99.7% with glucose and lactose, 73.9% with maltose, 66.2% with citric acid, and 70.8% with D-fructose, respectively. This fact suggested that both glucose and lactose could be effective additional carbon sources, while maltose, citric acid, and D-fructose rather decreased the degradation rate for kerosene. The degradation rates for kerosene with nitrogen sources such as NH4Cl, peptone, urea, and yeast were 76.68%, 60.51%, 73.4%, and 99.97%, respectively, suggesting that yeast could be a nitrogen source which could enhance the kerosene degradation by strain SHD-34. Specific investigation by kerosene compounds, in particular, showed that aromatic hydrocarbons, 1-ethyl-2-methyl benzene and 2-ethyl-p-xylene in kerosene were more rapidly degraded with additional carbon sources glucose and lactose, as well as with additional nitrogen source, yeast.

**B034**

**Characterization of Freshwater Microbial Community of Lake Soyang by Shotgun Metagenome Sequencing of Seasonal Samples**

Suhyun Kim, Ilnam Kang, and Jang-Cheon Cho*  
Department of Biological Sciences, Inha University

Lake Soyang is the largest reservoir in Korea. In a previous study based on 16S rRNA gene amplicon sequencing, the bacterial assemblage of Lake Soyang exhibited seasonal and depth-dependent variation. In this study, to understand the microbial community structure of Lake Soyang more thoroughly, we analyzed shotgun metagenomes for 8 water samples collected from two depths (1 m and 50 m) for four seasons. Metagenome sequencing yielded an average of 16 Gbp (64 million reads; 250-bp paired end) for each sample. Taxonomic profiling by MetaPhlAn showed the prevalence of the acl gene lineage and the LD12 clade and also revealed bacterial community shift following summer stratification and winter mixing, which were congruent with the results from 16S rRNA gene amplicon sequencing. Metagenome assembly by SPAdes produced more than 1.6 million contigs of 1 kb or more. Contig binning by MaxBin resulted in 224 metagenome assembled genomes (MAGs) of ≥ 90% completeness. The sizes and GC contents of the 224 MAGs ranged 1.36–7.85 Mbp and 31.5–70.8%, respectively. Fragment recruitment of the metagenome sequences was performed using the complete genome sequences of the four acl strains (previously isolated from Lake Soyang) and the results showed specific distribution patterns of the acl sub-clades.  

**B035**

**Genome Analysis of Marine Bacterial Strains belonging to the OM60/NORS Clade of Gammaproteobacteria**

Shan-Hui Li, Ilnam Kang, Yeon-Jung Lim, and Jang-Cheon Cho*  
Department of Biological Sciences, Inha University

The OM60/NORS clade of Gammaproteobacteria is of significant ecological importance since its high abundance in coastal waters. Organisms from this clade comprise up to 11% of the bacterial communities in the ocean surface, which include the aerobic anoxygenic phototrophs (AAnP), represented by *Congregibacter litoralis* KT71 and HTCC2080. In this study, three isolates from coastal surface waters, IMCC3088 (NORS-4 subclade), IMCC14385 (Halologobus sp.) and IMCC14734 (unclassified subclade), in the OM60/NORS clade were selected for whole genome sequencing. Two complete genomes of IMCC3088 and IMCC14385 comprised of 3,095,392 and 4,306,437 bases, 2915 and 3918 predicated ORFs, 51.7% and 56.7% G+C content, respectively. Draft genome of IMCC14734 containing 20 contigs comprised of 8,385,928 bases, 4418 predicated ORFs, and 54.2% G+C content, respectively. Unexpectedly, no puf genes for photosynthesis were harbored by those three strains. Interestingly, IMCC3088 and IMCC14734 possess proteorhodopsin (PR) gene instead of puf genes, indicating their different photo-lifestyles. These genomes not only can help us to elucidate the life strategy and ecotype differentiation of members of the OM60/NORS clade, but enrich the genetic materials for more comprehensive investigation on the adaptation of proteorhodopsin-containing strains in the nutrient-poor ocean surface.  

This study was supported by a grant from the Marine Biotechnology Program (PIT2000620) funded by the MOF, Korea.
unit-1. Various sulfate- (e.g., bacteria also contributed major nitrogen removal near the separator in (e.g., might function autotrophic denitrification using electron from electrode. (grown near the both anode and cathode. Heterotrophic nitrite reduction and nitrite oxidation (cathode, coupled with denitrification by several families (e.g., using Illumina MiSeq platform revealed that ammonia (compounds were removed in unit-1 and unit-2. Family group analysis (after 8 month-operation), respectively. The most of organic and nitrogen improved from 78% and 85% (initial 1 month-operation) to 85% and 94% as assemblies could achieve simultaneous removal of organic and nitrogen activity. Microbial carbonate formation can regulate element and energy cycle of Earth’s environment. This research is performed to know the relationship between environmental changes and biotic activities through a metagenomic analysis of microorganisms that is acquired from secondary calcite “Moonmilk” from Baeg-nyong(BN) cave, Korean CZO. Moonmilk is one of the carbonate precipitates. Recent researchers proposed that many kinds of bacteria play a major role of carbonate precipitation forming moonmilk. Therefore, our research shows that what kind of microorganism is a major inducer for the formation of moonmilk in BN cave and how they work on the mechanism forming moonmilk. First we collected moonmilk from two sites in the middle of the first branch in BN cave and analyzed microbial genomes through the metagenomic sequencing. As a result, we found that dominant microbial species in two moonmilk is the Lysobacter group. L.arseniciresistens have an ability to reduce toxic Arsenate (As), so we suggest that L.arseniciresistens might be the crucial bacteria in bioremediation of As. [This work is supported by NRF-2017R1A4A1041105 and NRF-2017R1D1A1A02061743]

In this study, we investigated the aerobic heterotrophic bacterial population densities and diversities of the soil communities of Dokdo and Ulleungdo islands from August 22 to 25, 2016. Additionally, we studied the soil bacterial communities composition of Dokdo and Ulleungdo islands by using pyrosequencing analysis. The results were as follows; The population densities of aerobic heterotrophic bacteria in the soil of Dokdo island were in the range of (1.7 ± 0.2) x 10^8~ (2.4 ± 0.3) x 10^8 CFU/g dry wt. and those of Ulleungdo island were in the range of (5.6 ± 0.3) x 10^8~ (3.8 ± 0.5) x 10^8 CFU/g dry wt. during investigation, respectively. And by statistical analyses, Proteobacteria was the most dominant phylum [Dokdo (41.83%) and Ulleungdo (38.59%)] and Alphaproteobacteria was the most dominant Class[Dokdo (22.45%) and Ulleungdo (23.68%)] in both of the soil samples. It indicated that bacterial diversity in the soil of Ulleungdo island was slightly higher than that of Dokdo island.

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Microbial Community Structure for Simultaneous Nitrification and Denitrification in Flat-panel Air-cathode Microbial Fuel Cells Treating Domestic Wastewater

Younghyun Park, Jaecheul Yu, Wonyoung Choi, and Taeho Lee* Pusan National University

A flat-panel air-cathode MFC (FA-MFC) consisted of two separator electrode assemblies could achieve simultaneous removal of organic and nitrogen compounds within short hydraulic retention time (HRT). The five FA-MFC units were connected to have a domestic wastewater flow in series and operated with an HRT of 2.5 h (0.5 h for each unit). Throughout 8 month-operation, the most of current was produced from the first unit received relatively high concentration of organics from the influent. By contrast, organics and nitrogen removal efficiencies were significantly improved from 78% and 85% (initial 1 month-operation) to 85% and 94% (after 8 month-operation), respectively. The most of organic and nitrogen compounds were removed in unit-1 and unit-2. Family group analysis using Illumina Miseq platform revealed that ammonia (Nitrosomonadaceae) and nitrite oxidation (Nitrospiraceae) using oxygen penetrated through cathode, coupled with denitrification by several families [e.g., Rhodocyclaceae] grown near the both anode and cathode. Heterotrophic nitrite reduction (Proteobacteria) and anoxic aerobic ammonium oxidation (Brocadaceae) bacteria also contributed major nitrogen removal near the separator in unit-1. Various sulfate- [e.g., Desulfovibulacaceae] and iron-reducing bacteria [e.g., GeoBacteraceae] were detected in the anode, indicating that it might function autotrophic denitrification using electron from electrode. [Supported by grants from NRF (NRF-2017R1A2B1A15054528.)]
The Effect of Long-term Fertilizations on Altering the Bacterial Community Composition and Its Consequences on Microbial Methane Cycling in Rice Paddy Soils

Ju-Hee An¹, Sang Yoon Kim², Chang Hoon Lee³, Sung Hwan Oh⁴, and Jaekyong Song⁵

¹Agricultural Microbiology Division, National Institute of Agricultural Sciences (NARS), Rural Development Administration (RDA), ²Soil and Fertilizer Division, National Institute of Agricultural Science, Rural Development Administration (RDA), ³Department of Functional Crop, National Institute of Crop Science, Rural Development Administration (RDA), ⁴Agricultural Microbiology Division, National Institute of Agricultural Sciences (NARS), Rural Development Administration (RDA)

Long-term repeated fertilizations may alter the microbial community composition in agricultural soils. However, the role of soil microorganisms and their interaction with methane-cycling remain unclear. We investigated the response of the soil bacterial community abundance and composition, and methane turnover under four different fertilization regimes (control, NPK, compost, and NPK + compost) in a 49-year old paddy field. The abundance of the 16S rRNA genes in the soils treated with compost was higher than that in the untreated soils. The soil bacteria community composition varied among the fertilization regimes, more pronounced after organic or inorganic fertilization as compared to the control. Organic fertilization increased bacterial richness and stimulated members belonging to the phyla Proteobacteria, Cyanobacteria, and Actinobacteria. In contrast, soils receiving inorganic fertilizer harbored distinct microbial communities such as Nitrospirae and Planctomycetes. Methane production potential decreased after mineral fertilizer application, but methane oxidation potential increased under the same conditions. Soil chemical properties including pH and labile carbon pools were the major contributing factors to altered bacterial community composition and impact the activity of methane-cycling microbes in the soils. Our findings suggest that inorganic fertilizer in combination with compost would be beneficial to potentially mitigate methane budget in rice paddy fields.

Wolbachia Inhabited by the Insect Vector of Pine Wilt Disease

Su-Kyong Shin¹, Hyerim Han², and Hana Yi¹

¹Department of Public Health Sciences, Graduate School, Korea University, ²Division of Forest Insect Pests and Diseases, National Institute of Forest Science, ³School of Biosystem and Biomedical Science, Korea University

Wolbachia, the obligate endosymbiotic bacteria influencing the reproduction of its host, is of interest in the role in the insects of pine wilt disease (PWD). To evaluate the possibility of Wolbachia-mediated pest control method in PWD, the infection status and diversity of Wolbachia in long-horned beetles, the insect vector of this disease, was investigated in this study. Two species of long-haired beetle, Monochamus alternatus and Monochamus saltuarius, were collected from diverse regional areas of Korea including PWD outbreak area and non-endemic area. Wolbachia infection status was determined by using multi-locus sequence typing and Wolbachia surface protein typing. The result showed that 13% (50 of 386 beetles) of examined long-horned beetles possessed Wolbachia. Three different singly infected Wolbachia sequence types were observed in Korean long-haired beetles. Two of them are newly discovered sequence types, but all of the three sequence types belonged to supergroup A. Depending on beetle species diversity, regional diversity, and gender of beetles, Wolbachia infection rate and dominant sequence type varied. However, no correlation was observed between Wolbachia infection and parasitic PWD nematode (Bursaphelenchus xylophilus) infection. [This work was supported by the Korea Forest Research Institute (Project: Characteristics of Insect Vector Microbiome according to Geographical Distribution of Pine Wilt Disease)]

The First Complete Genome Sequences of the aci Lineage, the Most Abundant Freshwater Actinobacterial Group

Ilam Kang, Suhyun Kim, and Jang-Cheon Cho⁶

⁶Department of Biological Sciences, Inha University

The aci lineage of the phylum Actinobacteria is the most abundant bacterial group in most freshwater environments. However, due to difficulties in laboratory cultivation, only two mixed cultures and some incomplete single-amplified or metagenome-derived genomes have been reported for the lineage so far. Here, we report the initial cultivation and complete genome sequences of four novel strains of the aci lineage from the tribes acl-A1, -A4, -A7, and -C1. The four acl strains, initially isolated by dilution-to-extinction culturing, eventually failed to be maintained as axenic cultures. However, the first complete genomes of the acl lineage were successfully obtained from these initial cultures through whole genome amplification applied to more than hundreds of cultured acl cells. The genome sequences exhibited features of genome streamlining and showed that the acl strains are aerobic chemoheterotrophs sharing central metabolic pathways, with some differences among tribes that may underline niche diversification within the acl lineage. Acrinobacteriopsis was found in all strains, but retinal biosynthesis was complete in only A1 and A4 tribes. [This study was supported by the National Research Foundation, Korea.]

Cultivation of Bacteria from Brackish Water by Using High-throughput-culturing Based on Cell-sorter Inoculation

Hyong Tae Jeon, Yeonjung Lim, Suhyun Kim, and Jang-Cheon Cho⁶

⁶Department of Biological Sciences, Inha University

In this study, we applied dilution-to-extinction high-throughput-culturing (HTC) based on cell-sorter inoculation to cultivate diverse bacterial strains from water samples collected inside and outside of the Saemangeum Seawall, Korea. Cells in water samples were analyzed using a FACS instrument, and were inoculated directly from the nozzle into low nutrient media aliquoted into multiwall plates, with a final cell density of 1 or 3 cells/well. After inoculation, culture plates were incubated at 18–20°C for 7 weeks, and microbial growth was measured using a flow cytometer. Among a total of 2,592 inoculated wells, 414 wells were determined to be growth-positive (10⁶ cells/ml). Further analyses by PCR and sequencing of 16S rRNA genes showed that 333 putatively pure cultures were obtained successfully. Bacterial strains cultured in this study were affiliated with 7 phyla, 12 classes, 27 orders, and 33 families. Dominant phylogenetic groups included Rhodobacteraceae (74 strains), the SAR11 clade (49), Pseudomonadaceae (39), Alteromonadaceae (32), and Flavobacteraceae (31 strains). These results showed that diverse marine and freshwater bacteria can be isolated from brackish water by using HTC based on cell-sorter inoculation. [This study was supported by a grant from the Marine Biotechnology Program (PJT200620) funded by the Ministry of Oceans and Fisheries, Korea.]
Primers for Amplification and Deep Sequencing of Alphacoronavirus Genomes

Jungmo Lee1, Sungmi Choi2, Su-Kyoung Shin1, Seil Kim1,2, Gun-Soo Park1,4, Gir Won Lee3, and Hana Yi1,3,5,6

1Department of Public Health Sciences, Graduate School, Korea University, 2School of Bioanalytical Sciences, Korea Research Institute of Standards and Science, 3Convergent Research Center for Emerging Virus Infection, Korea Research Institute of Chemical Technology, 4Division of Functional Food Research, Korea Research Institute of Chemical Technology, 5School of Biosystem and Biomedical Science, Korea University

Rapid full genome sequencing is essential for identification of unknown viral pathogens, but amplification-free shotgun metagenomic sequencing is generally not possible to recover the entire genome sequence. In this regard, universal primers are needed that allows amplification of viral nucleic acid to be deep-sequenced. In this study, we aimed to produce a universal primer set for the genomic amplification of Alphacoronavirus as part of our rapid identification method for new corona viruses. The primer sets common to the sequences of Alphacoronavirus HCoV-229E group were designed considering the degeneracy, Tm, and amplicon size. Nine pairs of primers were designed to produce amplicons (average length of 5 kb) stretched over the entire genome and the experimental procedure was optimized. The selected primer set was applied to Alphacoronavirus HCoV-229E and produced amplicons with expected size. The mixture of amplicons was sequenced using MinION portable sequencer. The sequencing results demonstrated that primer sets produced in this study amplify the best length and position to be sequenced. To evaluate the versatility of the primers, amplification and sequencing experiments are being conducted with respect to other Alphacoronavirus and Betacoronavirus. [This study was supported by the Korean Ministry of Environment and by the National Research Council of Science & Technology (NST) grant by the Korean government (MSIP) [No. CRC-16-01-KRICT]]

Studies on the Pathway and Enzymes Involved in the Degradation of Dibenzo-furan by Novosphingobium pentaromativorans US6-1

Hyeyun Na1,2, Sujung Yoo3,4, Yeonju Lee1,3, and Kaekyoung Kwon1,2,4

1Korea Institute of Ocean Science and Technology, 2Korea University of Science and Technology

Novosphingobium pentaromativorans US6-1 is a marine bacterium that was isolated from muddy sediments of Ulsan Bay, Korea and could utilize high-molecular-weight PAHs as a sole carbon and energy source. Degradation of dibenzo-furan (DBF) by strain US6-1 was compared with Sphingomonas wittichii RW1 - known as the outstanding DBF-degrading bacterium - and confirmed that degradation of DBF by strain US6-1 was faster than that by strain RW1. Phylogenetic analysis of α-subunits of the aromatic ring hydrogenase of the strains US6-1 and RW1 with other strains revealed that genes of the strain US6-1 were differentially clustered from those of strain RW1 which degrade DBF via angular dioxygenation process. Degradation of DBF by strain US6-1 was monitored with fluorescence spectrometer and metabolic intermediates were analyzed by GC-MS and LC-MS. As a result, 3(2H)-benzofuranone was detected and this implied that degradation of DBF by strain US6-1 was started with lateral dioxygenation process. [Supported by MOF [PITZ00620] & KIST [PE99514]]

Genomic Analysis Reveals Versatility of Anaerobic Energy Metabolism of Geosporobacter ferrireducens IRF9 of Phylum Firmicutes

So-Jeong Kim1, Heeji Hong2, Joo-Han Gwak2, Soo-Je Park3, and Sung-Keun Rhee4,5

1Freshwater Bioresources Utilization Division, Nakdonggang National Institute of Biological Resources, 2Department of Microbiology, Chungbuk National University, 3Department of Biology, Jeju National University

Here, we report the analysis of genome sequence of Geosporobacter ferrireducens IRF9, a strict anaerobe fermenting sugars and degrading toluene under iron-reducing conditions. Putative alkyl succinate synthase genes instead of typical putative benzyl succinate synthase genes were observed in this genome. Canonical genes associated with iron reduction were not observed in this genome either, although other candidate genes were found. The genome of IRF9 contained genes for acetogenesis with two types of Rnf complexes translocating H+ and Na+ ion, respectively. Two types of ATPase (Na+-dependent F-type ATPase and H+-dependent V-type ATPase) were encoded for full exploitation of ion gradients. The versatile energy conservation potential of strain IRF9 might help its survival in various environmental conditions. It can be exploited for bioremediation of oil contaminated site. [Supported by grants [NRF-2016R1C1B1010946]]

Comparative Metagenomic Methylome Analysis (COMMA): Metatagomic Analysis of Methylation in Different Microbial Communities

Hoon Je Seong, Kyu-Chan Lee, and Woo Jun Sul*

Department of Systems Biotechnology, Chung-Ang University

Bacterial DNA methylations functions as part of restriction modification systems, participating in various cellular processes including antiviral defense, cell cycle regulation and transcriptional modulation. Recently, advanced sequencing techniques has made detection of base modifications and sequencing of methylated DNA at high speed possible. Single molecule, real-time (SMRT) sequencing allows detection of N6-methyladenine and N4-methylcytosine, representative types of bacterial methylomes. Although previous studies investigated methylation patterns of single genome, we intended to understand how methylation patterns contribute from different environments. We present COMMA (Comparative Metagenomic Methylome Analysis), a novel framework for detecting a relationship between DNA methylation and microbial communities from the environment. Using seven metagenomic samples, we showed that the COMMA revealed different methylation patterns depending on the environment. COMMA is a new method, not an existing metagenomic analysis, by linking methylation analysis with microbial community and functional gene analysis. Overall, our findings show that the SMRT platform has tremendous potential to examine the exact nature of methylome and to significantly improve our understanding of the methylation function affected by the environment.
B047
Metagenomic Description of Lignocellulose-degrading Potentials in Soil near the Antarctic King Sejong Station Using Single Molecules Real-Time Sequencing
Han Na Oh1, Jae Wan Park1, Dockyu Kim2, and Woo Jun Sul1*
1Department of Systems Biotechnology, Chung-Ang University, 2Division of Life Sciences, Korea Polar Research Institute

Lignocellulose composed of complex carbohydrates and aromatic compound is considered as a biofuel stock, but its decomposition leads to emission of greenhouse gas such as carbon dioxide. Considering increase of global warming, stacks of lignocellulose in Antarctic soil is sources of greenhouse gas emission. We seek the microbial degradation of lignocellulose in extremely low temperature environment, especially specific microbes and their enzymes using metagenomics approaches. Microbial community analysis of soil collected near the King Sejong Station showed dominance of Proteobacteria, Bacteriodetes and Acidobacteria. We applied Single Molecules Real-Time Sequencing to determine microbes and functional genes responsible for the degradation of lignocellulose. The most abundant phylum, Actinobacteria, possessed functional genes with lignocellulolytic activities from the analysis with CAZy and Pfam. CAZy-based annotation identified 1,068 genes encoding 380 glycoside hydrolases families and 74 auxiliary activities families. Also, we identified the enzymes which were affiliated to 5 consensus plant biomass degradation modules, collection of enzymes breaking lignocellulosic materials. Combined results showed that the genetic capacity of lignocellulose-degrading bacteria was also found in Antarctic environments. As the temperature of the Antarctica increases, microorganisms degrading lignocellulose can promote the production of carbon dioxide.

[B049]
Distinct Soil Bacterial Distribution in Four Different Farming Management Soils
Jeong Myeong Kim, Shin Ae Lee, Jae-Hyung Ahn, Jaekyeong Song, and Hang-Yeon Weon*
National Academy of Agricultural Science, Rural Development Administration (RDA)

Despite the role of soil bacteria in sustainable crop production and ecosystem health of farming soils, little is known about the bacterial diversity in farming lands. Here we report the effect of different agricultural managements (greenhouse, orchard, paddy, and upland) on bacterial community. In total, 853 soil samples were collected across Korea and analysed using pyrosequencing approach. Bacterial richness and diversity were the highest in paddy soils, and lowest in upland soils. The bacterial diversity and richness of all four management soils were significantly correlated with soil pH. Non-metric multidimensional scaling (NMDS) ordination and distance-based redundancy analysis showed that bacterial community structures of paddy soils were different in those of other management types and communities in greenhouse soils were differentiated from those in orchard and upland soils. Indicator species analysis showed that Firmicutes was largely abundant in greenhouse soil, and Chloroflexi, Acidobacteria, β-Proteobacteria, and δ-Proteobacteria were prevalent in paddy soils. The results of this study indicate that bacterial community compositions and the distributions of individual bacterial taxa in farmland soils are mainly influenced by anthropogenic land management types.

[This research was supported by “Cooperative Research Program for Agricultural Science & Technology Development”, RDA]

B048
Comparative Analysis of Skin Microbiome between Young and Old Women
Jin Ju Kim, Hye-Jin Kim, and Woo Jun Sul*
Department of Systems Biotechnology, Chung-Ang University

Human skin undergoes dramatic age-related changes such as a reduction of epidermal thickness, laxity, non-elasticity, and color changes with functional deficit. However, relationship between the microbiome and skin aging has not yet been elucidated, and the role of skin microbiome during aging also remains unclear. To investigate difference in skin microbiome depending on the age, we recruited two groups: young (20~30s) and old (50~60s) Chinese women lived in Xi’an. PCoA of 73 skin microbiome depending on the age, we recruited two groups: young and old women. In network analysis with SPIEC-EASI, the old group showed lower density and transitivity and less complex network than the young group. Aging-associated skin microbiome can be suggested with the potential as a novel diagnostic and therapeutic target for skin aging and related diseases.

[B049]
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[This research was supported by “Cooperative Research Program for Agricultural Science & Technology Development”, RDA]

B050
Microbial Community Structure and Composition in Rhizosphere and Endosphere of Tomato Plants Cultivated in Greenhouses
Shin Ae Lee1, Jeong Myeong Kim1, Jae-Ho Joa2, Jaekyeong Song1, and Hang-Yeon Weon*
National Institute of Agricultural Sciences, National Institute of Horticultural & Herbal Science

The structure and composition of microbial community are related to microbial contribution to plant growth and differentiated according to plant tissues and compartments which are specific habitats for microbial colonization. To understand the complex microbial community associated with plant roots, tomato plants were collected from 26 geographically different greenhouses and analyzed by illumina MiSeq sequencing targeting 16S rRNA gene for bacteria and archaea, and internal transcribed spacer (ITS) region for fungi. Microbial richness and diversity were significantly low in the endosphere compared to the rhizosphere. Microbial communities were notably clustered by each compartment despite geographical differences. Only the rhizosphere communities of bacteria and fungi had correlation with soil chemical properties such as pH, electrical conductivity, and exchangeable cations. We identified distinct genera enriched at rhizosphere and endosphere regardless of geographical and plant genotype differences by using indicator species analysis. The co-occurrence analysis presented less complex network and fewer associations between different domains in the endosphere compared to the rhizosphere. These results provide information of microbial communities associated with tomato root growing in greenhouse, which contribute to phytobiome study for improving sustainable agricultural productivity.

[Supported by grant from RDA]
**Characterization of Spatial Distribution of the Bacterial Community in the South Sea of Korea**

Ji-Hui Seo1, Ilnam Kang1, Seung-Jo Yang2, and Jang-Cheon Cho1,*

1Department of Biological Sciences, Inha University; 2ChunLab, Inc., Seoul National University

The importance of spatial and environmental factors on the structure and diversity of bacterial communities was investigated in the littoral sea by pyrosequencing of 16S rRNA genes. Samples were prepared from seven different stations, and were divided into three groups according to distances from the coastline. Major bacterial lineages showed different niche preferences among three locational groups. Alphaproteobacteria showed a clear difference among the samples at the order levels. The SAR11 clade was more abundant in coastal waters while the Roseobacter clade prevailed at stations far away from the coastline. Furthermore, members of Actinobacteria and Cyanobacteria also exhibited spatial variability. The OM1 clade constituted a predominant fraction in coastal samples, but was essentially absent at the distal stations. In contrast, Synechococcus was the predominant taxon in the distal samples, but hardly detected in coastal waters. In Bacteroidetes, NS5 and NS9 groups tended to inhabit coastal waters while the genera Polaribacter and Ulvibacter were more abundant in distal stations. Clustering and principle coordinates analysis indicated that bacterial communities in the studied area were separated into three groups that coincided with locational grouping. Statistical analysis showed that phosphate and dissolved oxygen concentration had a significant influence on the bacterial community composition.

[Supported by grants from Marine Biotechnology Program PJT200620 and NRF]

**Isolation of a BTEX-degrading Bacterium, Rhodococcus sp. B23, from a Freshwater and Optimization of Biodegradation Conditions**

Hyun Mi Jin, Hye Kyeong Kim, So-Jeong Kim, Byung Gon Ryu, Young Su Kim, and Sang Chul Jeong*

Nakdonggang National Institute of Biological Resources

An enrichment culture was established using freshwater containing BTEX (benzene, toluene, ethylbenzene and xylene) compounds to isolate a BTEX (Benzene, Toluene, Ethylbenzene, o-, m-, p-Xylene) 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 B23, 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 B23 was also able to utilize 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. NH4Cl, NaH2PO4, cell mass and contaminant concentrations were used as independent variables to optimize the degradation of aromatics by strain B23 in a Nakdong-river and a statistically significant p<0.0001) quadratic polynomial mathematical model was suggested.

**Bacterial Community Structure of Surface Snow in Antarctica**

Ahnna Cho1,2, Yong-Joong Cho3, Jong Ik Lee1, and Ok-Sun Kim1,*

1Korea Polar Research Institute; 2Kangwon National University

In recent years, applications of molecular methods to study microbial ecology have allowed the extension of our knowledge that extreme environment contains unexpected high diversity of bacteria and their complex of community. Recently, a metagenomic study of snow suggested that snow bacteria can be adapted to photochemical reactions and oxidative stress in addition to cold stress, and therefore may form specific communities. In this study, we investigated the bacterial communities in Antarctic surface snow based on culture-dependent and -independent approaches. Total 13 samples were collected from November in 2015 to January in 2016 around Victoria Land (East Antarctica). A total of 8 strains belonging to either Actinobacteria or Firmicutes were isolated from two samples. On the basis of 165 rRNA gene by pyrosequencing, overall 13,504 sequence reads were obtained, and 412 operational taxonomic units (OTUs) were generated with 97% similarity cutoff. Gammaproteobacteria (0.2~70.1%), Actinobacteria (1.9~67.8%), Firmicutes (0.0~45.8%), and Alphaproteobacteria (0.5~22.9%) were dominant. The dominant genera such as Propionibacterium, Aerococcus, and Micrococcus may have been deposited on the snow surface from the atmosphere. In contrast, genus Enhydrobacter may be considered most abundant as endogenous Antarctic snow inhabitants. These findings can get closer to the snow ecosystem, which occupy over a third of land surface area.

[Supported by KOSEF (Grant PE17110)]

**Metagenomic Analysis of Relationships between Carbon and Nitrogen Metabolisms during Tannery Wastewater Treatment Undergoing a Microbial Bioaugmentation**

Woo-Jun Sul1, Chaeyoung Rhee2, HoonJe Seoung2, S. Aalfin Emmanuel2, and Sung-Cheol Koh1,*

1Chung-Ang University, Systems Biotechnology; 2Korea Maritime and Ocean University, Environmental Engineering

The goal of this study was to elucidate relationships between the inorganic nitrogen removal and the carbon source utilization in the metagenomic perspective. Metagenomic taxonomic and functional analysis were profiled using HUMAnN2. Shotgun metagenomic reads were mapped to ‘ChocoPhlan’ pan-genome database and MetaPhAn2 database for organism specific functional profiling. We then analyzed the gene families and pathways using the extended databases UniFrat Reference Clusters and MetaCyc metabolic pathway database. The functional analysis revealed that 32 metabolic pathways were involved in amino acid production whose pathways were dominantly found in the stage I and PA, and most of amino acid degradation pathways were also dominant in I, indicating that NH4 might be mostly released in these stages. However, nosZ gene was highly dominant in the process B where a significant removal of nitrogen and COD was observed. It was also revealed that l-arginine degradation is specifically linked to DNRA pathway. A linkage analysis between denitrification/DNRA and degradation of fatty acid and other organic acids will be also discussed in association with an efficient removal of nitrogen and COD (and hence sludge reduction). These metagenomic insights will contribute to a successful monitoring and operation of the eco-friendly tannery wastewater treatment system.

[This work was supported by NRF grant [No. 2012-0005136] and SMBA Grant No. 10047298]
To Study the Effect of Change in NaCl Concentrations on Virulence of Streptococcus parauberis

Durga Ray, Seongyong Yoon, Yunjeong Choe, Yeon Ha Kim, Moonjung Jeon, and Ho Young Kang

Department of Microbiology, Pusan National University, 
Department of Integrated Biological Science, Pusan National University

Many bacterial species, including a number of fish pathogens, such as Streptococcus parauberis and S. iniae are able to survive for a long time under conditions of starvation in the aquatic environment. Streptococcus parauberis strain SpOF3k used in this study was previously isolated from the kidney of a diseased olive flounder (Paralichthys olivaceus) collected from Geoje Island in 2013. S. parauberis strain studied in this work is a coccoid, nonmotile, alpha-hemolytic, Gram-positive bacterium of the Streptoccocaceae family. In the present work, the growth rates of S. parauberis were determined in BHI medium containing diverse array of saltiness based on environmental salt concentrations. As the concentration of salt increased, the growth rate decreased. Similar lag in growth rate was seen in nineteen other strains of S. parauberis and S. iniae. The culture at high salinity of 3 and 3.5% resulted in complete bacterial growth retardation indicating that the high salt condition is not optimally suited for its growth. Their ability to enter and recover from this state, were examined via drop plate method, through which it was found that after 60 hours of growth in presence of 3.5% NaCl, there was a steady decline in the cell number. Further, we are planning to do electron microscope examination and confirm differences in protein expression patterns by studying the sub-cellular fractions of the cells in presence and absence of 3.5% NaCl.

Control of Soil Community Structure with Oxygen and Organic Substrates

Jae Ho Han, Hye Yoon Na, and Kae Kyoung Kwon

Marine Biotechnology Center, KIOST, Major of Marine Biotechnology, UST

Large amounts of herbicide mainly including TCDD (Tetra-chlorinated dibenzo-p-dioxin) had been sprayed during Vietnam War. Due to chlorine atoms TCDD showed strong biological toxicity and recalcitrant to degradation. Therefore, dechlorination is critical step and known to be progressed under anaerobic conditions. For comparison of dechlorination and degradation of TCDD under aerobic, anaerobic, or anaerobic-aerobic sequential conditions, several microcosms were prepared. 20 ml slurry samples (50% v/v) of TCDD contaminated soil from A Luoi, Vietnam was distributed into 100 ml serum bottles and set four different conditions with 1) addition of organic acids, 2) supplementation of oxygen, and 3) addition of mono- or di-chlorinated aromatic compounds as supplementary substrates. Community structure of each treatment was analyzed after 5, 7, 9, and 15 weeks of incubation. As a result, microbial community was divided by type of treatments, briefly, oxygen and organic acids governing community structure. Under anaerobic conditions Classes Acidobacteria and Chloroflexi dominated with time, however, no diverse bacterial groups were competed in aerobic conditions. The results implied that microbial community structure could be guided with appropriate treatment. [Supported by UST & KIOST (PE99514)]

Exploring the Influence of Environmental Factors on Rock-associated Bacterial Communities in Northern Victoria Land, Antarctica

Jun Ho Cho, Jaein Lee, Ahnna Cho, and Ok-Sun Kim

Systems Biotechnology, Chungang University, Unit of Antarctic K-Route Expedition, Korea Polar Research Institute

Northern Victoria Land in Antarctica, mostly covered with ice-free deserts and rocky outcrops, has extreme environmental conditions for living organisms to survive. Under such circumstances, rocks can be favorable habitat for microorganisms to avoid environmental stress. Rock-associated bacteria in extreme environments play significant roles such as providing nutrients through weathering. Up to date, many researches have mainly focused on specific bacterial phyla, specific locations of inhabitation and/or specific types of rocks. Thus, the present study aimed to explore rock-associated bacterial community structures using various rock samples and to investigate correlation between bacterial community structure and environmental factors. Chemical properties of 56 rock samples, collected from northern Victoria Land, were determined using an ion chromatography, inductively coupled plasma mass spectrometry, inductively coupled plasma atomic emission spectroscopy. Illumina MiSeq amplicon sequencing was used to analyze bacterial community structures. The results showed that pH and latitude were significantly correlated to rock-associated bacterial community structures. As latitude increased, pH tended to decrease and Proteobacteria tended to become a dominant phylum, while Actinobacteria was the dominant phylum at lower latitudes. Microbial diversity was also found to be higher at relatively low latitudes. [Supported by Korea Polar Research Institute (Grant PM160301)].
Isolation, Identification and Growth-promotion Effect of Phosphate Solubilizing and Potassium Decomposing Bacteria Isolated from Mangrove Soil

Jiafa Wu, Xueping Lin, Yong Huang, Liting Huang, Xianyue Jiang, Lv Xiaofen, and Mingguo Jiang*

School of Marine Sciences and Biotechnology/Guangxi Key Laboratory of Utilization of Microbial and Botanical Resources, Guangxi University for Nationalities, Nanning 530008, P.R. China

*Corresponding author: mzxyjiang@163.com

The study aimed to obtain microorganisms that can convert insoluble inorganic phosphate and potassium to an accessible form to promote the growth of plant for increasing yield. Phosphate-solubilizing and potassium-decomposing bacteria were screened and isolated from mangrove soil in Shangkou Mangrove National Nature Reserve, Guangxi Province, China. Out of 300 colonies, 4 best phosphate-solubilizing and 4 potassium-solubilizing strains were isolated and purified, and the morphology, biochemical, and physiological characteristics were tested. Phosphate-solubilizing strain P225, P238, P379 and P402 were identified as Bacillus aerophilus, Bacillus cereus, Sphingomonas jaspii, and potassium-solubilizing strain K119, K355, K375, and K395 were identified as Bacillus marisflavi, Sphingomonas sediminicola, Bacillus vietnamsensis, and Halobacillus trueperi according to the results of polyphasic taxonomy. The sequences of eight strains were deposited in the GenBank nucleotide sequence database in NCBI with the accession numbers KY982790, KY982805, KY982816, KY982825, KY982843, KY982738, KY982746, and KY982739. The inorganic phosphate-solubilizing activity was determined by the molybdenum blue method at 30°C using NBRIP medium to measure calcium phosphate [Ca₃(PO₄)₂]-solubilizing activity, while NBRIP replaced with aluminum phosphate (AlPO₄) and iron phosphate FeSO₄ to measure AlPO₄ and FePO₄-solubilizing activities. The ability of dissolving potassium were determined with the atomic absorption spectrophotometer method. Under greenhouse conditions and no phosphorus and potassium applying, 8 isolates showed increased root and shoot length of maize and soybean in various degree. With phosphorus and potassium applying, 8 isolates showed increased root and shoot length of maize and soybean in various degree. With phosphorus and potassium applying, 8 isolates showed increased root and shoot length of maize and soybean in various degree.

B059 9th ASME

Diversity and Composition of Rumen Microbiome in Sika Deers (Cervus nippon yakushimae) from Yakushima Island, Japan

Misaki Eto, Tetsukazu Yahara, Arika Kuroiwa, and Natsuko Hamamura*

Department of Biology, Faculty of Science, Kyushu University, Japan

Rumen microbiome (RM) play a crucial role in digesting cellulolytic biomass for ruminant diet. Sika deer in Yakushima Island Japan, known as Yaku sika, graze a wide range of forage types, and have caused serious reduction of forest understorey vegetation in the island while increasing their population. Dietary shifts in the host, observed especially with Yaku sika at highly populated areas, are expected to influence community composition in RM. In this study, we characterized the RM of wild Yaku sika population by high throughput sequencing of bacterial 16S rRNA genes. Significantly higher diversity and distinct community structure were observed (P<0.05) in Yaku sika from high-density areas compared to those from decreasing population density areas. Furthermore, the comparative analyses of RM datasets from other ruminant animals revealed that Yaku sika contained significantly higher percentage of fibrolytic bacterial groups, such as Ruminococcaceae and Prevotellaceae, and also exhibited higher community diversity (P<0.001). Consequently, these features may contribute to the flexibility of Yaku sika’s dietary shift and maintain nutritional status under high density conditions.

B060 9th ASME

Species-specific Interaction Using Membrane Vesicles in Enterobacteria

Kotaro Takaki¹, Yusuke Hasegawa¹, Hiroyuki Futamata², and Yosuke Tashiro*²

1Department of Applied Chemistry and Biochemical Engineering, Shizuoka University, Japan, 2Research Institute of Green Science and Technology, Shizuoka University, Japan

A wide range of microbial species secrete membrane vesicles (MVs) and MVs play a role in the transfer of several compounds to other cells in microbial communities. However, little is known about the mechanism how MVs interact with microbial cells. In this study, we investigated the specificity of the MV-cell interactions and explore novel factors relative to the uptake of MVs in bacteria. MVs derived from an enterobacterium Buttaiauxella agrestis specifically interacted with Buttaiauxella spp. but interacted less specifically with those of other genera. Moreover, a mutant, which lacks a periplasmic protein contained in MVs, showed higher uptake of MVs as compared to the wild type in B. agrestis. Thus, we demonstrated that MVs selectively interacted with target bacterial cells and found a novel factor involved in uptake of MVs. These results would offer valuable insights for understanding microbial interaction via MVs.

B061 9th ASME

Species-specific Interaction Using Membrane Vesicles in Enterobacteria

Kotaro Takaki¹, Yusuke Hasegawa¹, Hiroyuki Futamata², and Yosuke Tashiro*²

1Department of Applied Chemistry and Biochemical Engineering, Shizuoka University, Japan, 2Research Institute of Green Science and Technology, Shizuoka University, Japan

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[This work support by JSPS KAKENHI Grant Numbers JP15K21043 and JP15H01315 to Y. T.]
**Poster**

### B062 9th ASME

**PCE/TCE-dechlorinating Activities of Dehalococcoides-enrichment Culture TUT2264 are Regulated by Different Two Stages Using Hydrogen**

Hiroya Ueda¹, Tomoharu Iikura¹, Shuji Yamamoto¹, Hiroyuki Morioka¹, Yusuke Tashiro¹, and Hiroyuki Futamata¹,², *¹

¹Department of Applied Chemistry and Biochemical Engineering, Graduate School of Engineering, Shizuoka University, Japan, ²Research Institute of Green Science and Technology, Shizuoka University, Japan

It is usually thought that a competitive inhibition occurred between Dehalococcoides and Methanogens under highly H₂ conditions, resulting in the decrease of chloroethene-dechlorinating activities. Actually, tetrachloroethene (PCE) and trichloroethene (TCE)-dechlorinating activities of Dehalococcoides-enrichment culture TUT2264 decreased significantly under the condition of highly H₂ concentration. However, it is known that the activity of reductive-dehalogenase depends on the activity of hydrogenase that is positive correlation with H₂ concentrations. Furthermore, although the concentration of H₂ was sufficient for PCE/TCE-dechlorination under highly H₂ conditions, why were PCE/TCE-dechlorinating activities inhibited? Reverse transcription-PCR was used to evaluate the transcriptional efficiency (rdhAs mRNA per rdhAs). These analyses revealed that three of eight reductive-dehalogenase genes in TUT2264 played major role in PCE/TCE-dechlorination and their transcriptional efficiencies were changed by H₂ concentrations. These results indicated that PCE/TCE-dechlorinating activities of Dehalococcoides in enrichment culture TUT2264 are regulated by different two stages using hydrogen.

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### B063 9th ASME

**Screening and Identification of Cellulolytic Microorganisms from Soils in Subtropical Forests of Southwest China**

Zheng Zhang¹, Shuang Wu¹, Jia-xun Feng², and Ming-guo Jiang¹, *¹

¹College of Marine Sciences and Biotechnology/Guangxi Key Laboratory of Utilization of Microbial and Botanical Resources, Guangxi University for Nationalities, Nanning Guangxi, 530008, P. R. China, ²College of Life Science and Technology, Guangxi University, Nanning, Guangxi, 530004, P. R. China

*Corresponding author: mzxyjiang@163.com

Cellulose is the most abundant biomass in nature which can be degraded to glucose through the synergistic action of cellulases. This work aims to isolate, screen and identify environmental microbes which can produce acidic cellulase to degrade microcrystalline cellulose, and clone cellulase genes from the strains with high cellulase activity.

In total, 480 soil samples were collected from sampling sites in virgin forests in 11 natural reserves in Guangxi and Yunnan Provinces, China. From these 363 samples, 327 fungal strains were isolated, using agar medium plates containing Whatman No. 1 filter paper as the sole carbon source. Of the 327 fungal strains, 36 were found to have Avicelase activity of more than 0.2 U/ml in liquid batch cultivation, compared with 0.39 U/ml for T. reesei Rut-C30 under the same cultivation conditions. Molecular analyses of the 36 strains based on internal transcribed spacer sequences revealed that 20 were Trichoderma and 16 were Penicillium species. Notably, 9 of the 36 strains were isolated from Huaping National Nature Reserve in Guangxi, which was the sample location that gave the highest number of strains with Avicelase activity of greater than 0.2 U/ml, and 5 of these 9 strains were identified as Trichoderma harzianum. Of the 36 strains, 8 were isolated from Xishuangbanna National Nature Reserve in Yunnan, which was the sample location with the second highest number of strains producing Avicelase activity greater than 0.2 U/ml, and 3 of these 8 strains were identified as T. harzianum. Strains of Trichoderma and Penicillium were the predominant cellulolytic fungi in subtropical and tropical forests in China.

Two of the best-performing isolates, Trichoderma koningiiopsis FCD3-1 and Hypocrea crenea BM48-3, had Avicelase activity of 0.37 and 0.36 U/ml, respectively, which did not differ statistically from that of Rut-C30. Interestingly, strain FCD3-1 produced a high level of β-glucosidase activity at 1.18 U/ml, which was approximately 17 times higher than that of Rut-C30. Glucose was the sole hydrolytic product of Avicel hydrolyzed by the crude cellulase produced by strain FCD3-1.

**Key Words:** cellulase; Avicel; screening; identification; Trichoderma
Mechanism of Growth-repression Induced by *Pseudomonas* sp. C8

Masahiro Honjo1, Kenshi Suzuki2, Tomoka Nishimura3, Yosuke Tashiro4, and Hiroyuki Futamata2,5*

1Department of Applied Chemistry and Biochemical Engineering, Shizuoka University, Japan, 2Graduate school of Science and Technology, Shizuoka University, Japan, 3Research Institute of Green Science and Technology, Shizuoka University, Japan

It is important for understanding complex microbial ecosystems to analyze interspecies interaction. In our previous study, a supernatant (CBS) retrieved from a chemostat culture of *Pseudomonas* sp. C8 supplied with phenol as sole carbon and energy source inhibits the growth of bacteria, *Ralstonia* sp. P-10, *Comamonas testosteroni* R2, *Escherichia coli* BW25113 and the like, which means the extension of lag time or the decrease of growth and growth rate. *E. coli* BW25113 consumed completely glucose under the presence of the CBS and the amount of growth was 20% to 50% less than that under absence of the CBS. KEIO library was used to unveil the growth-inhibiting mechanism of the CBS. Of them, 107 mutants were selected as non-growth inhibiting mutants, which defect genes encoding glucose metabolism, electron transfer, and transporters. These results suggested that the CBSP was not directly toxic compound to bacterial cells but affected indirectly through the change of metabolic process. Namely, series catabolic processes were enhanced by the CBSP, resulting in growth inhibition based on the imbalance between catabolic and anabolic processes.

Characterization of Extracellular Electron Transfer in *Desulfovibrio* sp. HK-II

Shota Ando1, Arashi Yui2, Miki Katagiri3, Yosuke Tashiro4, and Hiroyuki Futamata2,5*

1Department of Applied Chemistry and Biochemical Engineering, Shizuoka University, Japan, 3Research Institute of Green Science and Technology, Shizuoka University, Japan

Extracellular electron transfer (EET) is one of respiration using soluble or insoluble conductive compound as electron acceptor. We investigated whether sulfate-reducing bacterium *Desulfovibrio* sp. HK-II, which is isolated from the surface of anode in a microbial fuel cell (MFC), had an EET mechanism or not. After strain HK-II was incubated under sulfate-reducing condition with lactate as sole electron donor, the cells were inoculated in the anode chamber in an MFC. Electrochemical analysis indicated that the EET mechanism of strain HK-II was conducted with electron carriers at least, namely indirect EET. Strain HK-II converted lactate to acetate under the sulfate-reducing condition, in which acetate was accumulated. However, acetate was consumed completely under the MFC-condition, suggesting that metabolic pathways changed over in EET. SDS-PAGE analyses showed that proteins expressed only under the MFC-condition were observed and almost of all these proteins were located in outer membrane. These results demonstrated that *Desulfovibrio* sp. strain HK-II enables to exhibit the EET by dramatically changing metabolic pathways, suggesting the one of survival strategies under sulfate-free conditions.

Microbial Diversity on the Leaves of Japanese and Taiwan Teas

Takanori Satoh1, Haruka Iwata2, Hajime Nonochi3, Ai Hasegawa4, Mitsuki Fuji5, Kouta Nagai6, Shota Inoue7, and Kenji Akioyshi-Hiraoa2

1Graduated school of Science and Technology, Tokushima University, 2Faculty of Integrated Arts and Sciences, Tokushima University, 3Graduated school of Integrated Arts and Sciences, Tokushima University, Japan

There are various teas in East Asia, but potential microbial diversity has not been elucidated in many Japanese and Taiwan teas. Then, we explored bacteria on the leaves of six Japanese Awa Bancha teas (AB: fermented teas), and four Taiwan oolong teas (semi-fermented teas) by the clone library method of 16S rRNA gene. Firstly, we examined bacteria in six Japanese AB teas (A-F). Based on analyzed DNA sequence of totally 106 clones, three kinds of *Lactobacillus* were found in sample A, D and E, whereas clone to *Leuconostoc* were detected only in sample C. On the contrary, *Klebsiella* bacteria were detected in sample D and E. Next, we also explored bacteria in four kinds of Taiwan oolong tea (Tw1-4), and analyzed totally 64 clones (each 16 clones) of 16S rRNA gene. As results, various clones were detected in Taiwan teas, which corresponding to *Weissella*, *Paenibacillus*, *Propionibacterium* in Tw1, *Devisia* and *Chryseobacterium* in Tw2, *Aurantimonas* and *Pedobacter* in Tw3, *Brevundimonas*, *Edaphobacter*, *Propionicibacterium* and *Streptomyces* in Tw4, respectively. Therefore, these results on their microbial diversities might be useful information for utilizing them as bioresource.

Isolation and Preliminary Characterization of Bacterial Isolates on the Degradation of Natural Estrogens and an Estrogen-derived Pharmaceutical

Yung-Chun Hsu1, I-Chen Yang2*, and Shir-Ly Huang2*

1Department of Life Sciences, National Central University, 2Institute of Microbiology & Immunology, National Yang-Ming University, Taiwan

*Presenting student, **Corresponding author

Since 1990, the presence of endocrine-disrupting compounds in the environment displaying estrogen-like activities has become a major issue in environmental research. Both natural estrogen, estrone (E1), 17β-estradiol (E2), and synthetic estrogen used as a pharmaceutical, 17α-ethinylestradiol (EE2), reach the environment through discharge from sewage treatment plants (STP) and livestocks. EE2 displayed estrogen activity 30 times higher than E2, and 100 times higher than E1. *Pseudomonas* sp. SH101, *Gordonia terrae* SH102, *Pseudomonas* sp. SH104 were isolated from STP and Rhodococcus sp. SH11 and *Acinetobacter radiisiten* SH28 were isolated from pig manure. The estrogen degradation metabolites were analyzed by HPLC. All isolates were able to convert E2 to E1. Moreover, E2 degradation rates of strain SH11, SH28 and SH102 were above 70% and EE2 degradation rates of strain SH28, SH102 and SH104 were above 50% after 5-day incubation. The highest degradation rate of E2 (from 10 ppm) and EE2 (from 5 ppm) in 5 days were 90.8% and 64.6% by strain SH11 and SH102, respectively. *G. terrae* was first demonstrated to be a EE2 degrader. The degradation metabolites and mechanism are under investigating. [This work support by National funds]
In the tree phyllosphere, trunks have hardly attracted any attention as bacterial habitats and have been less studied in contrast to leaves. We here report culture-dependent and -independent studies on epiphytic bacteria inhabiting the trunk surfaces of angiosperms and gymnosperms trees (e.g., Fagus crenata, Quercus crispula, Magnolia obovata, and Larix kaempferi) growing in the Shirakami Mountains in Japan, a natural heritage site that is renowned for primeval beech forests. Based on an Illumina sequencing analysis of the bacterial 16S gene, bacterial communities on various tree trunk surfaces were predominantly of the phyla Proteobacteria, Acidobacteria, and Bacteroidetes, and the bacterial community compositions and their host species seemed to be weakly correlated. In our culture-dependent studies, several phylogenetically novel bacterial strains belonging to various phyla, including Proteobacteria, Actinobacteria, Deinococcus-Thermus, and Armatimonadetes, were isolated, especially from trunk surfaces of beech trees. From among these isolates, few strains were subjected to further characterization studies.

Application of Entrapped Mixed Microbial Cells as the Pretreatment for Drinking Water Treatment Plant

Yi-Ching, Cheng, Haon-Yao Chen, Cheng-Fang Lin, and Chang-Ping Yu*

Graduate Institute of Environmental Engineering, National Taiwan University

The clean source water is crucial for the drinking water treatment plant to supply high-quality tap water to the general public. The polluted source water may cause problems in the drinking water treatment and pose a risk to drinking water safety. In this study, using entrapped mixed microbial cells (EMMC) as the pretreatment of micro-polluted source water was examined in Nangang, Matsu, a small island lied in the northwestern Taiwan Strait. The pilot scale EMMCs were constructed and placed in two drinking water treatment plants, receiving different micro-polluted source water, as the pretreatment before the source water enters the water treatment system. To evaluate the influence of the treatment of EMMCs to the source water, the differences in chemical parameters between influent and effluent were compared under two hydraulic retention time (HRT). The results showed that in two pilot plants, the EMMCs were able to remove the total organic carbon (TOC), total nitrogen (TN) the chlorophyll a in the source water. The removal efficiency of TOC was about 14 - 27%. For the TN, the removal efficiency was about 30 - 41%. EMMC could achieve 53-93% removal of chlorophyll a from the source water. We found that HRT, concentrations of TOC, TN, and chlorophyll a in the source water would affect the removal efficiency in EMMC systems. According to the results, when the concentrations of the TOC, TN and chlorophyll a in the influent were above 3 mg/L, 0.5 mg/L and 5 μg/L, respectively, the EMMC systems have demonstrated excellent performance in pretreatment of micro-polluted source water.

Development of Continuous Flow Membrane-less Microbial Fuel Cells for Decentralized Wastewater Treatment

Chao-Chin, Chang1, Wade Kao2, and Chang-Ping Yu*1

1Graduate Institute of Environmental Engineering, National Taiwan University, 2Department of Mechatronic Engineering, National Taiwan Normal University

Microbial fuel cells are promising technology which can generate electrical power from organic matter through oxidation-reduction reactions using microorganisms as catalysts, allowing for wastewater purification and renewable electricity production. In MFCs, lots of factors affect its performance. However, electrode materials play important roles in deciding the performance and cost of MFCs. High surface area, good conductivity, and biocompatibility are common characteristics of electrode materials used for MFC, which improve bacterial adhesion and provide efficient electron transfer between bacteria and electrode surface. In this study, four commonly used electrode materials, including carbon nanotube-coated sponge, graphite granules, carbon fiber brush and carbon felt are used in anode to investigate their ability to treat wastewater and generate electricity in continuous mode for a period of 80 days in eight novel membrane-less MFCs, which were designed for decentralized wastewater treatment. With 468 ± 61.5 mg/L of COD and 15.8 ± 3.3 mg/L of NH4+-N in the influent, the MFCs using carbon nanotube-coated sponge could effectively remove 81 ± 0.1% COD and 76 ± 0.2% NH4+-N with 175.8 ± 80.6 mV open circuit voltage, and around 77.6 ± 47.5 mV under load of 1 kΩ. The MFCs using graphite granules could effectively remove 91 ± 0.1% COD and 86 ± 0.2% NH4+-N with 289.3 ± 78.4 mV open circuit voltage, and around 150.2 ± 55.3 mV under load of 1 kΩ. The MFCs using carbon fiber brush could effectively remove 81 ± 0.1% COD and 77 ± 0.2% NH4+-N with 246.7 ± 97.9 mV open circuit voltage, and around 165.6 ± 92.7 mV under load of 1 kΩ. The MFCs using carbon fiber felt could effectively remove 82 ± 0.1% COD and 73 ± 0.2% NH4+-N with 240.4 ± 115.1 mV open circuit voltage, and around 157.8 ± 93.0 mV under load of 1 kΩ. The cathode limitation can be improved by coating iron (II) phthalocyanine (FePc) to increase cathode performance and therefore enhance voltage production in the membrane-less MFCs. The polarization curve for each MFC was constructed and internal resistance was estimated. Ongoing experiments are examining the addition of entrapped-mixed-microbial-cell (EMMC) in the cathode to improve the total nitrogen removal. Last but not least, a power management system (PMS) prototype is designed for energy harvesting from MFCs in order to promote future application.

Studies on the Epiphytic Bacteria from the Trunk Surface of the Trees Growing in the Shirakami Mountains in Japan

Juan Li1 and Akio Tonouchi1,2*

1The United Graduate School of Agricultural Sciences, Iwate University, Japan, 2Faculty of Agriculture and Life Science, Hirosaki University, Japan

In our culture-dependent studies, several phylogenetically novel phyla communities on various tree trunk surfaces were predominantly of the Bacteroidetes, Proteobacteria, Acidobacteria, and Bacteroidetes, and the bacterial community compositions and their host species seemed to be weakly correlated. In our culture-dependent studies, several phylogenetically novel bacterial strains belonging to various phyla, including Proteobacteria, Actinobacteria, Deinococcus-Thermus, and Armatimonadetes, were isolated, especially from trunk surfaces of beech trees. From among these isolates, few strains were subjected to further characterization studies.
Multiple Advanced Cultivation Techniques for Isolation of Fastidious Microorganisms Associated with the Marine Sponge

Dawoon Jung1, Koshi Machida2, Yoichi Nakao2, Tomonori Kindaichi3, Akiyoshi Ohashi3, and Yoshiteru Aoi1**

1Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Japan; 2Department of Chemistry and Biochemistry, School of Advanced Science and Engineering, Waseda University, Japan; 3Department of Civil and Environmental Engineering, Graduate School of Engineering, Hiroshima University, Japan

Marine sponges contain dense and diverse microbial communities, which are well known for a source of bioactive secondary metabolites. However, most bacteria from environmental samples have resisted cultivation with conventional method, and the bacteria associated with marine sponges are no exception. Here, we employed multiple approaches, diffusion chamber (DC; in-situ cultivation device), I-tip (trap device that allows entering of microbes) and continuous-feeding (CF; feeding medium and discharging supernatant continuously) cultivation in prior to standard cultivation for enrichment of fastidious microorganisms associated with the marine sponge. Total 60 strains per each method (DC, I-tip, CF, and standard cultivation) were isolated, and their 16S rRNA gene sequences were comparatively analyzed. The results show that advanced techniques allowed us to obtain a different culture collection larger, richer, and more novel than that obtained by standard agar plating method. In addition, there are very few overlap in the species among the isolates from each method. Our results indicate that the use of advanced techniques is effective to expand the cultivable microorganisms from sponges.

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Pseudoalteromonas aliena Strain EH1 Producing Cold-active Amylases, Isolated from Chukchi Sea

EunGyeong Heo1, Dockyu Kim1, Hyunjun Kim2, and Eungbin Kim1**

1Department of Systems Biology, Yonsei University, 2Korea Polar Research Institute

A Gram-negative rod and aerobic bacterium, Pseudoalteromonas aliena strain EH1 was isolated from the Chukchi Sea, a marginal of the Arctic, which is located between Beaufort (north of Alaska) and East Siberian Seas. P. aliena strain EH1 produces cold-active extracellular enzymes including amylases and proteases, some of which have potential for industrial applications. Here, we present the 4,594,697-bp complete genome sequence of EH1. The annotation was done by merging the results obtained from the RAST (Rapid Annotation using Subsystem Technology) server and COG (Cluster of Orthologous Groups) database. Sequence analysis showed that the G+C content is 39.06% and that the sequence contains 3,988 coding sequences (CDSs). Additionally, 104 tRNA genes and 1D rRNA operons were predicted in the circular chromosome. [Supported by grants from KOPRI]
Synthetic Small Regulatory RNA-based Efficient Gene Knockdown in Cladostrium acetobutylicum

Kyeong Rok Choi1, Changhee Cho1, and Sang Yup Lee1,2,3,4*
1Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering, KAIST, 2BioProcess Engineering Research Center, KAIST, 3Bioinformatics Research Center, KAIST

Cladostrium is a promising industrial microorganism for the production of valuable chemicals. However, current inefficient genetic manipulation techniques retard metabolic engineering of Cladostrium. Here, we report a gene expression regulation system for Cladostrium acetobutylicum based on synthetic small regulatory RNA (sRNA). Escherichia coli MiCC scaffold-based anti-Evoglow sRNA was found to knock down the expression of Evoglow although the sRNA does not bind to the native C. acetobutylicum Hfq (Cahfq). In contrast, E. coli Hfq (EChfq) forms complex with sRNA and allows stronger repression of Evoglow. Heterologous expression of E. coli MiCC scaffold-Hfq system to knock down adhE1 in C. acetobutylicum led to 40% decrease in butanol production (2.5 g/L). Moreover, knockdown of pta using the sRNA system in the in-bulk mutant C. acetobutylicum strain PJC4BK (PJC4BK (pPta-HfqEco)) allows production of 16.9 g/L of 3-AP. Optimization of PPC expression level by using synthetic promoter and RBS sequences increased the titer up to 3.94 g/L. Native promoter of the acs gene was replaced with strong trc promoter to reduce acetic acid accumulation and the fed-batch culture of this final strain produced 32.5 g/L of 3-AP in 39 h with an overall yield and productivity of 0.135 g/L/h, respectively. This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557).

Production L-arginine by Metabolically Engineered Corynebacterium glutamicum

Cindy Pricilla Surya Prabowo1, Seok Hyun Park1, Hyun Uk Kim1,2, Tae Yong Kim1,2, Jun Seok Park1, Suok-su Kim1, and Sang Yup Lee1,2,3,4*
1Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering, KAIST, 2Bioinformatics Research Center, KAIST, 3BioProcess Engineering Research Center, KAIST

Corynebacterium glutamicum was metabolically engineered for the production of L-arginine. First, to increase tolerance to L-arginine, random mutagenesis was performed on C. glutamicum, resulting in inactivation of Arginine operon repressor proteins. Then, PPP flux was strengthened by downregulating the pgi gene and overexpressing the oprA, pgl, tal, fht, and zwf genes. It was followed by the inactivation of the Ncgl1221 gene encoding L-glutamate exporter in order to channel L-glutamate to L-arginine. Also, optimization for argF and carAB gene expression levels were done to convert L-ornithine to L-citrulline effectively. At last, the argGH operon was overexpressed. Fed-batch fermentation of the final developed strain resulted in 81 g/L of L-arginine production in a 1,500 L bioreactor. These approaches described in this report will be useful in developing strains of Corynebacteria for the production of arginine as well as its derivatives.

Establishment of a Biosynthesis Pathway for Producing 5-Aminolevulinic Acid in Escherichia coli

Jiha Kim1, Yoosung Ko1, Sol Choi1, Jae Won Jang1, Dong In Kim1, Hyun Uk Kim1,2, Si Jae Park1, and Sang Yup Lee1,2,3,4*
1Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 Plus program), KAIST, 2Bioinformatics Research Center, KAIST, 3Department of Environmental Engineering and Energy, Myongji University

Escherichia coli W3110 strain was metabolically engineered to produce 5-aminolevulinic acid (ALA). First, Rhodobacter sphaeroides hemA gene was codon-optimized and cloned into high copy number plasmid pKE112. Second, plasmid pKE112hemA was introduced into the lacI-deleted WL3110 strain; the WL3110 (pKE112hemA) strain produced 0.249 g/L of ALA. Third, in silico knockout simulation was carried out to identify additional gene knockout-targets to further improve ALA production. The gcvTHP genes (glycine cleavage system) were predicted as knockout targets. The JW01 strain (WL3110 (pSCgcvTHP pKE112hemA) produced 1.17 g/L of ALA, which was 4.7 times higher than that obtained with the base strain. Finally, in order to increase the succinyl-CoA pool, the glyoxylate shunt flux was enhanced by the deletion of the icdR and sdhAB genes, while the TCA cycle flux was reinforced by the deletion of ptsG gene. The JW03 strain (JW01 (ΔicdR ΔsdhAB ΔptsG pKE112hemA)) was able to produce 1.72 g/L of ALA. Fed-batch culture of the JW03 (pKE112hemA) strain resulted in production of 5.77 g/L of ALA in 41 h.

Production of 3-Aminopropionic Acid by Metabolically Engineered Escherichia coli

Jiha Kim1, Chan Woo Song1,2, Jungmin Lee1,2, Yoo-Sung Ko1,2, and Sang Yup Lee1,2,3,4*
1Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 Plus program), KAIST, 2BioProcess Engineering Research Center, KAIST, 3Bioinformatics Research Center, KAIST

3-Aminopropionic acid (3-AP) is an important platform chemical for manufacturing acrylamide and acrylonitrile. Escherichia coli strain was metabolically engineered to produce 3-AP. 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 to strengthen the aspartase-catalyzed reaction, the native promoter of the aspA gene was replaced with the strong trc promoter. The aspA and phosphoethanolpyruvate 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 of 3-AP. Optimization of PPC expression level by using synthetic promoter and RBS sequences increased the titer up to 3.94 g/L. Native promoter of the acs gene was replaced with strong trc promoter to reduce acetic acid accumulation and the fed-batch culture of this final strain produced 32.5 g/L of 3-AP in 39 h with an overall yield and productivity of 0.135 g/L/h, respectively. This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557).
Polyhydroxyalkanoates (PHAs) are bio-based polyesters accumulated in many bacteria. The production of PHAs has a great interest in that they are biodegradable and biocompatible thermoplastics. Especially, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] is a meaningful copolymer with its lower melting point and better flexibility compared to commercial polymers. However, addition of second auxiliary carbon source was the drawback for production of P(3-HB-co-3HV) should be followed. Second auxiliary carbon makes it hard to maintain the balance between cell growth and P(3HB-co-3HV) production with its toxicity. Thus, we developed the system in which the E. coli can stably synthesize 3HB-CoA and 3HV-CoA with controlled ratio from glucose without exogenous auxiliary carbon source. Two different metabolic pathways for the production of propionyl-CoA from 2-ketobutyrate were constructed via conversion to propionate as an intermediate. This metabolically engineered strain can synthesize P(3HB-co-3HV) efficiently without feeding any exogenous auxiliary carbon source.

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

Polyhydroxyalkanoates (PHAs) are bacterial polyesters accumulated in various microorganisms such as Ralstonia and Pseudomonas. Previously, we have metabolically developed E. coli strains for the production of 2-hydroxyacids containing PHAs such as lactate (LA) and 2-hydroxybutyrate (2HB)-containing PHAs from structurally unrelated carbon sources including glucose and xylose.

In this study, new metabolic engineering strategies using recombinant Ralstonia eutropha strains are introduced to synthesize PHAs containing 2-hydroxyacids as monomers. With the feature that Ralstonia eutropha is native producer of poly(3-hydroxybutyrate), this could be achieved by changing the inherent R. eutropha phaC gene of which enzyme shows negligible activity toward LA-CoA to either the R. eutropha phaC S506G A510K gene or the Pseudomonas sp. MBEL 6-19 phaC1437 gene. The R. eutropha phaAB genes in the chromosome were also replaced with the Clostridium propionicum pct540 gene. Furthermore, introduction of the Escherichia coli ldhA gene to engineered R. eutropha strains made production of poly(3-hydroxybutyrate-co-lactate) [P(3HB-co-LA)] available from glucose as the sole carbon source.

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

JiHyun Kim, Do-Bu Lee, Sun-Young Park, Su-Jin Ku, and Mun-Cheol Paek*
Korea Materials and Analysis Corporation

We developed a novel molecular diagnostic platform, called Ampli & Array system, which integrating Real-time PCR and DNA microarray. Probes identifying mutation genes and genotyping of TB/NTM are immobilized on the surface of K-CAP™ platform. We can recognize clinically important target genes, rpoB, katG and inhA that are related with rifampin and isoniazid by using these probes. We measured 10 copies of MTB in Real-time PCR process, and detected NTM genotypes and mutation genes of rpoB, katG and inhA specifically. In hybridization, we screened 5 types of NTM and also classified the mutation related to MDR, rifampin and isoniazid related genes. The results of analytical showed that precision of the A&A™ TB/MDR/NTM(5) qK-CAP™ was 96%. In addition, as a result of our experiments, all of reproducibility per gene of A&A™ TB/MDR/NTM(5) qK-CAP™ showed the results that meet more than 90% of the judgment criteria. In clinical tests, results showed that sensitivity, accuracy and precision of the A&A™ TB/MDR/NTM(5) qK-CAP™ were 91.07%, 79.45% and 83.61%, respectively, when compared with the culture method. As described above, this kit is evaluated good diagnostic performance for detection of TB and NTM. Therefore, the A&A™ TB/MDR/NTM(5) qK-CAP™ kit is expected to be applicable to various molecular diagnostics in the near future.

[This study was supported by the Technology Innovation Program (10042215) funded by the MOTIE.]

Metabolic Engineering of Escherichia coli for Production of Bio-isoprene

Seon-Yeong Jo, Murali Kannan Maruthamuthu, Myeong-Seok Cha, Myeong-Seok Yoo, Re Anthony Sanjoro, and Seon-won Kim*
Gyongang National University

Isoprene (2-methyl-1,3-butadiene) is a C5 terpenoid with the formula CH$_3$=C(CH$_3$)CH=CH$_2$. Isoprene is a colorless, highly volatile compound and soluble in ether and hydrocarbon solvents. Isoprene is an important feedstock for commercial production of synthetic rubber. Moreover, isoprene has a higher energy content than other biofuels and is convertible to biofuel blend stocks, such as C10 gasoline, C15 diesel, and jet fuels. Isoprene can be synthesized by isoprene synthase from dimethylallyl diphosphate (DMAPP), which is derived from the mevalonate (MVA) or meythlyerythritol phosphate pathway. Bio-isoprene from renewable source is attractive alternative because of high purity, sustainability and cost-effectiveness. In this study, we constructed plasmids containing isoprene synthase genes (ispS) from Populus trichocarpa, Puerulus montana (kudzu), and Populus alba and introduced them into E. coli to produce isoprene. We analyzed and confirmed isoprene production via GC-FID analysis from recombinant E. coli. To enhance isoprene production, we introduced isoprene synthesis plasmid containing codon optimized ispS for conversion of DMAPP to isoprene and mevalonate (MVA) pathway plasmid to supply DMAPP.

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Investigation of Prebiotics Nanoparticles as Enhanced Antibacterial Property of Probiotics against Pathogens through Internalization

Whee soo Kim$, Bijay Singh$, Sang Kee Kang$, Yunjae Choi $^1$ and Chong-su Cho$^*$
$^1$Department of Agricultural Biotechnology, Seoul National University, $^2$United States of America, $^3$Institutes of Greenbioscience Technology, Seoul National University

Prebiotics is widely used as for improvement of animal health and growth. It is only digested by gut microbes of mammals, and stimulates the growth and activity of the advantageous bacteria. Polymeric nanoparticles have been used for biomedical application because of their interesting characteristics to overcome various biological barriers. In this study, we synthesized prebiotic nanoparticles (PN) as prebiotics by conjugation with hydrophobic groups with hydrophilic prebiotics and we compared antimicrobial activity of probiotic bacteria against pathogens between prebiotics and PNs. The PNs indicated that the nanoparticle sizes decreased with an increase of hydrophobic groups. The PNs were internalized to probiotics, and occurred size and the energy dependently. PNs influenced antimicrobial activity of probiotic bacteria in vitro. PN-loaded probiotics had higher antimicrobial property against gram-positive and negative pathogens than prebiotics-treated ones by the upregulation of antimicrobial peptide. To the best of our knowledge, this is the first report of improved antimicrobial activity by the PNs compared to prebiotic itself. These results suggest that prebiotic nanoparticle-treated probiotics will have potentials for development of a new prebiotic through a new concept.

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C012

Soluble Expression of Artificially Designed Recombinant Protein through Conjugating Fusion Protein in Escherichia coli

Ho-Bin Lee1, Chong-Su Cho2, Sang-Kee Kang3, and Yun-Jaie Choi1,2,3*
1Department of Agriculture Biotechnology, Seoul National University, 2Research institute for Agriculture and Life Science, Seoul National University, 3Institute of Green-Bio Science & Technology, Seoul National University

Foot-and-mouth disease (FMD) has severe implication on livestock industries as it causes an acute disease to cloven-hoofed animals such as pigs and cattle. FMD virus (FMDV) is difficult to be eradicated because of its rapid mutation and variation. Vaccination with subunit vaccine based on multiple epitopic protein can be a promising strategy to protect animals from various FMDV variants. However, when producing recombinant proteins in bacteria, some hurdles can be blocked. Fusion proteins can be a solution to the problems. We designed a chimerial multi-epitopic recombinant protein (SBT), which is comprised of tandem repeats of five B cell epitopes and one T cell epitope derived from FMDV. To increase solubility and stability of SBT, it was conjugated with 8mpB, the membrane protein B of Brachyspira hyodysenteriae as fusion protein (BSBT). Our results showed that SBT was susceptible to degradation by host protease and produced with fraction of inclusion body. The stability and solubility of SBT was greatly increased by conjugating to 8mpB. We also carried out in vivo immunization into mouse model. The FMD specific antibodies were detected in serum of our vaccine candidate as well as groups vaccinated with commercial vaccine. The results offer insight about next generation vaccine model and producing recombinant protein in bacteria such as bioreactor.

This work was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, grant#: PJ01106201), RDA, Korea.

C013

Antimicrobial Activity Test with Lactobacillus plantarum Derived from Kimchi and Pig Feces

Soo Jin Kim1, Gwi-Deuk Jin1, Jongbin Park1, Jihyun Won1,2, Inhwan You1, and Eun Bae Kim1,3,4*
1Department of Animal Life Science, College of Animal Life Science, Kangwon National University, 2Institute of Animal Resources, College of Animal Life Science, Kangwon National University, 3Division of Applied Animal Science, College of Animal Life Science, Kangwon National University

Lactobacillus plantarum is a Gram-positive bacteria belonging to the genus Lactobacillus known as a lactic acid bacteria. It has been used as a probiotic because it improves the balance of the gut microbiota and has antimicrobial activities to inhibit pathogens. Here, we tested the antimicrobial activity of Lactobacillus plantarum. In total, 164 L. plantarum strains were isolated from kimchi and pig feces. (43 strains from kimchi, 121 strains from pig feces). CILP-133, a commercial strain, was used as a positive control and Salmonella and Escherichia coli were used as target pathogenic bacteria. The antimicrobial activity of Lactobacillus plantarum against Salmonella and E. coli was confirmed by the clear zone formed around paper disks that were loaded with L. plantarum culture. We selected 11 L. plantarum strains with high antimicrobial activity that have larger diameter than positive control. In this study, we confirmed the possibility of Lactobacillus plantarum as alternative feed additives for antibiotics used to improve the performance of livestock animals.

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C014

Use of Volatile Metabolites of Pichia anomala MZ-02 to Induce Pochazia shantungensis and Use as Flat Traps

Jae-Jun Oh1, Heung-Sun Shim2, Young-Kun Shim1, and Doo-Soo Yoon1,2*
1Microzyme R&D Department, 2Chosun College of Science & Technology, Dept. of Bioenvironmental & Chemical Engineering

Previous studies have shown that P. anomala miz-02 isolated from native fermented food produces a fermented product that has an attractant effect on pests that damage crops. The results showed that the induction effect of OFM was 93.3% for the Pochazia shantungensis, which are widespread in various plants including fruit trees, and have a low productivity and sooty mold black mildew. The active ingredients of the fermented composition are Phenyl acetaldehyde, Phenetyl alcohol, and Phenethyl acetate, and they are established as effective ingredients by appearing as various components of fruit and flower like honey, rose, lily, banana and the like. In order to increase the commercial value of the fermentation composition, various formulation and manufacturing methods have been studied and manufacturing process of attracting flat traps has been established which is utilized as a component contained in a flat trap widely used in domestic agricultural pest control market. The Prototype was manufacture under the above conditions and applied to various damage areas in honam area. As a result, the capture efficiency of P. shantungensis was 300% or more on average compared to the control plate trap.
The TOR Pathway Governs Growth and Pathogenicity of Fungal Meningitis Pathogen Cryptococcus neoformans

Yee-Seul So1, Giuseppe Ianiri2, Alex Idnurm2, and Yong-Sun Bahn4*
1Departments of Biotechnology, College of Life Science and Biotechnology, Yonsei University, 2Departments of Molecular Genetics and Microbiology, Medicine, and Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA, 3Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri-Kansas City, MO 64110, USA, 4Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

The TOR pathway has been implicated in regulating cellular responses to nutrients, proliferation, translation, transcription, and autophagy. Here we identified two homologues of S. cerevisiae Tor protein, CNAG_O6642 (Tor1) and CNAG_05220 (Tlk1, TOR-like kinase 1), in Cryptococcus neoformans. Both Tor1 and Tlk1 have rapamycin-binding (RB) domains but Tlk1 has truncated RB form. To study the TOR-signaling pathway, we attempt to construct the tor1 and tlk1 deletion mutant. Although we fail to construct the tor1 deletion mutant, we successfully construct the tlk1 deletion mutant. The tlk1 deletion mutant does not exhibit any discernible phenotypes, suggesting that Tlk1 is dispensable in C. neoformans. The essentiality of TOR1 is independently confirmed by constructing the TOR1 promoter replacement strain by using a copper transporter 4 (CTR4) promoter and the TOR1/tor1 heterozygous mutant in diploid C. neoformans strain background followed by sporulation analysis. To further analyze the function of Tor1, we construct TOR1 overexpression mutant using a constitutively active histone H3 in C. neoformans. We find that the Tor1 overexpression mutant is resistant to rapamycin but the tlk1 deletion mutant does not exhibit any altered resistance to rapamycin. Furthermore, we found that Tor1 is involved in response to diverse stresses, including genotoxic stress, oxidative stress, thermo-stress, antifungal drug treatment, and production of melanin.

Metabolic Engineering of E. coli for Production of Fragrant α-Santalene

Hyo-jung Han1,2, Chonglong Wang1,2, Sinyoung Kim1,2, Ju-eon Park1,2, and Seon-Won Kim1,2*
1Gyeongsang National University, 2Division of Applied Life Science (BK21 Plus) and PMBBRC

Sandalwood oil possesses a very pleasant and long-lasting scent and is widely used in food and cosmetic industries. It is obtained from the heartwood and roots of mature sandalwood trees via steam distillation. This traditional method suffers from low yields and over-harvesting of the trees. α-Santalene represents up to 50% of natural sandalwood oil. It is derived from isopentenyl diphasphate (IPP) and dimethylallyldiphosphate (DMAPP), which are generated via either the MEP pathway or the MVA pathway. Farnesylyrophosphate (FPP) synthase then catalyzes the assembly of IPP and DMAPP to the linear FPP, which undergoes rearrangement and cyclization by santalene synthase to form santalene. Here, we constructed a heterologous biosynthesis pathway for α-santalene production. Manipulation of ribosome binding sites (RBS) or other RNA regulators offer a very effective alternative to tune the expression of multiple genes. A set of synthetic RBSs were utilized to modulate diverse expression levels of FPP synthase and α-santalene synthase for the pathway optimization. By this approach, we have successfully produced α-santalene of > 400 mg/L. Indole synthesis pathway was also removed because indole has been known to inhibit isoprenoids production and have unpleasant odor. Thus, we could produce pure santalene with an enhanced yield. [This work was supported by a grant (NRF-2016R1A2B2010678) from the National Research Foundation, MSIP, Korea.]

Metabolic Redesign of Corynebacteria for Isoprenoid Production Based on Carotenoids

Sinyoung Kim1,2, Hyo-jung Han1,2, Ju-eon Park1,2, and Seon-Won Kim1,2*
1Gyeongsang National University, 2Division of Applied Life Science (BK21 Plus) and PMBBRC

Isoprenoids, composed of IPP as building block, is the largest compound class in nature which has been known above 50,000. Isoprenoid technology is powerful and extensible which can produce a number of high-value compounds by combining stem technology producing IPP and branch technology consisting of target product pathway. IPP is generated either via the MEP pathway or the MVA pathway. As Corynebacteria, a popular industrial strain, harbors endogenous synthesis pathway of carotenoids belonging to the class of isoprenoids. The bacteria will be metabolically engineered to produce various valuable isoprenoids based on metabolic redesign of the carotenoids synthesis pathway. In this study, we enhanced the production of the building block IPP by overexpressing box gene of the endogenous MEP pathway or by introducing the whole MVA pathway into Corynebacterium glutamicum. We constructed lycopene production plasmid by putting heterologous crteBII operon and idl gene into pSGT208 shuttle vector derived from pCES208. We transformed each plasmid into C. glutamicum strain lacking its own carotenoid decaprenoxanthin biosynthesis and used as a lycopene-production strain. By this approach, we have successfully produced carotenoid including lycopene. In addition, co-expressing crtx and BCMO gene into the lycopene-production plasmid was able to produce retinal of >4.3 mg/L. [This work was supported by a grant (NRF-2016M1A2A2924237) from the National Research Foundation, MSIP, Korea.]
**Antibacterial Compound Produced by Bacteria Isolated from Soil**

Gayoung Hong, Sora Kim, Eunjung Roh, Jae-Gee Ryu, Seungdon Lee, and Kyuseok Jung*

*Microbial Safety Team, National Institute of Agricultural Sciences, Rural Development Administration*

These days, antibiotics have been used in agriculture for control of plant diseases and increase the productivity. The abuse of antibiotics, however, can run the risk of creating resistant forms of bacterium. There are many studies to solve the problem of antibiotic resistance. One of them is to discover new antibacterial substance(s) produced by bacteria. Because there are significant number of soil microorganisms which have not studied yet, soil microorganisms have potential to find new antibacterial substance(s).

In this study, the antibacterial activity test of the substance produced by the bacteria (SR111) isolated from the tomato cultivated soil was carried out. Antibiotics were isolated from the culture broth and have been used for stability against heat, pH, Enzyme by spot on the lawn method to characterize the antibiotics. The antibacterial activity was found to be stable at 60°C-100°C for 30 min and in the pH range of 2-10. The antibacterial substance was not affected by carbohydrase.

**Genetic and Biochemical Characterization of a Lignin Degrading Bacterium Ochrobactrum anthropi Strain AM3**

Kishor Sureshbhai Patil1, Seung Je Lee2, and Jong-Chan Chae1*

1Division of Biotechnology, Chonbuk National University, 2Jeonbuk Institute for Food-Bioindustry

Given their immense environmental adaptability and biochemical versatility, bacteria could be used as valuable tool for the rapid degradation of lignin. In an effort to discover aerobic bacteria capable of lignin degradation, we isolated *Ochrobactrum anthropi* strain AM3 from compost which is able to utilize alkali lignin as a sole carbon source under mesophilic conditions. The ligninolytic capability of the isolate was assessed by growth on lignin associated aromatic compounds and degradation of lignin mimicking dyes. Further, ligninolytic potential of strain AM3 was confirmed by enzyme assays for laccase, manganese peroxidase (MnP), lignin peroxidase (LiP).

Using PacBio RS II system, genome sequencing was performed to understand lignin degradation in genetic level. As a result, we reported 5.11 Mb draft genome with G+C content of 56.2%. The genome contained genes encoding oxidases, peroxidases, catalase, laccases, oxygenase and hydrolytic enzymes that are possibly involved in lignin degradation.
Microbial Distribution and Potential Abundances of Anammox Bacteria in the Rice Paddy Soil

Anamika Khanal1,2, Seul Lee2, Hyeri Lee2, Ahyeon Cho2, and Ji-Hoon Lee1,2*
1Chonbuk National University, 2Department of Bioenvironmental Chemistry

An aerobic ammonium oxidation (anammox) bacteria, responsible for mediating unique biochemical process of oxidizing ammonia into dinitrogen gas has been studied for only the past 25 years. These bacteria belonging to phylum Planctomycetes have been reported to play important role in nitrogen production in agricultural soil environments and in oceanic environments. In this study, we have identified the presence of anammox bacteria in the rice paddy soil. The detection, confirmation and evolutionary relationship of anammox bacteria were done on the basis of their genetic composition using molecular techniques such as phylogenetic analysis of 16S rRNA gene through clone library construction and metagenomic analysis. Among the 19 clones taken from the clone library, all of them showed sequence similarities with uncultured bacteria ranging from 78% to 99%. Some clones showed high sequence similarities with Planctomycetes ranging from 97% to 98% and anammox bacteria ranging from 93% to 97%. Clone (PA-20) showed 96% similarity specifically with "Candidatus Kuenenia stuttgartiensis", 90% with "Ca. Brocadia fulgida" and "Ca. Anammoxoglobus propionicus". Microbial community analysis of soil samples (RPS1, RPS2) was performed by using Illumina MiSeq sequencing of bacterial 16S rRNA. The distribution of bacteria at phylum level showed unclassified bacteria was the most abundant at 31.7% and 42.4%, followed by Proteobacteria at 22% and 18% and Planctomycetes at 13% and 7% respectively.

Non-ureolytic Calcium Carbonate Precipitation by Lysinibacillus sp. YS11 Isolated from the Rhizosphere of Miscanthus sacchariflorus

Yun Suk Lee and Woojun Park*
Laboratory of Molecular Environmental Microbiology, Department of Environmental Science and Ecological Engineering, Korea University

Microbially induced calcium carbonate precipitation (CCP) occurs through different metabolic pathways. Although CCP through ureolysis has been widely considered in environmental engineering fields, urea utilization showed sequence similarities with Plasymoebae ranging from 78% to 99%. Some clones showed high sequence similarities with uncultured bacteria ranging from 78% to 99%. Some clones showed high sequence similarities with Plasymoebae. The detection, confirmation and evolutionary relationship of anammox bacteria were done on the basis of their genetic composition using molecular techniques such as phylogenetic analysis of 16S rRNA gene through clone library construction and metagenomic analysis. Among the 19 clones taken from the clone library, all of them showed sequence similarities with uncultured bacteria ranging from 78% to 99%. Some clones showed high sequence similarities with Planctomycetes ranging from 97% to 98% and anammox bacteria ranging from 93% to 97%. Clone (PA-20) showed 96% similarity specifically with "Candidatus Kuenenia stuttgartiensis", 90% with "Ca. Brocadia fulgida" and "Ca. Anammoxoglobus propionicus". Microbial community analysis of soil samples (RPS1, RPS2) was performed by using Illumina MiSeq sequencing of bacterial 16S rRNA. The distribution of bacteria at phylum level showed unclassified bacteria was the most abundant at 31.7% and 42.4%, followed by Proteobacteria at 22% and 18% and Planctomycetes at 13% and 7% respectively.

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Yun Suk Lee and Woojun Park*
Laboratory of Molecular Environmental Microbiology, Department of Environmental Science and Ecological Engineering, Korea University

An Integrative Approach for de novo Genome Annotation in Escherichia coli BL21(DE3)

Sinyeon Kim1, Haeyoung Jeong2, Eun-Youn Kim3, Ji Hyun F. Kim4, Sang Yup Lee5, and Sung Ho Yoon6*
1Department of Bioscience and Biotechnology, Konkuk University, 2Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 3School of Basic Sciences, Hankuk National University, 4Department of Systems Biology and Division of Life Sciences, Yonsei University, 5Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 Plus Program), BioProcess Engineering Research Center, Center for System Biotechnology, Konkuk University, 6School of Basic Sciences, Konkuk University

Escherichia coli BL21(DE3) is one of the most widely studied laboratory strains of bacteria in the field of biotechnology. Here, our study present the latest genomic annotation of E. coli BL21 (DE3). The transcriptome structure has been determined by using this strain for the first time. Genomic sequences were annotated by multiple automatic pipelines including RefSeq, IMG and RAST. We combined and compared the products with up-to-date genome annotation of closely related E. coli K-12. Also, high-resolution tiling array analysis from different stages of growth in a complex and minimal medium focused on determination and/or identification of the transcriptome structure and supportive evidence of the ORFs. An integrated analysis of E. coli BL21(DE3) genome and transcript structure contributed the correction of translation initiation sites for 88 coding sequences and provided up-to-date information on the majority of genes. Moreover, 36 putative genes and 67 putative non-coding RNAs were discovered. The cutting edge genomic reannotation and transcriptome studies of E. coli BL21(DE3) will be used as an essential resource for system modeling and functional genomic analysis and will be applied for biological and industrial technology to enhance the ability of this strain in the near future.

Ahyeon Cho, bacterium might cause environmental problems. In this study, a non-ureolytic CCP widely considered in environmental engineering fields, urea utilization different metabolic pathways. Although CCP through ureolysis has been studied for only the past 25 years. These bacteria belonging to phylum Planctomycetes have been reported to play important role in nitrogen production in agricultural soil environments and in oceanic environments. In this study, we have identified the presence of anammox bacteria in the rice paddy soil. The detection, confirmation and evolutionary relationship of anammox bacteria were done on the basis of their genetic composition using molecular techniques such as phylogenetic analysis of 16S rRNA gene through clone library construction and metagenomic analysis. Among the 19 clones taken from the clone library, all of them showed sequence similarities with uncultured bacteria ranging from 78% to 99%. Some clones showed high sequence similarities with Planctomycetes ranging from 97% to 98% and anammox bacteria ranging from 93% to 97%. Clone (PA-20) showed 96% similarity specifically with "Candidatus Kuenenia stuttgartiensis", 90% with "Ca. Brocadia fulgida" and "Ca. Anammoxoglobus propionicus". Microbial community analysis of soil samples (RPS1, RPS2) was performed by using Illumina MiSeq sequencing of bacterial 16S rRNA. The distribution of bacteria at phylum level showed unclassified bacteria was the most abundant at 31.7% and 42.4%, followed by Proteobacteria at 22% and 18% and Planctomycetes at 13% and 7% respectively.
Substitution of the Native Promoter of an rRNA Operon in *Escherichia coli* to an rRNA Promoter of *Vibrio natriegens* for Accelerate Growth

Kitae Kim, Soon-Kyeong Kwon, and Jihyun Kim*
Yonsei University

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 9.6 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 *E. coli*, we substituted the natural promoters of rRNA operons in *E. coli* K-12 MG1655 with the rna promoter of *V. natriegens*. The lambda red recombination system was used to introduce the rna promoter of *V. natriegens* to *E. coli*; it was recombined to the promoter sites of *E. coli* rna, rmb, rmg, rhm, rna and rmg, or rmb and rmg. The growth rate of the rna 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* rna promoter in rmg. 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.

Optimization of Cultural Conditions for Phosphate Solubilizing by *Kluyvera* sp. CL-2

Ki Moon Bong¹, Min-Kyoung Seo¹,², JaeYoung Song³, and Pyoung Il Kim*¹⁺
¹Bio Control Research Institute, Jeonnam Bioindustry Foundation, ²School of Biological Sciences and Technology, Chonnam National University, ³Department of Agricultural Biology, National Institute of Agricultural Sciences, Republic of Korea

Effective microorganisms are utilized for crop growth promoting, soil development, immune activity and pest control in the agricultural fields. In this study, we investigated culture conditions for high phosphate solubilizing by *Kluyvera* sp. CL-2 in liquid state fermentation. Effective microorganism, *Kluyvera* sp. CL-2, was kindly provided by Korean Agriculture Collection (KACC), Rural Development Administration (RDA), Republic of Korea. Ten carbon sources including glucose were tested to optimize the culture condition and examined the biochemical characteristics of *Kluyvera* sp. CL-2. Also, the amount of phosphoric acid solubilized was measured by vanado molybdc acid method using spectrophotometer. Plackett-Burman design was employed for medium optimization. Optimal medium was ten-fold cheaper than control (tryptic soybean broth, TSB) and the phosphate solubilizing rate in the optimized culture were better than in the TSB culture. [Supported by grants from Rural Development Administration (Project No. PJ012825)]

Optimization of Cultural Conditions for Multifunctional Metabolite Production by *Bacillus velezensis* GH1-13

Min-Kyoung Seo¹,², Ki Moon Bong¹, Kong-Min Kim¹, Sang Yoon Kim¹, Jaekeyeong Song¹, and Pyoung Il Kim*¹⁺
¹Bio Control Research Institute, Jeonnam Bioindustry Foundation, ²School of Biological Sciences and Technology, Chonnam National University, ³Department of Agricultural Biology, National Institute of Agricultural Sciences, RDA, Republic of Korea

For the mass production and field application of *Bacillus* strains in agriculture, it is important to cultivate in a lower cost and higher efficacy using the economical carbon and nitrogen sources. *Bacillus velezensis* GH1-13 was previously reported as multifunctional agent for biocontrol against various phytopathogens and plant growth promoting (PGP) in agriculture. Agricultural bacterium, *Bacillus velezensis* GH1-13, was kindly provided by Korean Agriculture Culture Collection (KACC), Rural Development Administration (RDA), Republic of Korea. The purpose of this study was to develop an economical culture medium for the optimal cell growth and endospore forming by submerged fermentation of *B. velezensis* GH1-13. The optimal carbon and nitrogen sources were determined as maltose and yeast extract, respectively. *Bacillus velezensis* GH1-13 was mass cultivated at 37°C, pH 7.0 for 36 h in 500 L submerged fermenter using the optimal medium. The results showed that bacterial cells of 1.0 × 10^10 cfu/ml and sporulation yield of 90%. The functional metabolites including indole-3-acetic acid (IAA), cytokinin, fengycin, iturin, and surfactin were also investigated from cell-free supernatant of *B. velezensis* GH1-13 after mass culture for 36 h. [This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ012467)” Rural Development Administration, Republic of Korea.]

Protective Effect of BPP-turmeric against *S. Gallinarum* in Chicks

June Bong Lee, Seon Mi Wi, Se Kye Kim, and Jang Won Yoon*
College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University

Our previous studies demonstrated that a bioprocessed polysaccharide (BPP) isolated from *Lentinus edodes* mushroom mycelia cultures supplemented with black rice bran can protect mice against *Salmonella* lipopolysaccharide-induced endotoxemia and reduce the mortality from *S. Typhimurium* infection through upregulated Th1 immunity. Here we report that a BPP from *L. edodes* mycelia cultures supplemented with turmeric (referred to as BPP-turmeric) alters chicken macrophage responses against avian-adapted *S. Gallinarum* and protects chicks against a lethal challenge from *S. Gallinarum*. *In vitro* analyses revealed that the water extract of BPP-turmeric changed the protein expression/secretion profile of *S. Gallinarum* although it was not bactericidal, reduced the phagocytic activity of the chicken-derived macrophage cell line HD-11 when infected with *S. Gallinarum*, and significantly activated the transcription expression of interleukin (IL)-1β, IL-10, tumor necrosis factor-α (TNF-α), and inducible nitric oxide synthase (iNOS), whereas repressed that of IL-4, IL-6, interferon (INF)-γ, and INF-α. We also found that BPP-turmeric (0.1 g/kg feed) as a feed additive provided significant protection to 1-day-old chicks infected with a lethal dose of *S. Gallinarum*. Collectively, these results imply that BPP-turmeric contains biologically active component(s) that protect chicks against *S. Gallinarum* infection. [Supported by the Bio-Industry Technology Development Program No. 314019-3, MAFFA].
**C031**

**Induction of Wolfiporia cocos Fruit Bodies in Different Temperature Conditions**

Jinmo Koo, Sung-Hak Lee, Eun-Hee Kim, Min-Gu Kang, and Woo-Sik Jo*

Gyeongbuk Province Agricultural Technology Administration

Wolfiporia cocos is a widely known traditional medicine in China, Japan, Korea, and other Asian countries due to its various medicinal effect. Aiming to determine the optimum condition for W. cocos fruit body induction, we cultured 10 strains of W. cocos on potato dextrose agar medium (PDA) in different temperature conditions (12, 16, 20, 24, 28°C). KFRI 1105s exceptionally formed fruit body in low temperatures (12 and 16°C), but in other strains, fruit body induction was restricted. In 12 and 16°C, fruit body formation started at 20°C, and formation rate increased proportionally with the temperature increase. 5 strains induced fruit body in 20°C, 7 strains in 24°C, and 9 strains in 28°C. This is the first research to identify a fruit body formation in vitro cultured W. cocos in Korea. This investigation will enable further studies of W. cocos physiology and breeding.

**C032**

**The Conversion of BTEX Compounds by Mixed Microbial Cultures to Polyhydroxyalkanoates**

Yu-Yang Ni, Jang Hun Jung, Dong Hoon Jung, and Young Ha Rhee*

Department of Microbiology and Molecular Biology, Chungnam National University

The current study describes the biosynthesis of polyhydroxyalkanoates (PHAs) from Benzen, toluene, ethylbenzene, and xylene (BTEX) compounds by using a mixed microbial culture (MMC). The enrichment of PHA-accumulating MMC was achieved by periodic feeding with BTEX in a sequencing batch reactor starting from activated sludge. DGGE analysis revealed that the enriched MMC was dominated by the genera Pseudomonas and Sphingobium. The enrichment of BTEX-utilizing bacteria during the SBR process was also supported by PCR detection of some oxygenase gene sequences (xylA, todc1, tbmD). The enriched MMC was inoculated into a mineral medium containing each BTEX compound as the sole carbon substrate. The composition of PHAs synthesized from benzene, toluene, ethylbenzene, and o-xylene were copolymers of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV). Contrarily, PHAs from m- and p-xylenes were polymers consisting of 3HB, 3HV and medium length 3-hydroxyalkanoates. The amounts of PHAs accumulated in the cells from these substrates were in the range of 8-34 wt% and 16% of dry cell weight. In subsequent fed-batch experiments where a mixture of BTEX was constantly supplied to the fermentor at a flow rate of 0.4 ml/L/h, the enriched MMC produced a PHA copolyester consisting of 3HB (74 mol%) and 3HV (26 mol%) with a PHA yield of 1.27 g/L. The present results suggest that the use of MMC is a promising candidate for the biotechnological conversion of BTEX to valuable biopolymers.

**C033**

**Screening and Identification of Bacillus Species Isolated from Traditional Fermented Foods as Potential Probiotics**

Myeong Seon Ryu, Jeong Seon Eom, Hee-Jong Yang, Su-Ji Jeong, Seong-Yeop Jeong, and Do-Youn Jeong*

Microbial Institute for Fermentation Industry (MIFI)

The purpose of this study was to improve the quality and to given potential probiotics properties of traditional fermented soybean product by selected Bacillus strain. We have isolated and selected Bacillus strains with high activities of extracellular enzymes from traditionally fermented food that did not contain gene for Bacillus cereus toxins including cer, hblC, bceT, entFM, nheA, cytK and not produce biogenic amines. They were confirmed as safe resources because of their non-hemolytic activities and non-production of harmful β-glucuronidase, urease, indole, phenylpyruvic acid. These selected strains containing hydrophobicity were survived in acidic (at pH 2.0) and bile salts (at concentration of 0.3%, 0.6% oxgill), and were inhibited antibacterial activity against pathogenic bacteria such as B. cereus and Staphylococcus aureus. The isolated strains were finally identified as Bacillus subtilis and Bacillus amyloliquefaciens by sequence analysis of 16S ribosomal RNA. These results suggest that the strains have high potential for application in functional fermented foods and health-related fermented products.

**C034**

**Inhibitory Effects of Novel Probiotic Bacillus Species from Traditional Fermented Foods against Infection by Pathogenic Bacteria**

Jeong Seon Eom, Myeong Seon Ryu, Hee-Jong Yang, Su-Ji Jeong, Seong-Yeop Jeong, and Do-Youn Jeong*

Microbial Institute for Fermentation Industry (MIFI)

Bacillus spp. produces broad-spectrum antibiotics with a variety of structures such as bacteriocin-like peptide and antimicrobial lipopeptides, and the genetic and biochemical properties of this organism are well understood. The antimicrobial substances produced by Bacillus spp. have low toxicity and high biodegradability, and the organism has been granted generally recognized as safe (GRAS) status. Due to these beneficial properties, Bacillus spp. has widely been used to inhibit the growth of foodborne pathogens, particularly in the production of fermented foods. Thus, Bacillus spp. have been used as probiotics for treating various diseases, including intestinal disorders, and as biological preservatives in the food and agricultural industry. In this study, we investigated the effects of a potential probiotic of antimicrobial substances-producing Bacillus spp. isolated from traditional Korean fermented food, and the inhibition of the growth of foodborne pathogenic bacteria in intestinal epithelial cells. The results showed antimicrobial activity and probiotic characteristics of Bacillus spp. and co-incubation of pathogenic bacteria such as S. Typhimurium and Bacillus spp. with intestinal epithelial cells suppressed S. Typhimurium infection. These data indicated that Bacillus spp. has probiotic properties, and can inhibit pathogenic bacterial infection of intestinal epithelial cells.
Isolation of *Paenibacillus amyloticus* JS3-20 with Antimicrobial Activity

Eui Jin Chung, Ahyoung Choi, Jung Moon Hwang, and Gang-Guk Choi*

Nakdonggang National Institute of Biological Resources

The goal of this research is a selection of antimicrobial bacteria against antibiotics-resistant bacteria by isolation bacteria in soil. The bacterial community were examined by culture-dependent analyse. Soil sample was collected in the Geumoreum on Jeju Island. Total 33 bacteria were isolated from the sample. The analyses of 16S rRNA gene sequences showed that the bacteria were composed of 29 genus and 33 species. The antimicrobial activity was determined by an in vitro bioassay on Nutrient agar. Strain JS3-20 showed antimicrobial activity against methillin-resistant *Staphylococcus aureus* KCCM 40510 with inhibition zones from 2 to 3 mm. Strain JS3-20 grew optimally at 20–35°C and pH 7.5–10.5. Comparative 16S rRNA gene sequence analyses showed that the strain was most closely related to *Paenibacillus amyloticus* NRRL NRS-290 (99.4% 16S rRNA gene sequence similarity). We will perform a study on culture condition standardization of strain JS3-20 for purification of antimicrobial compound.

Development and Evaluation of rDNA-NTS/CRE-based Gene Integration Vectors as A Multiple Marker-Recycling System in *Saccharomyces cerevisiae*

Gyu Hun Sim, Hye Yun Moon, and Hyun Ah Kang*

Department of Life Science, College of Natural Science, Chung-Ang University

In this study, we developed the rDNA-NTS/CRE system, by combining the rDNA-NTS integration system with the Cre-lox system, and evaluated its potential as a marker-recycling multiple integration system in *Saccharomyces cerevisiae*. The rDNA-NTS/CRE vectors, rDNA-NTS-lox-ThMG1 and rDNA-NTS-lox-NNV-CP, were constructed to contain the expression cassettes for a truncated 3-hydroxyl-3-methylglutaryl-CoA reductase (tHMG1) and a capsid protein of red-spotted grouper necrosis virus (RGNNV-CP), respectively. With the multiple integrant of rDNA-NTS-lox-ThMG1, all the ura3 markers were pop out efficiently during cultivation on YPD plates, which allows a basic level expression of Cre recombinase from the GAL1 promoter. In contrast, with the multiple integrant of rDNA-NTS/lox-NNV-CP, the removal of multiple ura3 markers was possible only by prolonged cultivation in the presence of galactose. However, the pop-out of the multiply integrated expression cassettes, mostly leaving only one or two copies, was also observed in both ThMG1 and RGNNV-CP integrants. Optimization of Cre expression by cultivation on different concentrations of galactose was shown to be as a strategy to partly overcome this problem. Altogether, our results suggest that despite efficient removal of multiply integrated selection markers, the use of Cre-lox for marker recycling in the rDNA-NTS integration system is limited due to high frequency of simultaneous pop-out of expression cassettes integrated at the rDNA cluster.

Construction of Conditional Cell-wall Mutants by Modulating OCH1 and CHS3 Expression in *Saccharomyces cerevisiae*

Van Trinh Luu, Hye Yun Moon, and Hyun Ah Kang*

Department of Life Science, College of Natural Science, Chung-Ang University

Saccharomyces cerevisiae is widely used as host for production of recombinant proteins and metabolites with industrial potential. However, its thick and rigid cell wall presents problems in recovery of products. In this study, we modulated the expression of OCH1, encoding α-1,6-mannosyltransferase, responsible for outer chain biosynthesis of N-glycans, and CHS3, encoding chitin synthase III, required for synthesis of the majority of cell wall chitin, by exploiting the repressible MET3 promoter. The constructed mutant strains showed increased sensitivity to Hygromycin B, high temperature, and Calcofluor white (CFW) when methionine was added, while the growth defects were fully recovered by supplementation with 1M sorbitol. The osmotic lysis of the conditional mutants, especially ScMET3(p)-Scoch1 and ScMET3(p)-Scoch1/ScMET3(p)-Scchs3 constructs, was evident in the presence of methionine. The alteration of cell wall integrity in the mutants was also observed by CFW staining. The mutant strains were shown to release significantly the intracellularly expressed recombinant capsid proteins when cultured with methionine. Furthermore, the extraction of squalene by osmotic lysis was achieved with a high efficiency in the mutant yeast cells expressing N-terminal-truncated HMG1-CoA reductase. Our data support that the conditional cell-wall mutants, based on the modulation of OCH1 and CHS3 expression, are useful hosts with enhanced release of recombinant proteins and metabolites.

Analysis of the Secretome of *Pediococcus pentosaceus* SL4, a Kimchi Lactic Acid Bacterium

Jin-young Lee, Min-Jung Kwak, and Jihyun F. Kim*

Department of Systems Biology and Division of Life Sciences, Yonsei University

Pediococcus pentosaceus SL4 was isolated from kimchi, a Korean fermented vegetable product, and is a member of lactic acid bacteria that are classified as generally regarded as safe. Its whole-genome sequence was reported recently, to reveal that it possesses a 1,789,138-bp circular chromosome with 1,709 coding sequences and a number of plasmids. In this study, the genome sequence of *P. pentosaceus* SL4 was analyzed using SignalP 4.1 (DTU CBS), and 57 kinds of signal peptides were detected (score > 0.45). Its secretome separated from the culture supernatant was analyzed using LC-MS/MS, and proteins having high levels of expression and secretion were identified. The secretory signal peptides from these proteins were analyzed for their capabilities to secrete heterologous proteins. The secretory signal peptides of *P. pentosaceus* SL4 that have been elucidated from this study will be useful for development of lactic acid bacteria as a vehicle in the drug delivery system. [This work was supported by the WC300 R&D program (S2416717) funded by the Small and Medium Business Administration (SMBA, Korea)]
Engineered Attenuated Salmonella Kills Cancer Cells through Activating Endothelium Reticulum Stress

Shuang Gao1,2, Shunemi Lin2, Sangyong Lin2, and Hyon E. Choy1,3*
1Department of Microbiology, Chonnam National University Medical College, 2Research Division for Biotechnology, Korea Atomic Energy Research Institute

Since current chemo- and radiation-therapeutics for cancer patients have limitations including toxicity, poor tumor targeting, and inadequate tissue penetration, Salmonella cancer targeting techniques have been considered as alternative therapeutic agents. However, in order to use Salmonella as a cancer targeting weapon, it should be attenuated enough to be safe for the patient and has an efficient delivery system of anticancer molecules in the site of tumors. We first constructed a highly attenuated Salmonella strain LT IrΔptsIcr (KST0650) using the combination of genetic manipulation and radiation mutation technology. In mice sepsis model, we found that its LD50 in mice sepsis model was about 1×10^6 CFU which was slightly less than Salmonella ΔppgG strain (KST0651) which has been most widely used in cancer therapy. PCR, its invasion and replication in cancer cells was significantly higher than KST0651 indicating that its ability to manipulate in the site of tumor might more efficient than KST0651 strain. We found Salmonella pathogenesis island (SPI)-2 expression of KST0650 was actively detected as much as the level of WT strain. In addition, KST0650 preferentially accumulated in tumor tissue as compared with normal organs. Thus, we constructed a well-known apoptosis inducing protein, ATF6 fused with a SPI secretory signal peptide, SspH1 and examined its secretion and induction of apoptosis in cancer cell line.

The Study on Pathogenic Bacteria in Coral, Scerlonepthya gracillimum

Seonock Woo
Korea Institute of Ocean Science & Technology

Over the last 30 years, coral reef assessment provided an extensive description of certain responses at population and community levels. However, with only these descriptive approaches for assessment are incapable of identifying the causes of deterioration of coral reef ecosystems. Most of physiological measurements do not identify the stressors or the underlying molecular mechanisms controlling a response. Some microorganisms have been identified as pathogenic agents responsible for various outbreaks of coral disease. But most of diseases have been implicated within a subset of corals, leaving differential in our knowledge of the host range and geographic extent of a given pathogen. In this study, we purposed the rapid and inexpensive detection of pathogens in coral species using PCR-based assays. Total 4 pathogen-specific 16S rDNA primer sets and 1 Vibrio cholerus Lux R gene primer set were designed for detecting coral pathogenic disease. The pathogenic bacteria used in this study are Aurantimonas coralicida, Serratia marcescens, Vibrio shiloni, Vibrio corallilyticus, and Vibrio carlheriae. The assay was applied to coral samples from the Jeju Island, Japan and Taiwan (Jeju Island -Sunggan, Munsum, Japan - Wokayama, Kochi, Taiwan - Green Island, Kenting). After the identification of infected samples, the PCR-based assay is to be evaluated for detection of presence of pathogenic bacteria in corals.

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High Prevalence of Mycobacterium avium subspp. paratuberculosis in Wild Ducks in the Middle Area of South Korea

Haerin Rhim1, Ji-Eun Bae2, Hong-Chul Kim2, Yong-Hi Cho3, Hye-Jin Jang3, Ki-Jeong Na3, and Jae-ik Han1*
1Laboratory of Wildlife Medicine/Diseases, College of Veterinary Medicine, Chungbuk National University, 2Department of Animal Science and Technology, Sunchon National University, 3Veterinary Laboratory Medicine, College of Veterinary Medicine, Chungbuk National University

Since the first description of Mycobacterium avium subspecies paratuberculosis (MAP) in 1895, it is known to cause paratuberculosis or Johnne’s disease in domestic and wild ruminants as well as wild Canidae, Mustelidae, and Herpestidae. Recent studies have also indicated the potential etiological role of MAP in the pathogenesis of Crohn’s disease in human, suggesting the zoonotic potential of the organism. Fecal samples were collected from 49 wild Spot-billed ducks and 79 Mallard. To demonstrate the presence of the MAP genome in the samples, quantitative real-time PCR assays specific for the IS900, F57, and ISMAP02 genes of MAP, respectively, was performed. Samples with positive signals in the assays were re-evaluated in 3 ways (gel electrophoresis, sequencing and nested PCR targeting different region of ISMAP02 gene). A type strain of MAP (ATCC 19698) was used as positive control. All tests revealed that 44 of the samples (34.4%) were positive for the MAP. When considering that the MAP is transmitted by ingestion of the organism from the feces of infected animals or contaminated food and water, the results of this study suggest that the wild ducks can be a reservoir of the disease transmission to human or other animals because of the long latent period between infection and the first signs of disease becoming apparent.

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Essential Oils and Eugenols Inhibit Biofilm Formation and the Virulence of Escherichia coli O157:H7

Yong-Guy Kim, Jin-Hyung Lee, and Jintae Lee*
School of Chemical Engineering, Yeungnam University

Enterohemorrhagic Escherichia coli O157:H7 (EHEC) has caused foodborne outbreaks worldwide and the bacterium forms antimicrobial-tolerant biofilms. We investigated the abilities of various plant essential oils and their components to inhibit biofilm formation by EHEC. Bay, clove, pimento berry oils and their major common constituent eugenol at 0.005% (v/v) were found to markedly inhibit EHEC biofilm formation without affecting planktonic cell growth. In addition, three other eugenol derivatives isoeugenol, 2-methoxy-4-propylphenol, and 4-ethylguaiacol had antibiofilm activity, indicating that the C-1 hydroxyl unit, the C-2 methoxy unit, and C-4 alkyl or alkane chain on the benzene ring of eugenol play important roles in antibiofilm activity. Interestingly, these essential oils and eugenol did not inhibit biofilm formation by three laboratory E. coli K-12 strains that reduced curli fimbriae production. Transcriptional analysis showed that eugenol down-regulated 17 of 28 genes analysed, including curli genes (csgABDFG), type I fimbriae genes (ftmCDH) and ler-controlled toxin genes (espD, esc, escR, and tir), which are required for biofilm formation and the attachment and effacement phenotype. In addition, biocompatible poly(lactic-co-glycolic acid) coatings containing clove oil or eugenol exhibited efficient biofilm inhibition on solid surfaces. In a Caenorhabditis elegans nematode model, clove oil and eugenol attenuated the virulence of EHEC.

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D005

Inhibition of Human Cytomegalovirus Immediate-early Gene Expression and Replication by Ethyl Acetate (EtOAc) Fraction of Elaeocarpus sylvestris in vitro

So-Hee Bae, Jung-Eun Kim, and Yoon-Jae Song*
Gachon University

We have previously reported that seventy percent ethanol extract of Elaeocarpus sylvestris (ESE) inhibits human cytomegalovirus (HCMV) replication in vitro. In the present study, we investigated the solvent fraction of ESE that inhibits HCMV replication by using activity-guided fractionation. Among the tested solvent fractions, the EtOAc fraction of ESE significantly reduced HCMV lytic gene expression and viral replication in vitro without exhibiting any significant cytotoxic effect against human foreskin fibroblasts (HFF). Furthermore, the EtOAc fraction of ESE negatively affected HCMV major immediate-early (MIE) enhancer/promoter activity. These data indicate that the EtOAc fraction of ESE contains active constituents that inhibit HCMV MIE enhancer/promoter activity and viral replication.

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D006

Increased Uptake of Chloramphenicol by 4-Hydroxybenzaldehyde in Acinetobacter baumannii

Bora Shin1, Chulwoo Park2,3, James A Imlay2, and Woojun Park1,4*
1Laboratory of Molecular Environmental Microbiology, Department of Environmental Sciences and Ecological Engineering, Korea University, 2Department of Microbiology, University of Illinois, Urbana, Illinois, USA

Bacterial metabolism modulated by environmental chemicals could alter antibiotic susceptibility. 4-Hydroxybenzaldehyde (4-HBA), which cannot support the growth of Acinetobacter baumannii, exhibited synergism only with amphenicol antibiotics including chloramphenicol (CAM) and thiamphenicol. Interestingly, this synergistic effect was not observed with other growth-supporting, structurally similar compounds such as 4-hydroxybenoate. Transcriptomic and qRT-PCR analyses demonstrated that genes involved in protocatechuate metabolism (pca genes), osmotic stress (bet genes) were significantly up-regulated by 4-HBA and CAM treatment. The 14C-labeled CAM influx was lower in a pcaK1 (encoding a transporter of protocatechuate) deletion mutant and was higher in the pcaK1 overexpressing cells relative to that in the wild type upon 4-HBA treatment. The amount of 4-HBA in the culture supernatant was, however, unaffected during the test conditions, validating that it was not metabolized by the bacteria. CAM resistant A. baumannii cells derived by serial passages through CAM-amended media exhibited lower level of pcaK1 gene expression. These results led us to conclude that the activation of PcaK1 transporter is probably linked to cellular CAM susceptibility. This is the first report showing a relationship between CAM influx and aromatic compound metabolism in Acinetobacter baumannii.

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D007

Development of MERS-Cov Spike and Nucleocapsid-based Recombinant Proteins and Their Application to Indirect and Blocking ELISA System

Ji Yeong Noh1,2, Min-Ju Ahn1,2, Min-Chul Jung1,3, Sun-Woo Yoon1,3, Hye Kwon Kim1, and Dae Gwin Jeong1,4*
1Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, 2Department of Veterinary Medicine, Chungbuk National University, 3Department of Bioscience, University of Science and Technology

The first case of MERS in South Korea occurred on May 2015. Subsequently, 186 confirmed cases and 38 deaths were reported. Development of the serologic diagnosis can be a good solution for inexpensive and simple diagnostic techniques to monitor the risk of recurrence of MERS. In this study, we amplified MERS-Cov spike (RBD, S1, and S2) and nucleocapsid (ΔNC) genes by PCR and cloned into vectors. Cloned pET28a (+)-ΔNC gene was expressed in E. coli BL21(DE3) Rosetta with 0.2 mM IPTG. Cloned pAcGP67a-RBD, S1 and S2 genes were expressed in Baculovirus. These antigens were used for optimize the condition of ELISA kits. By using human sera from MERS patients, these kits were compared with a commercial one.

In indirect ELISA, the optimized conditions are RBD 100ng with 1/40 diluted human serum, S2 200ng with 1/40, and ΔNC 400 ng with 1/20. 12 μg/ml of 2nd anti-human IgG(HRP) is required. In blocking ELISA, the optimized condition is 200 ng of ΔNC with 1/10 diluted human serum and HRP conjugated anti-ΔNC mouse IgG 25 ng/ml. The comparison result showed that the detection capability of newly developed ELISA kit based ΔNC is 4 times higher that of the commercial one. Therefore, this study demonstrated the recombinant proteins and ELISAs were effective to estimate serological responses against MERS-Cov infection. They can be further validated with human clinical samples to apply to the clinical field.

[This study was supported by KNIH (grant no. 2016-ER4B01-00).]
Shiga toxins (Stxs) produced by Shigellaserotype 1 and select serotypes of E. coli are most potent known virulence factors. To date, although numerous studies have been reported defining the apoptotic responses to the Stxs, the potential significance of Stxs-induced apoptosis following the intoxication are unknown in human retinal epithelial (RPE) cells. We explored the use of the RPE cells as an in vitro model of Stx-induced retinal damage. Here, we firstly report, to the best of our knowledge, that intoxication of RPE cells with Stxs activates apoptotic cell death signaling via ER stress response. In live-cell analysis, fluorescently labeled Stx B-subunits were internalized and routed to ER. RPE cells were sensitive to wild type Stxs by 72 h, while the cells survived enzymatically deficient mutant toxins challenge. Upon exposure to Stxs, RPE cells showed activation of caspase-dependent apoptotic cell death causing lowering of mitochondrial transmembrane potential (ΔΨm) with increased ER stress sensors. Finally we demonstrated that treatments of the RPE cells with Stxs resulted in activation of JNK and p38 MAPK, suggesting ribotoxic stress response may be triggered. Thus, these data support the ocular involvement in Stxs-induced apoptosis. In the RPE cells, evaluating the basis of the apoptotic responses to the Stxs may reveal unique functional patterns of the cytotoxic actions of these toxins on ocular system.

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Effects of Probiotics and Sodium Butyrate on Atopic Dermatitis

Jeong A Kim1, In sung Kim1, Da Yoon Yu1, Yeon Hee Hong1, Sung Hak Kim2, and Kwang Keun Choi*1
1Department of Animal Resources Technology, Gyeongsang National University of Science & Technology, 2Department of Animal Science and Biotechnology, Chonnam National University

To investigate the effects of probiotics and sodium butyrate on atopic dermatitis, the experimental animals were randomly divided into 5 groups: the control group (C), the negative control group (N), the probiotics group (T1), the sodium butyrate group (T2), and the probiotics + sodium butyrate group (T3). The effects of probiotics and sodium butyrate on atopic dermatitis relief showed decreased ear thickness, reduced plasma histamine, and increased serum IL-10. In addition, the probiotics and sodium butyrate increased the differentiation of TH1 and Treg cells and decreased the differentiation of TH2 and TH17 cells. The analysis of intestinal microorganisms by pyrosequencing showed that the ratio of Firmicutes/Bacteroidetes in the probiotics and sodium butyrate groups increased. Eubacterium belonging to Clostridia clust XIVa increased in the T3 group as compared to the control, while Clostridium belonging to Clostridia clust XIVa and IV increased in T1 and T3 groups as compared to the control. The qPCR analysis revealed that faecalibacterium prausnitzii spp. increased in T2 group as compared to the control, and Bifidobacterium spp. and Weissella cibaria increased in the probiotic and sodium butyrate groups. Therefore, our results demonstrate that the probiotics and sodium butyrate have immunomodulatory effects through the changes in intestinal microorganisms.

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

Mi Seon Jang1, Manki Song2, Yun-ji Jang3, Seung Hyun Han1, and Jae Seung Yang1*
1Clinical Immunology, Clinical Research Lab. Science Unit, International Vaccine Institute, 2Department of Oral Microbiology and Immunology, DRI, and BK21 Plus Program, School of Dentistry, Seoul National University

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. Interestingly, colonization of wild type V. cholerae O139 was not affected by growth condition. However, capsule-deficient strain of O139 grown anaerobically showed significant enhancement of colonization in epithelial cells, suggesting that capsule may interfere with exposure of adhesion factor induced by anaerobic growth. Collectively, our data indicate that V. cholerae upregulate expression of colonizing factors in anaerobic condition similar to intestinal environment.

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Identification of Anti-TB Agent from Newly Assembled Drug Library

Jinsun Jeong¹, Seung Heon Lee¹, Vincent Delorme¹, and Jihan Jang¹*

¹Molecular Mechanisms of Antibiotics, Division of Applied Life Science (BK21plus program), Gyeongsang National University, ²Korean Institute of Tuberculosis, ³Tuberculosis Research Laboratory, Institut Pasteur Korea

Tuberculosis (TB) is a strong infectious disease mainly caused by tubercle bacilli. However, current drug regimen is not effective to eradicate the bacilli that are showing several anti-TB drug resistance such as multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB). Therefore, novel drug discovery that can treat MDR/XDR TB is urgently needed for public health. In this study, we tried to find new drug candidate against drug resistant Mycobacterium tuberculosis. For this, a novel drug library set was assembled and screened by resazurin microtiter assay (REMA) plate method. In our screen, we could narrow down 54 hits and identify novel 9 hits that have not been reported yet. Our novel hit (REMA) plate method. In our screen, we could narrow down 54 hits and identify novel 9 hits that have not been reported yet. Our novel hit candidates exhibited excellent bacterial killing activity in vitro and intracellular model of infection. Furthermore, our hits also showed very good killing activity to MDR/XDR clinical isolates.

Verification of Proper Vaccination Time for Porkers with Foot-and-mouth Disease (FMD) Vaccine

Ah-Young Kim¹, Hyejin Kim¹, Minhee Kwon¹, Soohyun Bae¹, Byounghan Kim¹, and Young-Joon Ko¹*
¹Animal and Plant Quarantine Agency, ²College of Veterinary Medicine & Animal Disease Intervention Center, Kyungpook National University

Double vaccination with an interval of 4 weeks at ages between 2 and 3 months has been recommended for FMD-susceptible domestic animals in Korea. However, fattening pigs are vaccinated only once between 8 and 12 weeks of age because of the side effect such as injection-induced granulomas. Therefore, this study was aimed to verify the most proper time of single vaccination for fattening pigs with the currently used FMD vaccine consisting of 2 strains of serotype O virus. Pigs were divided into four groups and vaccinated at the ages of 8 weeks, 10 weeks, 12 weeks, and 14 weeks, respectively. All pigs were regularly bled after vaccination until 24 weeks. Antibody levels against FMDV were detected using commercial type O enzyme-linked immunosorbent assay. Although pigs vaccinated at 8 weeks (group I) had the highest maternally derived antibody level at the vaccination point, they exhibited higher vaccine-induced antibody level than the other groups (p< 0.05). Double vaccination is required to completely protect finishing pigs from FMD virus infection with the current FMD bivalent vaccine. However, the age of 8 weeks can be considered as the proper time for piglet single vaccination.

Evaluation for Inactivation of Foot and Mouth Disease Virus

Jae-Seok Kim¹, Ah-Young Kim¹, Hyejin Kim¹, Jung-Min Lee¹, Minhee Kwon¹, Soohyun Bae¹, Byounghan Kim¹, and Young-Joon Ko¹*
¹Animal and Plant Quarantine Agency, ²College of Veterinary Medicine & Animal Disease Intervention Center, Kyungpook National University

Inactivation of the foot-and-mouth (FMD) virus was done by formalin initially. However, it was revealed that the formalin treatment is not highly effective for virus inactivation. Subsequently, binary ethyleneimine (BEI) was found as an effective inactivation reagent for FMD virus. The validation of BEI is essential to ensure the quality of the inactivating agent and the validity of the process. In this experiment, the inactivation kinetics of FMD virus O (Jincheon strain) were determined for different concentrations of BEI (0 mM, 0.5 mM, 1 mM, 2 mM, and 3 mM), reaction times (1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 24 h) and temperature (37°C and 26°C). The result showed that FMD virus inactivation times were reduced depending on the increases of BEI concentration. The difference according to the temperature was also appeared. BEI at 3 mM was able to completely inactivate the FMDV within 8 h in 37°C. On the other hand, in 26°C, FMD virus was start to be inactivated from 15 h. This study can be used as a guideline for routine procedures for validating the quality of BEI and the inactivation process.

A Comparison of Methods for Purification and Concentration of Foot-and-mouth Disease Virus

Hyejin Kim¹, Ah-Young Kim¹, Jae-Seok Kim¹, Jung-Min Lee¹, Minhee Kwon¹, Soohyun Bae¹, Byounghan Kim¹, Choyu Park², and Young-Joon Ko¹*
¹Animal and Plant Quarantine Agency, ²College of Veterinary Medicine & Animal Disease Intervention Center, Kyungpook National University

Foot-and-mouth disease (FMD) is a highly contagious viral disease, which cause substantial economic loss to livestock. However, little is known about the efficient method to study the purification and concentration of FMD virus-like particles, 146S, including the removal of non-structural proteins (NSPs). The 146S particles of FMD virus (FMDV) were concentrated by dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The purified 146S particles were confirmed by transmission electron microscopy (TEM) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The results of the current study revealed that 146S particles concentrated by PEG showed the highest purity. Thus, PEG concentration is proposed to a reliable and efficient method for FMDV purification.
Chromatographic Analysis of Foot and Mouth Disease Virus

Jung-Min Lee1, Ah-Young Kim1, Hyejin Kim1,2, Jae-Seok Kim1, Minhee Kwon1, Soohyun Bae1, Byounghan Kim1, and Young-Joon Ko1

1Animal and Plant Quarantine Agency, 2College of Veterinary Medicine & Animal Disease Intervention Center, Kyungpook National University

To distinguish the infected animals with vaccinated ones, non-structural protein (NSP) should be eliminated from the FMD vaccines. Herein, different types of columns were tested to find the suitable column for 146S isolation. In this study, we used five different columns for analyses of foot and mouth disease virus purification. Five types of columns were used for AKTA purifier150 chromatographic system. With resultant fractions of each column, 146S quantitative sucrose density gradient analysis was done. Among five columns used for chromatographic analyses, a few columns exhibited marked isolation between NSP and structural protein (146S). Each fraction was concentrated to confirm if 146S was found on the SDS page. Other columns were not available because both NSP and SP were detected in the same fractions. Herein, chromatographic purification of 146S from crude FMD viruses was confirmed using a suitable column (confidential before publication) with general phosphate buffer. It will be applicable to the foot and mouth vaccine production process.

Lipoteichoic Acid of Lactobacillus plantarum Inhibits Poly I:C-induced IL-8 Production in Porcine Intestinal Epithelial Cells

Seok-Seong Kang

Department of Food Science and Biotechnology, Dongguk University

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. This study demonstrated that lipoteichoic acid of Lactobacillus plantarum (Lp.LTA) confers anti-inflammatory responses in porcine small intestinal epithelial cell line, IPEC-J2 cells. A synthetic analog of double-stranded RNA of viruses, poly I:C significantly induced IL-8 production at both mRNA and protein levels in IPEC-J2 cells. A synthetic analog of double-stranded RNA of viruses, poly I:C significantly induced IL-8 production at both mRNA and protein levels in IPEC-J2 cells. Poly I:C-induced IL-8 production from L. plantarum, exclusively suppressed poly I:C-induced IL-8 production. Comparing with LTA from other probiotic Lactobacillus strains, Lp.LTA had more potential to suppress poly I:C-induced IL-8 production. In addition, dealanoylated Lp.LTA did not suppress poly I:C-induced IL-8 production, suggesting that D-alanine of Lp.LTA is responsible for the inhibitory potential. Furthermore, Lp.LTA attenuated the phosphorylation of ERK and p38 kinase as well as the activation of NF-κB, resulting in. Taken together, Lp.LTA is revealed as an effector molecule to attribute to the inhibitory effect of viral-induced inflammatory responses in porcine small intestinal epithelial cells.

Recombinant BCG to Avoid Innate Immunity in Bladder Cancer

Subin Jin, Young Mi Whang, and In Ho Chang

School of Urology, Chung-Ang University

BCG is an attenuated live vaccine that has been reported to reduce recurrence rate of bladder cancer in 1976 and has been widely used for nonspecific immunotherapy. However, BCG is recognized as a pathogen in the aseptic environment of the bladder, which activates the innate immune response and induces the degradation of BCG by increasing the expression of antimicrobial peptides such as HBD2, HBD3, and CAMP. We have selected three genes from other bacteria that are resistant to the antimicrobial peptide. It is the dltA, ideS and sic gene.

We inserted the three genes into the pMV306hsp vector, which can be expressed in BCG, and transfected into BCG using an electroporator. Recombinant BCG was cultured in 7H9 both media containing kanamycin, and only recombinant BCG was selectively cultured. We are confirmed expression of each gene by extracted RNA in recombinant BCG. First, the extracted RNA was synthesized with cDNA, and it processed with PCR using specific primers of each gene. Recombinant BCG inhibited growth of bladder cancer cells compared with BCG and enhanced secretion of IL-6 which related to the immunotherapy of BCG. In addition, we are confirmed that the internalization of bladder cancer cells was further increased compared to BCG.

We have made recombinant BCG resistant to antimicrobial peptides. Recombinant BCG is expected to be an effective treatment for patients with bladder cancer by administrating small amounts of BCG and reducing side effects.

Lipid Composition of Membrane and Bacteria-host Interaction

Soo-Kyoung Kim1,2, Xi-Hui Li1,2, and Joon-Hee Lee1,2

1Department of Pharmacy, 2College of Pharmacy, Pusan National University

Omithine lipids (OLs) are bacteria-specific lipids that are widely found in outer membrane of many Gram (-) bacteria, but not detected in Eukarya and Archaea. Some bacteria produce OL to change the membrane composition in certain unusual situations like phosphate-limiting condition. Pseudomonas aeruginosa has olsBA operon for the OL biosynthesis to meet the phosphate limitation. We addressed how the OL production and resulting change of the lipid composition modulate the virulence of P. aeruginosa during the infection to host cells. The elevated level of OL in membrane of P. aeruginosa increased hydrophobicity and positive charge of cell surface, which resulted in significant change of susceptibility of P. aeruginosa cells to antibiotics and host immunity, such as antimicrobial peptides and macrophages. OLs reduced the production of inflammatory factors such as iNOS, COX-2, PGE2, and NO production in host cells. Also, OL can increase the resistance to antimicrobial peptides such as LL-37, magainin, and defensin. The increase of OL content in P. aeruginosa modifies virulence by changing the cell surface property.
Biofilms are microbial sessile communities characterized by cells that are attached to a substratum or interface or to each other, are embedded in a self-produced matrix of extracellular polymeric substances and exhibit an altered phenotype compared to planktonic cells, such as high infectivity, antibiotic resistance, and strong survivability. Currently, most persistent bacterial infections are associated with antibiotic-resistant biofilms of pathogenic bacteria. Quorum sensing (QS) is a key regulation system that induces a large number of virulence genes. Pseudomonas aeruginosa is an opportunistic human pathogen whose biofilm formation and QS regulation cause great losses in many industrial facilities and serious infections in humans. Therefore, controls of the P. aeruginosa biofilm and QS response are a very important issue in medicine, public health, and industry. We have screened a series of novel synthetic compounds for anti-biofilm and anti-QS activities. We found that several synthetic compounds such as MHY384, MHY1329, MHY1339, and so on had a significantly modulating activity on the biofilm formation and QS of P. aeruginosa. We suggest that these novel biofilm- and quorum sensing-modulating compounds are promising molecules for inhibition/disruption of biofilms and virulence factors formed by P. aeruginosa.

Probiotic Effect of Attenuated Pseudomonas aeruginosa in Brine Shrimp

Tae-Hyeon Kim, Mi-Nan Lee, Soo-Kyoung Kim, Xi-Hui Li, and Joon-Hee Lee
1Department of Pharmacy, 2College of Pharmacy, Pusan National University

Pseudomonas aeruginosa, an opportunistic pathogen causes various infections in plants, animals, and humans by expressing various virulence factors. However, here we show that if attenuated, P. aeruginosa could have a probiotic effect on an animal, providing better growth and resistance to pathogen. In this study, brine shrimp (Artemia salina) was used to a host model organism to find out probiotic effect of the virulence-attenuated P. aeruginosa. Both P. aeruginosa and Vibrio vulnificus have significant virulence to brine shrimp, killing the nauplii in a few days in a dose-dependent manner, but the virulence of V. vulnificus to brine shrimp was stronger than that of P. aeruginosa. We found that the brine shrimp surviving from the infection with P. aeruginosa or V. vulnificus were grown better and developed faster than no infection control. A protease IV-deficient mutant (piv mutant) of P. aeruginosa that has much less infectivity was also able to endow brine shrimps with better growth and faster development. When fed brine shrimps with the piv mutant before the V. vulnificus infection, the pre-fed brine shrimps became resistant to V. vulnificus significantly. These results strongly suggest that P. aeruginosa can play a probiotic role in the brine shrimp gut and the feeding brine shrimps with attenuated P. aeruginosa may be beneficial.

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

Yoontak Han, Eunna Choi, and Eun-Jin Lee
Department of Genetic Engineering and Graduate School of Biotechnology, Colleges of Life Sciences, Kyung Hee University

Salmonella Typhimurium which belongs to enterobacteriaeae is the rod shaped, flagellate, aerobic, gram-negative bacterium and the reason that cause 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 fljC gene. 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 fljC 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 fljC expression. Especially, Type 1 FlIC differs from type 2 FljB in that the former is performed at the time that site-specific inversion occur 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.

Quorum Sensing-dependent Extracellular Activation of Proteases in Pseudomonas aeruginosa

Xi-Hui Li, Jungmin Oh, Soo-Kyoung Kim, and Joon-Hee Lee
1Department of Pharmacy, 2College of Pharmacy, Pusan National University

Protease IV (PIV), a key virulence factor of Pseudomonas aeruginosa is a secreted lysyl-endopeptidase whose expression is induced by quorum sensing (QS). We found that PIV expressed in QS mutant has severe reduction of activity in culture supernatant (CS), even though it is overexpressed to high level. PIV purified from the QS mutant (M-PIV) had much lower activity than the PIV purified from wild type (P-PIV). We found that the propeptide cleaved from prepro-PIV was co-purified with M-PIV, but never with P-PIV. Since the activity of M-PIV was restored by adding the CS of QS-positive and PIV-deficient strain, we hypothesized that the propeptide binds to and inhibits PIV, and is degraded to activate PIV by a QS-dependent factor. In fact, the CS of the QS-positive and PIV-deficient strain was able to degrade the propeptide. Since the responsible factor should be a QS-dependently expressed extracellular protease, we tested QS-dependent proteases of P. aeruginosa and found that LasB (elastase) can degrade the propeptide and activate M-PIV. We purified the propeptide of PIV and confirmed that the propeptide can bind to and inhibit PIV. We suggest that PIV is post-secretionally activated through the extracellular degradation of the propeptide by LasB, a QS-dependent protease.
**D030**

**Next Generation Sequencing to Predict Drug Resistance of Mycobacterium tuberculosis Isolates in Korean TB Patients**

Ji Lee¹, JS Lee¹, BY Jeon², JS Kim³, JH Kim¹, SN Cho²* and SC Kim¹*  
¹International Tuberculosis Research Center, ²Department of Biomedical Laboratory Science, College of Health Science, Yonsei University, ³Ministry of Food and Drug Safety, ⁴School of Food Science and Technology, College of Biotechnology & Natural Resource, Chung-Ang University, ⁵Department of Microbiology and Institute for Immunology and Immunological Disease, Yonsei University College of Medicine

Whole genomes of 123 clinical isolates which consisted of 34 drug susceptible isolates, 14 non-MDR drug resistant, 31 MDR-s and 44 XDR-TB isolates (based on pDST results) were sequenced. Beijing genotype was most abundant (85.4%, 105/123), followed by Euro-American Sub-lineage (8.9%, 11/123) - Table 1. There were 19,538 alterations throughout 123 isolates, and 59 alterations showed in all 123 isolates. These alterations might be common in most of clinical isolates at least Korea or common in other genetic classes, too (specific alterations only in H37Rv). It was necessary to find out those common alterations which show throughout most of genetic clades and exclude from analysis - Table 2.

Table 3 shows different concordances with pDST results in 'High confident resistant' call and 'Any alterations in known drug resistant related genomic regions' call from PhyreSE SNP analysis. This may suggest that some of SNPs which were not included in PhyreSE high confidence SNP calls would be considered their roles in drug resistance and need to be confirmed for their biological functions.

Table 4 shows drug resistant mutations in Korean isolates and shows complicated patterns of mutations including double-, triple mutations.

**D031**

**Complete Genome Sequence of Vibrio coralliilyticus 58 Isolated from Pacific Oyster (Crassostrea gigas) Larvae**

Ji Hyung Kim¹, Hyoun Joong Kim¹, and Se Chang Park*  
¹Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, ²Laboratory of Aquatic Biomedicine, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University

Vibrios are ubiquitous marine bacteria, certain species of which are known to be associated with diseases of marine organisms. Among these, *Vibrio coralliilyticus* has been identified as the causative agent of coral bleaching and bacteriolytic necrosis in oysters. *V. coralliilyticus* 58, formerly reported as *V. splendidus* biovar II 58, was originally isolated from inactiv Pacific oyster (Crassostrea gigas) larvae in Japan. Here, we sequenced and assembled the complete genome of this strain, identifying two chromosomes and one plasmid. The virulence-associated cytolsin/hemolysin and metalloprotease genes were present on the chromosomes of strain 58. In addition, the former was also detected on plasmid pVs58, suggesting that this virulence plasmid may also be associated with the pathogenicity of *V. coralliilyticus*. These data will provide important insights into the biodiversity of this organism and valuable information for the study of virulence factors, facilitating the control of *V. coralliilyticus* infections in aquaculture.

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**D032**

**The Gene Expression by Set1 is Required for Virulence of Candida albicans**

Jueun Kim¹, Shinae Park², Yong-Joon Cho², and Jung-Shin Lee*  
¹Department of Molecular Bioscience, College of Biomedical Science, Kangwon National University, ²Division of Life Sciences, Korea Polar Research Institute

*Candida albicans* is the most common fungal pathogen in human. Although the C. albicans is normal flora for healthy people, it can cause opportunistic infection. The mortality rate of candidemia is about 50 percent even though a patient is taken the antifungal drugs. Because the expression patterns of numerous genes are changed in pathogenesis of *C. albicans*, it is required for the study in terms of transcriptional regulation. Set1 is the histone methyltransferase for H3 at lysine 4 which is indicated for active transcription. It is previously described that set1 deletion mutant shows attenuated virulence and pathogenesis in *C. albicans*. However, it is unclear why Set1 is important for virulence of *C. albicans*. In this study, we performed RNA-sequencing of wild-type and ∆set1 strain to identify the role of Set1 in *C. albicans* pathogenesis. In ∆set1, the 156 genes are down-regulated more than 2-fold. The GO enrichment analysis revealed that the quite number of these genes have oxidoreductase activity. Indeed, the ∆set1 strain is more sensitive to hydrogen peroxide (H₂O₂) or menadione which induces oxidative stress. The survival assay in macrophages indicated that the survival rate of ∆set1 in macrophages is less than wild-type strain. These results show that the Set1 is required for the survival in host cells by regulating the expression of genes whose products defend against an oxidative stress.

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**D033**

**Gpp2 is Required for Cell Wall Integrity and Fungal Virulence in Cryptococcus neoformans**

Won-Hee Jung¹, Jin-Ju Kim², Ye-Eun Son², Sang-Hun Oh², Hye Shin Kim², Jin-Hwan Kwak², Joseph Heitman², Maria E. Cardenas³, and Hee-Soo Park*  
¹School of Food Science and Biotechnology, Kyungpook National University, ²School of Life Science, Handong Global University, ³Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA

Calcineurin is required for temperature stress survival and virulence of human fungal pathogen Cryptococcus neoformans. Previously, we identified 46 calcineurin substrates in human fungal pathogen *C. neoformans* by employing phosphoproteomic analysis. In this study, we characterized one of putative calcineurin substrates Gpp2, a glyceral-3-phosphatase that involved in glycerol biosynthetic pathway. Growth of the Δgpp2-deletion mutant was attenuated compared to the wild type strain in the murine inhalation model. Genetic epistasis analysis discovered that Gpp2 deletion mutant shows attenuated virulence in macrophages compared to the wild-type strain. These results show that the Set1 is required for the survival in host cells by regulating the expression of genes whose products defend against an oxidative stress.
Mon1, subunit of a guanine nucleotide exchange factor, is essential for vacuole trafficking and autophagy processes in eukaryotic system. Herein, we identified and characterized the function of CaMon1, an orthologue of Saccharomyces cerevisiae, in human fungal pathogen Cryptococcus neoformans. Mutation of the mon1 gene resulted in hypersensitivity to thermal stress. In addition, the mon1 deletion mutant exhibited increased sensitivity to SDS and DTT. However, the mon1 deletion mutant showed more resistance to antifungal agent fluconazole. In vivo studies demonstrated that the mon1 deletion mutant was attenuated compared to the wild type strains in Galleria mellonella insect model. Moreover, the mon1 deletion mutant was avirulent in the murine inhalation model. These results propose that Mon1 plays a crucial role in stress survival and pathogenicity in C. neoformans.

D034
The Role of CaMon1 in Fungal Virulence and Stress Survival in Cryptococcus neoformans

Ye-Eun Son1, Hyun-Ji Kim2, Won-Hee Jung3, Joseph Heitman2, Maria E. Cardenas2, and Hee-Soo Park4,5
1School of Food Science and Biotechnology, Kyungpook National University, 2Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA

The Role of Mkt1 in Sexual Reproduction in Cryptococcus neoformans

Ye-Eun Son1, Won-Hee Jung2, Chi Fu3, Tae-Jin Eom3, Joseph Heitman2, Maria E. Cardenas2, and Hee-Soo Park4
1School of Food Science and Biotechnology, Kyungpook National University, 2Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA

Oral Treatment with Probiotics Inhibits the Atopic Dermatitis in Mice

Jin Yi Hyeon, Bo Ram Keum, So Hui Choe, Eun Young Choi, and In Soon Choi
Department of Life Science, Silla University

Allergic diseases have been increasing worldwide over the past several decade. Atopic dermatitis (AD), one of allergic disease, is a chronic and relapsing inflammatory skin disease. To investigate the effects of probiotics mixture on atopic dermatitis, experimental model was induced by atopy-induced cream. After induction of atopic dermatitis, probiotics mixture were administered orally every day for 4 weeks until the end of the study. The AD model increase weight loss, ear thickness, total IgE levels and histopathological inflammation score. In addition, expression of inflammatory cytokine and transcription factor were increased. On the other hand, Oral application of probiotics mixture can attenuated the development of atopic dermatitis in AD mice by suppressing production of the inflammatory cytokines and expression of Transcription factor such as T-bet, GATA-3 and c-maf. The results of this study demonstrated that probiotics mixture exhibit an protective effect in atopic dermatitis-like mouse model.

D036
Oral Treatment with Probiotics Inhibits the Atopic Dermatitis in Mice

Jin Yi Hyeon, Bo Ram Keum, So Hui Choe, Eun Young Choi, and In Soon Choi
Department of Life Science, Silla University

Allergic diseases have been increasing worldwide over the past several decade. Atopic dermatitis (AD), one of allergic disease, is a chronic and relapsing inflammatory skin disease. To investigate the effects of probiotics mixture on atopic dermatitis, experimental model was induced by atopy-induced cream. After induction of atopic dermatitis, probiotics mixture were administered orally every day for 4 weeks until the end of the study. The AD model increase weight loss, ear thickness, total IgE levels and histopathological inflammation score. In addition, expression of inflammatory cytokine and transcription factor were increased. On the other hand, Oral application of probiotics mixture can attenuated the development of atopic dermatitis in AD mice by suppressing production of the inflammatory cytokines and expression of Transcription factor such as T-bet, GATA-3 and c-maf. The results of this study demonstrated that probiotics mixture exhibit an protective effect in atopic dermatitis-like mouse model.

D037
Understanding the Mechanism of Action of the Anti-dandruff Agent Zinc Pyrithione against Malassezia restricta

Minji Park1, Yang Won Lee2,3, and Won Hee Jung4
1Department of Systems Biotechnology, Chung-Ang University, 2Department of Dermatology, School of Medicine, Konkuk University, 4Research Institute of Medical Science, Konkuk University

Dandruff is a global skin disease that has affected almost 50% of population and is known to be associated with Malassezia restricta, which is a lipid dependent yeast and the most frequently isolated fungus from the human skin. To treat the disease, zinc pyrithione (ZPT) has been used as an anti-dandruff ingredient in various anti-dandruff shampoo. There have been several studies investigating the mechanism of ZPT but mainly used a non-pathogenic model yeast Saccharomyces cerevisiae and a different Malassezia species M. globosa. In the current study, we aimed to understand how ZPT inhibits the growth of M. restricta and found that ZPT treatment dramatically increased intracellular zinc levels along with a small increase of intracellular copper. Analysis of transcriptome changes in ZPT treated M. restricta cells were also performed and it suggested that ZPT inhibits Fe-S cluster synthesis in M. restricta, which was similar to what were shown in the study using S. cerevisiae. Apart from above findings, we also observed that ZPT treatment caused significant reduction of expression of lipases, activities of which were thought to contribute to the survival and virulence of M. restricta on the human skin. Overall, the results of our study suggest that at least three inhibitory mechanisms may be associated with action of ZPT against M. restricta: i) increase of intracellular zinc levels, ii) inhibition of Fe-S cluster synthesis, and iii) reduction of lipase expression.

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We isolate and identify bacteria from seawater collected from Jeju coast, to evaluate the antimicrobial activity against the fish pathogenic bacteria. 14 bacterial strains were isolated and identified using physiological, biochemical and molecular tools. Antibacterial activity of all the 14 isolates were screened against four major fish pathogens namely, two Gram-positive: S. iniae, S. parauberis and two Gram-negative: V. anguillarum, E. tarda. Results revealed that among the 14 isolates, MK-11 was found to have antibacterial activity against S. iniae, S. parauberis, V. anguillarum. Particularly, S. iniae was susceptibility with the MIC value of 250 μg/ml. The biochemical and physio-chemical results reveal that MK-11 had the sugar-alcohol disassemble ability of the D-sorbitol and D-mannitol. Also, the utilization of the yeast extract, sorbitol and di-potassium phosphate were noted to be high. The optimum culture condition such as pH and temperature was recorded as pH 6.0, 25°C and along with 1% NaCl which differs from the previous reports particularly in nutrient resolutions. As results of the analysis of 16S rDNA sequences, MK-11 show the high similarity with Paenibacillus polymyxa, P. jamilae, P. brasilensis 99.78, 99.43, 99.39%. In the study, the isolated Paemibacillus sp. MK-11 from Jeju seawater possesses the antibacterial activity against fish pathogens and it could be used as a new antibiotic agents against the gram positive fish pathogens.
**D042**

**Rapid Identification of Nosocomial Infection Bacterial by 4-Plex Polymerase Chain Reaction Assay**

Eui-Suk Jeong1, Min-Kyung Park1, Hye Lim Hong1, Dae-Yong Han2, Woo Suk Koh3, Yang-Kyu Choi4, and Choong-Yong Kim1,5,

1Laboratory Animal Center, Daegu-Gyeongbuk Medical Innovation Foundation, 2Department of Laboratory Animal Medicine, College of Veterinary Medicine, Konkuk University

Nosocomial infections occur worldwide and affect both developed and resource poor countries. Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients. The major pathogens causing nosocomial infection were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. We were trying to find the objective, rapid and accurate method for major pathogens causing nosocomial infection. In this study, conventional PCR was used for rapid identification. The results, under optimal PCR conditions, the 4-plex PCR assay simultaneously yielded a 207 bp from *K. pneumoniae*, a 581 bp from *S. pneumoniae*, a 581 bp from *S. aureus* and a 726 bp from *P. aeruginosa*. All four bacteria were successfully identified. *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae* are often found in mice, rats, guinea pigs, dogs, pigs and primates. Our 4-plex PCR assay can be applied to hospitalized patients as well as laboratory animals and economic animals. And, our 4-plex PCR assay will be used rapid diagnosis in hospitalized patients as well as improve quality control in laboratory animal facilities. **Key words**: Nosocomial infections, identification, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. pneumoniae*.

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**D043**

**The mRNA Involved in Uptake of Hexose Phosphates is Regulated by a Salmonella Virulence Protein**

Jin-Seob Lee, Eunna Choi, and Eun-Jin Lee*

Department of Genetic Engineering and Graduate School of Biotechnology, Colleges of Life Sciences, Kyung Hee University

The living environment is very important for all organisms. Because of all nutrients and chemicals, needed for organisms, exist in there. *Salmonella Typhimurium* lives in poor environment after, it had consumed by macrophages. But *S. Typhimurium* overcomes this critical situation with help from virulence protein (VP). One of the methods that *S. Typhimurium* surmounts crisis is to take hexose phosphates into cytoplasm with transporter proteins (TP), to use it as carbon sources. Our previous research revealed that, when virulence protein exists, the mRNA of certain protein involved in hexose phosphates transport is increased. Therefore, we studied what component interacts with virulence protein directly among many proteins involved in to take up hexose phosphates, using bacteria two hybrid system and qRT-PCR. It is one interesting point that a substitution mutant that could not bind to the virulence protein increases its own transcription, suggesting that this interaction acts as a negative regulator in the interaction. Furthermore, these results suggest that not only interaction with virulence protein but also another protein is needed to control mRNA expression of transporter protein.

**D044**

**Extension of O-linked Glycans is Essential for Cell Wall Integrity Signaling and Virulence of the Human Pathogenic Yeast Cryptococcus neoformans**

Eun Jung Thak, Dong-Jik Lee, Jung Ho Kim, and Hyun Ah Kang*

Department of Life Science, Chung-Ang University

The human pathogenic yeast *Cryptococcus neoformans* assembles two types of O-glycans with and without xylose on its proteins. In this study, we report that the *CAP6* gene encodes an α,1,2-mannosyltransferase responsible for the second mannose addition to the minor O-glycans with xylose. Thus, the *CAP6* deletion along with the deletion of *KTR3*, the gene involved in the α,1,2-mannose addition at the second position of the major O-glycans, resulted in the shift of all peaks of O-glycans to the single mannose M1 peak in HPLC analysis. The *ktr3Δcap6Δ* strain exhibited increased sensitivity to SDS, NaCl, and high temperature. O-mannosylation of T-cell antigen MP88, and putative cell wall sensors Wsc1p and Wml1p were shown to be dramatically reduced in *ktr3Δcap6Δ*. Particularly, the absence of O-glycan extension in Wsc1p and Wml1p rendered instability of these surface proteins. Quite interestingly, the phosphorylation of *Mpk1* was greatly decreased in *ktr3Δcap6Δ* compared to the wild-type strain upon tunicamycin treatment, indicating that O-glycans with extended structure are essential for Mpk1-mediated-cell wall integrity signaling. Moreover, *ktr3Δcap6Δ* showed fully attenuated virulence in a mouse model of cryptococcosis, suggesting that the O-glycan extension is critical for pathogenicity of *C. neoformans*. The non-ospionic phagocytosis of *ktr3Δcap6Δ* was comparable to that of WT indicating that O-glycans may be important in the late steps in interaction of *C. neoformans* with macrophages.

**D045**

**Structural Study of a PadR-like Transcriptional Regulator from Bacillus cereus**

Sun Cheol Park1, Kang Cheon Lee2, and Sung-ill Yoon1,2,*

1Division of Biomedical Convergence, College of Biomedical Science, Kangwon National University, 2Institute of Bioscience and Biotechnology, Kangwon National University

The PadR family is a large family of bacterial transcription factors and plays key roles in the transcriptional regulation of metabolism, toxin production, and antibiotic resistance. The PadR family is classified into subfamily-I and subfamily-II. Numerous apo structures and ligand-bound structures of subfamily-II members have been presented to date in diverse bacterial species. However, in the subfamily-I, only one apo structure from *Vibrio cholerae* was reported. Thus, structural studies are still required to reveal the regulatory mechanism of the subfamily-I. We have determined the crystal structure of a *Bacillus cereus* PadR-like protein (bcPLP) that belongs to the subfamily-I. bcPLP consists of N-terminal and C-terminal domains. The N-terminal domain contains a winged helix-turn-helix motif that is characterized with a positive patch, which would provide a DNA-binding site. The C-terminal domain possesses two long α-helices that are specific to the subfamily-I and contribute to dimerization. Our structural study of bcPLP would provide a foundation stone for the future researches on PadR subfamily-I-mediated transcriptional regulation.
Gastric cancer is one of the leading causes of cancer-related mortality in Korea. Helicobacter pylori infection is recognized as the main risk factor for gastric cancer, and it functions as a trigger for the chronic atrophic gastritis, which is the first sequential stage in gastric carcinogenesis. More complex microbial community in the stomach would contribute to the progression to the later stages of cancer development including the progression to intestinal metaplasia and gastric dysplasia. We aimed to characterize the microbial communities of the gastric mucosal tissue and gastric juice from patients who have intestinal types on progression to cancer or who are diagnosed with gastric cancer. Microbial compositions of gastric juice were less similar than those of stomach tissue samples from two mucosal parts, antrum and corpus. H. pylori-negative patients have more diverse gastric microbiotas, and the proportions of Actinobacteria and Clostridia are more abundant than the H. pylori-infected group. Composition of microbiotas depends on the diseased stage such as atrophic gastritis, intestinal metaplasia, and gastric cancer. As it progresses to gastric cancer, the levels of Epsilonproteobacteria and Bacteroidia increased, and Fusobacteria, Bacteroidia, and Betaproteobacteria decreased. Access to the gastric microbiotas allows us to identify their dynamics during disease development, as well as microbes possibly contribute to carcinogenesis besides H. pylori.

Recently, due to the environmental factors such as changes in aquatic ecosystem and climate change, the number of diseases in marine organisms is increasing. *Streptococcus iniae* is one of the pathogens causing streptococcosis in marine organisms. It is known that growth of the *S. iniae* depends greatly on the temperature and the nutrition ingredient in the water. Especially, it survives well in the lower water temperature until 10°C and the more nutrition of water condition. Based on these, we measured growth of *S. iniae* in three temperatures. It was cultured in Brain Heart Infusion(BHI) medium at three temperatures based on sea water condition, low temperature (2°C), middle temperature (27°C) and high temperature (37°C). The aim of this study was to determine how temperatures affect the variable physiological features of *S. iniae*. In this study, we identified differences in whole cell protein (WCP) and Extracellular protein (ECP) of *S. iniae* at the set temperatures by SDS-PAGE. We extracted those proteins and analyzed them by MALDI-TOF. In addition, we examined antigen related in virulence using antibody from olive flounder infected *S. iniae*. Further, we will analyze putative virulence factors using MALDI-TOF and identify genes coding putative virulence factors. As a result, Identifying these factors and genes coding these factors will have positive effect on treatment and prevention of streptococcosis.

[Supported by grants from Ministry of Oceans and Fisheries.]

**Analysis of Gastric Bacterial Communities for the Study of Gastric Carcinogenesis**

Soon-Kyeong Kwon¹, Jun Chul Park², Yong Chan Lee³, and Jihyun F. Kim¹*  
¹Department of Systems Biology and Division of Life Sciences, Yonsei University,  
²Division of Gastroenterology, Severance Hospital, Institute of Gastroenterology, Yonsei University College of Medicine

**Peroxisome Proliferator-activated Receptor α Activation Promotes Lipid Catabolism and Fatty Acid β-Oxidation in Macrophages during Mycobacterial Infection**

Yi Sak Kim¹,², Hye-Mi Lee¹, Tae Sung Kim¹,², Jin Kyung Kim¹,², Jin Ho Choe¹,², Soo Yeon Kim¹,², Sup Kim¹,², and Eun-Kyeong Jo¹,²*  
¹Department of Microbiology, Chungnam National University School of Medicine,  
²Department of Medical Science, Chungnam National University School of Medicine

The peroxisome proliferator-activated receptor α (PPAR-α) is a nuclear receptor that plays a variety of biological functions, largely involved in metabolism and inflammation. However, the roles of PPAR-α in innate immune activation is fully uncharacterized. We investigated whether PPAR-α activation contributes to the reduction in fatty acid–rich lipid body formation in BMDM infected with *M. tuberculosis* or BCG. Importantly, the PPAR-α–mediated reduction in lipid body formation was absent in Ppara⁻/⁻ BMDM during *M. tuberculosis* or BCG infection. We then quantified the cellular oxygen consumption rate as a measure of mitochondrial respiration and FAO in unstimulated and GW7647- or Wy14643-treated BMDM. PPAR-α agonist treatment increased the basal and maximum oxygen consumption rates, both of which were significantly decreased in Ppara⁻/⁻ BMDM. In addition, PPAR-α agonist treatment upregulated the expression of genes involved in lipid uptake, lipolysis, and FAO in *M. tuberculosis*-infected macrophages. Collectively, these data indicate that PPAR-α activation is required for lipid catabolism, increased mitochondrial respiratory function, and upregulation of FAO in BMDM during mycobacterial infection.

**Effect of Different Temperatures on Streptococcus iniae**

Moonjong Jeon¹, Yunjeong Choe², Yeonha Kim¹, Druga Ray², Seongyong Yoon¹, and Ho Young Kang*  
¹Department of Integrated Biological system, Pusan National University,  
²Department of Microbiology, Pusan National University

*S. iniae* is a known bacterial fish pathogen, which is a major causative agent of streptococcosis in Olive flounder and Oplegnathus fasciatus. Nonetheless, there have been few reports on the biological characteristics of *S. parauberis*. The aim of this study was to investigate comparative growth patterns and protein expression of the *S. parauberis* under different temperatures. To examine the changes in the biological characteristics of *S. parauberis* in different temperatures, it was cultured in BHI medium with different temperature condition based on according to climate change in sea water temperature such as low temperature (17°C), middle temperature (27°C) and high temperature (37°C). The difference in growth rate showed that low temperature had slower growth rate when compared with middle temperature. However, there was no discernable growth at high temperature condition. In addition, in order to find out specific factors in related with different bands in protein expression according to temperature, we selected fractions of total protein and extracellular protein in each temperature condition resolved with SDS-PAGE these bands were further identified by MALDI-TOF. We identified the factors for the individual protein bands difference caused by changes in temperature. Furthermore, searching for these virulence factors by identifying the proteins would be as well to begin overcome the streptococcosis in fish.

[Supported by grants from the Ministry of Oceans and Fisheries.]
**D050**

**Effect of Salinity on the Pathogenicity of Streptococcus iniae**

FP5228

Seongyong Yoon1, Yunjeong Choe1, Durga Ray1, Yeonha Kim1, Moonjung Jeun1, and Ho Young Kang1*  
1Department of Integrated Biological Science, Pusan National University,  
2Department of Microbiology, Pusan National University

Streptococcus iniae is a Gram-positive bacterium that causes serious disease in a wide range of fish species. Because of this feature, it is one of the major pathogens that affect aquaculture and fishery industry. Especially in a narrow and dense aquaculture, disease spreads quickly and causes enormous damage. It was thought that the salinity was effective for the pathogenicity of S. iniae. The salinity of seawater is about 3.5%, which varies according to the season and weather, and varies depending on the degree of freshwater inflow in brackish water, resulting in changes in the aquatic ecosystem. And the salinity concentration in marine environments, in other words the host body, is about 0.9%, which is very different from the salinity of seawater. In addition, we determined growth curves by incubating S. iniae in BH medium by setting three different salinity concentrations (0.9%, 2.0%, or 3.5%). And viable cell count was decreased at the concentration of 3.5% NaCl. In this study, we aimed to identify morphological and protein expression differences according to salinity in S. iniae, to identify pathogenic factors through protein analysis such as MALDI-TOF, and to construct a vaccine model.  
[Supported by grant from Ministry of Oceans and fisheries.]

**D052**

**The Contribution of Pseudomonas aeruginosa DesB to Its Pathogenicity in Host**

Jimyoun Ha1, Sejeong Kim2, Yohan Yoon1, and Kyoung-Hee Choi1*  
1Sookmyung Women’s University, 2Wonkwang University

Pseudomonas aeruginosa, which is widely found in a variety of environments, can cause harmful chronic lung damage in immunocompromised patients. In previous study, it was shown that an aerobic desaturase (DesB) of P. aeruginosa exerted significant effect on virulence determinants. The objective of this study was to analyze the effect of DesB on host-interaction. For the in vitro experiments, cells and supernatants of P. aeruginosa wild-type (WT) or its desB mutant were collected by centrifugation and diluted with F-12 supplemented with 10% FBS, and the diluents were added to A549 cell monolayer for determining cell viability, invasion assay, and/or immune response. For the in vivo experiments, 6 weeks old ICR mice were infected by endotracheal intubation with 6 log CFU/ml bacterial cell. After mice sacrifice, the survival rate of each strain in lung was measured. Also the histopathology of lung tissue was observed. desB mutant exhibited lower cytotoxicity to A549 cells than WT. Also, desB mutant stimulated secretion of more pro-inflammatory cytokine and chemokines compared to WT. In mouse model, the survival rate of desB mutant was higher than WT in the lungs. The results suggest that P. aeruginosa DesB affects the pathogenicity and microbe-host interaction.  
[This study supported by grants from Basic Science Research Program through the NRF funded by the Ministry of Science, ICT & Future Planning (2014-0811).]

**D051**

**Adaptability of Pseudomonas aeruginosa in High Osmotic Stress Mediated by Desaturase**

Sejeong Kim1, Jimyoun Ha2, Yohan Yoon1, and Kyoung-Hee Choi2*  
1Sookmyung Women’s University, 2Wonkwang University

Pseudomonas aeruginosa can be exposed to various stressful environments, and it was generally known that proper membrane fluidity can be maintained by adjusting the ratio of saturated fatty acid and unsaturated fatty acid, and the control of the membrane fluidity plays an important role on bacterial adaptation in stress environments. Therefore, this study investigated the role of an aerobic desaturase of P. aeruginosa (DesB) on salt resistance. The growth of bacteria was compared, and transcriptional levels of P. aeruginosa PAO1 (wild type, WT) and desB mutant were compared using qRT-PCR analysis. The role of DesB on salinity adaptation was phenotypically confirmed using betaine. The growth of all strains was inhibited under the exposure of 0.5M or 1.0M NaCl. Especially, desB mutant displayed more impaired growth compared to WT and other mutants, suggesting the role of DesB as a player on salt stress. Comparative transcriptional analysis showed that genes involved in the synthesis of osmoprotectants (trehalose, NAGGN, and hydrophilin) were highly expressed in WT in response to high salt, whereas rarely expressed in desB mutant. Further, decrease in osmoprotectant of desB mutant was partially complemented by the addition of betaine. These results indicate that P. aeruginosa DesB contributes to the adaptability to high salinity by positively regulating the synthesis of osmoprotectants.  
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Effect of Intermittent PTH Administration on Diabetes Rats with Periodontitis

Aeri Kim¹,², Ji-hye Kim³, Aeryun Kim¹, Yun Hui Choi¹, Jeong-Heon Cha¹,²,³, Eun-Jung Bak¹, and Yun-Jung Yoo¹,²

¹Department of Oral Biology, Yonsei University College of Dentistry, ²Department of Applied Life Science, The Graduate School, Yonsei University, ³BK21 PLUS Project, Yonsei University College of Dentistry

Parathyroid hormone (PTH) with an anabolic effect on bone is in a worldwide used for treatment of osteoporosis. Periodontitis is accompanied with diabetes shows severe alveolar bone loss. We investigated the effect of intermittent PTH administration on alveolar bone in diabetes with periodontitis.

Rats were divided into control (C), periodontitis (P), diabetes with periodontitis (DP), and diabetes with periodontitis treated with PTH (DP+PTH) groups. To induce diabetes, rats were injected with streptozotocin and after 7 days, periodontitis was induced by ligature of the mandibular first molar for 30 days. The DP+PTH group was injected with PTH (40 μg/kg) three times per week after ligature. Rats were injected with calein (10 mg/kg) and alizarin red (20 mg/kg). At day 30 after ligation, mandible and tibia were collected for histological, fluorescent labeling, and micro-CT analyses.

The DP+PTH group showed lesser alveolar bone loss than the DP group and showed a similar tendency in micro-CT, but there was no significant difference. The DP group showed lesser osteoid formation on alveolar bone than other groups, otherwise the DP+PTH group showed greater osteoid formation than the DP group. The DP+PTH group has more mineral deposition on the alveolar bone and tibia than the DP group. These results suggest that intermittent PTH administration inhibits alveolar bone loss and increases bone formation in diabetes with periodontitis.

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**D055**

**Amphiregulin and EGF are Involved in *Helicobacter pylori* Induced Cell Elongation via EGF Receptor**

Youngmin A Hong, Yun Hui Choi, Jinmoon Kim, and Jeong-heon Cha*

Department of Oral Biology, Oral Science Research Center, Department of Applied Life Science, The Graduate School, BK21 Plus Project, Yonsei University College of Dentistry

*Helicobacter pylori* is a well-known pathogen that colonizes in gastric mucosa, causing chronic gastritis, peptic ulcers, and gastric cancer. *H. pylori* carries several virulence factors such as cytotoxin-associated gene protein A (CagA), vacuolating cytotoxin A (VacA) and bacterial outer membrane proteins (OMPs) such as BabA and OipA. Among them, CagA has been thought to play a central role in cell morphological change. CagA protein is translocated into host cells via Type IV secretion system (T4SS), phosphorylated by the host cell kinase and induce cell morphological change. There was a study EGFR and cell elongation is correlated (Tegtmeyer et al., 2008). However, detail about how EGFR is associated with elongation was not studied. This study was conducted in an effort to determine the relationship between EGFR and cell morphological change induced by *H. pylori* in the human gastric cell. In this study, we examined that *H. pylori* infection induced epidermal growth factor (EGF) family ligands and receptors expression. Out of four isoforms of EGF family receptors, mRNA expression of EGFR1 was induced conspicuously in CagA dependent manner and out of ligands that bind to EGFR1, the induced ones include EGF, amphiregulin, HB-EGF and transforming growth factor-alpha. The inhibition of EGFR1 reduced cell elongation phenotype significantly. Also, within the ligands, we found two ligands, amphiregulin and EGF, are responsible for cell elongation. These mRNA expressions were also induced in CagA dependent manner. We suggest that amphiregulin and EGF plays major role in *Helicobacter pylori* induced cell elongation via EGFR.

**D056**

**Antiviral Activity of a Novel Biphenyl Amid p38 MAPK Inhibitor against Hepatitis B Virus**

So-young Kim1, Sang-Won Kim1, Hong Kim2, Junsu Ban1, Bum-Joon Kim1,***, Nam-Jung Kim2,** and Kyung-Soo Inn1*  

1,2Department of Pharmaceutical Science, College of Pharmacy, Kyung Hee University, 1Department of Biomedical Sciences, Microbiology and Immunology, and Liver Institute, College of Medicine, Seoul National University

Hepatitis B virus (HBV) causes a series of HBV-related liver disease such as viral hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC). However, current therapies such as antivirals and interferon have limitations including drug intolerance, kidney toxicity and require long-term medication. Thus, the development of new therapeutic agent for HBV infection is urgently needed.

*p38* mitogen-activated protein kinase (MAPK) activity is known to be critical for HBV antigen production and replication. A series of biphemyl amides have been synthesized as novel p38 MAPK selective inhibitors. In this study, we should that the suppression of HBsAg production by these compounds was positively correlated with the p38 MAPK inhibitory activity. Among these compounds, NJK14047 displayed the most potent p38 inhibitory and HBsAg suppressive activities. We further investigated the antiviral role of NJK14047 against HBV. NJK14047 efficiently suppressed the secretion of HBsAg and HBsAg in a dose-dependent manner. In addition, NJK14047 treatment resulted in decreased levels of pgRNA and cccDNA in HBV-harboring cells. These results suggest NJK14047 as a novel antiviral therapeutic agent for HBV related liver disease.
**E002**

**Phase Determination of Iron-dependent Homogentisate Dioxygenase from Acinetobacter oleivorans**

Seung-A HwangBo1 and Suk-Youl Park1,2, *+*

1Pohang Accelerator Laboratory, Pohang University of Science and Technology, 2Department of Chemistry, College of Natural Sciences, Chonnam National University

In aromatic compounds mineralizing bacterium Acinetobacter oleivorans, homogentisate 1,2-dioxygenase (HGO) catalyzes the conversion of homogentisate to 4-maleylacetoacetate by aromatic ring scission using zinc ions, in the breakdown of tyrosine and phenylalanine. To determine the molecular background of the enzymatic mechanism of HGO in this iron-resistant organism, DH17_10945 encoding HGO of Acinetobacter oleivorans was cloned, and the expressed protein was purified. The protein was crystallized in 0.2 M sodium chloride, 25% (w/v) polyethylene glycol 3350 and 0.1 M Bis-Tris at pH 6.5. X-ray diffraction data were collected to 1.5 Å resolution using synchrotron radiation. The crystal structure of HGO, determined at a resolution of 1.9 Å, showed that the enzyme has a canonical α/β hydrolase fold with an α-helical cap domain and a catalytic triad consisting of Ser96, Asp220, and His248. Interestingly, the active site of the structure of HGO is occupied by a peracete molecule, which is the product of perhydrolysis of acetate. This result suggests that HGO may have perhydrolyase activity. The activity assay showed that HGO has significant perhydrolyse and esterase activity with respect to short-chain p-nitrophenyl esters (≤ C8), naphthyl derivatives, phenyl acetate, and glyceryl tributyrate. However, the S96A single mutant had low esterase and perhydrolyase activity. Surprisingly, immobilized HGO was found to not only retain 200% of its initial activity after incubation for 1 h at 80°C, but also retained more than 60% of its initial activity after 20 cycles of reutilization. This research will serve as basis for future engineering of this esterase for biotechnological and industrial applications.

**Synopsis:** We report the crystallization, collection of X-ray diffraction data, and phase determination of iron-dependent homogentisate 1,2-dioxygenase from Acinetobacter oleivorans.

**E003**

**Functional Implications of Hexameric Assembly of RraA Proteins**

Daeyoung Kim1, Saemee Song1, Jinyang Jang2, Minho Lee1, Ji-Hyun Yeom1, Nohra Park2, Nam-Chul Ha2, and Kangseok Lee1,*

1Department of Life Science, Chung-Ang University, 2Department of Agricultural Biotechnology, Seoul National University

RNase E, Protein inhibitors RraA and RraB control the enzymatic activity of RNase E, which has a pivotal role in the degradation and processing of RNAs in Escherichia coli. In this study, we report that the oligomer formation of RraA proteins, RraA homologs of Vibrio vulnificus, affects binding efficiency to RNase EV as well as inhibitory activity on RNase EV action. The hexameric structure of RraA1 was converted to an octamer and tetramer when the Cys 9 residue was substituted with an Asp residue (RraA1-CD9), showing decreased inhibitory activity of RraA1 on RNase EV in vivo. These results indicated that the intermolecular disulfide linkage contributed critically to the hexamerization of RraA1 for its proper function. On the contrary, the RraA2 that existed in a trimeric state did not bind to or inhibit RNase EV. An in vitro cleavage assay further showed the reduced inhibitory effect of RraA1-C9D on RNase EV activity compared to wild-type RraA1. Based on structural and functional comparison of RraA homologs, we suggest that hexameric assembly of RraA homologs may well be required for their action on RNase E-like proteins.

**E004**

**The Histone Arginine Methylation Is Important for HM Silencing Maintenance in S. cerevisiae**

Soolin Yeom1,2 and Jung-Shin Lee1,2,*

1Department of Molecular Bioscience, Kangwon National University, 2Critical Zone Frontier Research Laboratory (CFRL), Kangwon National University

Gene silencing is one of important concepts for the epigenetic gene regulation. Histone modifications are critical factors for the maintenance of gene silencing in eukaryote. However, none of histone modifications as epigenetic silencing markers is conserved in Saccharomyces cerevisiae. To identify novel 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 found that several arginine residues are important for the maintenance of yeast mating type. Also we found one, arginine HMT called yHRMT, by screening using yeast single-gene knock out library. To identify yHRMT’s functional role for HM silencing, we analyzed the interactome of yHRMT. This result tells us that yHRMT can interact with H4 and it means that H4 is potential as a substrate of yHRMT. Also, we checked that Sir2 localization differ from ΔyHRMT and WT. Finally, we suggest that arginine residue is important for the HM silencing among others.

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The Regulation of the glpFKD Operon Involved in Glycerol Utilization in Mycobacterium smegmatis

Hyon-Ju Bong and Jeong-II Oh*
Department of Microbiology, Pusan National University

The glpFKD operon, which is induced under hypoxic conditions and in the presence of glycerol, is involved in glycerol metabolism in Mycobacterium smegmatis mc2155. Immediately upstream of the glpFKD operon is an open reading frame that is 48% identical to GyIR from Streptomyces coelicolor A3(2). To examine the role of GyIR, we constructed the gyIR inframe deletion mutant (ΔgyIR) of M. smegmatis. The expression of the glpFKD operon in the ΔgyIR mutant was abolished and the introduction of the intact gyIR gene into the mutant complemented the ΔgyIR phenotype, suggesting that the glpFKD operon is positively regulated by GyIR. The ΔgyIR mutant showed a slower growth than the wild type when glycerol was supplied as a sole source of carbon and energy, indicating the importance of the glpFKD operon in glycerol metabolism. Nucleotide sequence analysis on the control region of the glpFKD operon, revealed that a putative SigF-promoter consensus sequence and a putative CRP (cAMP receptor protein)-binding site, and three putative GyIR-binding sites (IR1, IR2, and IR3) with the consensus sequence GKTGCGR-N4-GYCAGMC. IR3 was demonstrated to be essential for glpFKD expression by the GyIR activator and that two neighboring GyIR-binding sites (either IR1 and IR2 or IR2 and IR3) are required for the binding of GyIR to the control region. Herein we propose the model explaining the regulation of the glpFKD operon by GyIR.

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The Iron Uptake Repressor Fep1 in the Fission Yeast Binds Fe-S Cluster through Conserved Cysteines

Hyo Jin Kim, Kang Lok Lee, and Jung Hye Roe*
School of Biological Sciences and Institute of Microbiology, Seoul National University

Iron homeostasis is tightly regulated since iron is an essential but toxic element in the cell. The GATA-type transcription factor Fep1 and its orthologs contribute to iron homeostasis in many fungi by repressing genes for iron uptake when intracellular iron is high. Even though the function and interaction partners of Fep1 have been elucidated extensively in Schizosaccharomyces pombe, the mechanism behind iron-sensing by Fep1 remains elusive. It has been reported that Fep1 interacts with Fe-S-containing monothiol glutaredoxin Grx4 and Grx4-Fra2 complex. In this study, we demonstrate that Fep1 also binds iron, in the form of Fe-S clusters. Spectroscopic and biochemical analyses of as isolated and reconstituted Fep1 suggest that the dimeric Fep1 binds Fe-S clusters. The mutation study revealed that the cluster-binding depended on the conserved cysteines located between the two zinc fingers in the DNA binding domain. EPR analyses revealed [Fe-S]-specific peaks indicative of mixed presence of [2Fe-2S], [3Fe-4S], or [4Fe-4S]. The finding that Fep1 is an Fe-S protein fits nicely with the model that the Fe-S-trafficking Grx4 senses intracellular iron environment and modulates the activity of Fep1.

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Structural Study on a Putative Ribonuclease Z from Bacillus subtilis

Byeol Nam-gung and Sung-il Yoon*
Division of Biomedical Convergence, College of Biomedical Science, Kangwon National University

The precise cleavage of both the 5’ and 3’ extensions in precursor tRNA by specific nucleases is a vital step in the maturation of tRNA. tRNase Z belongs to the metallo-β-lactamase superfamily and has been identified as an important endonuclease that cleaves the 3’ extension from various tRNA precursors. tRNase Z enzymes are ubiquitously found in both bacterial and archaeal organisms, as well as in eukaryotes. Bacillus subtilis contains the YhfI gene, which is considered as tRNase Z on the basis of sequence homology. To understand the function and structural properties of bsYhfI required for tRNA maturation, we solved the crystal structure of bsYhfI at 2.15 Å resolution by X-ray crystallography. bsYhfI crystals were obtained in PEG conditions and belonged to space group I4122. bsYhfI folds into the typical α/β/α sandwich structure of the metallo-β-lactamase superfamily and possesses a shallow active-site groove containing two divalent zinc ions that are flanked by flexible loops.

Structural Study on a Butanol Dehydrogenase from Bacillus subtilis

Ho jeong Hong, Mi-sun Nam, and Sung-il Yoon*
Division of Biomedical Convergence, College of Biomedical Science, Kangwon National University

Butanol dehydrogenase (BDH) catalyzes the conversion of butyl aldehyde to butanol using NAD(P)⁺. Horse liver BDH have been intensively studied and was shown to require structural changes for its enzymatic reaction. However, it remains unknown how BDH from Bacillus subtilis (bsBDH) catalyzes the butanol formation. To define the biochemical activity and structural features of bsBDH, we have performed expression, purification and X-ray crystallographic studies. bsBDH was overexpressed in an Escherichia coli expression system and purified by chromatographic methods. The rod-shaped crystals of bsBDH in complex with NAD⁺ were generated by vapor diffusion method in a solution containing ammonium sulfate and PEG 3350 at pH 6.5, and X-ray diffraction data were obtained up to 1.55 Å resolution. The crystals belonged to space group P2₁ with two molecules in the asymmetric unit. The bsBDH structure was determined by molecular replacement. Furthermore, we will present the structural features of bsBDH and its interaction with NAD⁺. This study will enhance our understanding of the enzymatic mechanism of BDH at the atomic level.
**Characterization of the FruBKA Operon Regulator FruR in Vibrio cholerae**

Chang-Kyu Yoon, Young-Ha Park, Yeon-Ran Kim, and Yeong-Jae Seok*
Department of Biological Sciences and Institute of Microbiology, Seoul National University

Fructose repressor (FruR), which belongs to the GalR/LacI family of transcriptional regulators, regulates the expression of genes involved in the transport and utilization of fructose through direct binding to the cognate DNA sequence upstream of the fruBKA operon. In *Escherichia coli*, FruR has been elucidated as a global regulator responsible for controlling the carbon metabolic flux through repression or activation of mRNA expression of approximately 60 genes in 24 operons. *Vibrio cholerae* also has a FruR ortholog (vcFruR) that shares 47% amino acid sequence identity with *E. coli* FruR (ecFruR). vcFruR has unique features compared to the ecFruR. First, the oligomeric state of ecFruR is a homotetramer whereas vcFruR exists in a dimeric state in vitro. In addition, the gene encoding vcFruR is located adjacent to and divergently transcribed from the fruBKA operon. Lastly, only three sites are expected to be the target sites of FruR in the entire *V. cholerae* genome and these sites are located in the intergenic region between fruB and fruK. These findings indicate that vcFruR might have regulatory mechanism different from that of ecFruR. In this study, we investigated the transcriptional regulation of FruR on the expression of the fruBKA operon. By binding to each target site in different combinations, vcFruR is considered to regulate the transcriptional level of the fruBKA operon and itself in *V. cholerae*.

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**RNA Activation-independent DNA Targeting of the Type III CRISPR-Cas System by a Csm Complex**

Kwang-Hyun Park, Yan An, Woo-Chan Ahn, In-Young Baek, and Eui-Jeon Woo*
Disease Target Structure Research Center, Korea Research Institute of Bioscience and Biotechnology,

The CRISPR-Cas system is an adaptive and heritable immune response that destroys invading foreign nucleic acids. The effector complex of the Type III CRISPR-Cas system targets RNA and DNA in a transcription-coupled manner, but the exact mechanism of DNA targeting by this complex remains elusive. In this study, an effector Csm holocomplex derived from *Thermococcus onnurineus* is reconstituted with a minimalistic combination of Csm1, 2, 3, 4, 5, and shows RNA targeting and RNA-activated single-stranded DNA (ssDNA) targeting activities. Unexpectedly, in the absence of an RNA transcript, it cleaves ssDNA containing a sequence complementary to the bound crRNA guide region in a manner dependent on the HD domain of the Csm1 subunit. This nuclease activity is blocked by a repeat tag found in the host CRISPR loci. The specific cleavage of ssDNA without a target RNA suggests a novel ssDNA targeting mechanism of the Type III system, which could facilitate the efficient and complete degradation of foreign nucleic acids.

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Biochemical Sciences and Institute of Microbiology, Seoul National University

Bacteria respond to various stresses by modulating the level of alarmone (pppGpp (guanosine tetraphosphate and pentaphosphate)) through a process called the stringent response. In Escherichia coli, the level of (pppGpp is regulated by stringent factors, RelA and SpoT proteins. RelA catalyzes the synthesis of (pppGpp, whereas SpoT catalyzes both the synthesis and hydrolysis of (pppGpp, serving as a bifunctional enzyme. SpoT is particularly important in balancing the intracellular level of (pppGpp since it is the only enzyme responsible for (pppGpp hydrolysis. However, the underlying mechanisms for the (pppGpp hydrolytic activity regulation of SpoT still remain unknown. In this study, we conducted ligand-fishing experiment and found Rsd as a novel interaction partner of SpoT. Furthermore, we confirmed that the (pppGpp hydrolytic activity of SpoT could be directly stimulated by Rsd. We recently reported that HPt is dephosphorylated and interacts with Rsd when a favorable carbon source, such as glucose, is available. In this study, we demonstrate that only dephosphorylated form of HPt, but not phosphorylated HPt, can sequester Rsd from SpoT to antagonize its stimulatory effect on SpoT. Based on these data, we suggest that SpoT-mediated stringent response can be regulated by Rsd in a glucose-dependent manner, proposing a novel stringent response mechanism in E. coli.

Jae-Woo Lee1, Young-Ha Park2, and Yeong-Jae Seok1,2*
1Dept. of Biophysics and Chemical Biology, Seoul National University; 2Dept. of Biological Sciences and Institute of Microbiology, Seoul National University

Our previous work indicated that Phanerochaete chrysosporium (ATCC20696) induced depolymerization of synthetic lignin polymer and production of lignin derived-aromatic and acid compounds under the addition of reducing agents. Transcriptomic analysis of P. chrysosporium provided information about various enzymes related to lignin degradation and the aromatic catabolic pathway. Especially, among the extracellular enzymes, seven genes of lignin peroxidase were up regulated by addition of synthetic lignin.

Based on these results, we analyzed the full-length of lignin peroxidase encoding gene (pclip2 gene) by using RACE-(Rapid amplification of cDNA Ends) PCR, and investigated expression of various lignin peroxidase by proteomic analysis. At first, in RACE PCR result, 235-bp amplification product was obtained, and full-length coding sequence of pclip2 resulted in cloning the 1,354-bp fragment. And then, for analyzing the expression of lignin peroxidase from P. chrysosporium depending on the addition of synthetic lignin, extracellular enzymes was obtained through the amicon ultra centrifugal filter. Concentration of extracellular enzymes was determined by Bradford method. Proteomic analysis by LCMS is underway. Consequently, this study is expected to support importance of lignin peroxidase in lignin depolymerization.

Chang-Young Hong, Su-Yeon Lee, Sun-Hwa Ryu, and Myungkil Kim*
Division of Wood Chemistry & Microbiology, Department of Forest Products, National Institute of Forest Science

Deinococcus radiodurans is a polyextremophilic bacteria that is capable of withstanding up to 15 kGy gamma radiation, several weeks of desiccation, 500 J/m² UV-C radiation, and various DNA damaging agents such as mitomycin C (MMC), with almost no loss of viability. Bioinformatic analysis of the D. radiodurans R1 genome has revealed specific expansion of certain protein families compared with other organism. D. radiodurans encodes at least 13 DinB/YfiT homologs, which greatly outnumber those found in related Gram-positive bacteria. DinB is a DNA damage-inducible protein, and YfiT protein is induced by general stress. In the previous study, we found that the expression level of DinB2 protein is extremely increased after 5 kGy irradiation. Toward the first step to understand the regulatory mechanism underlying DinB2 and the radiation resistance, we determined the crystal structure of DinB2 protein by single-wavelength anomalous dispersion based on zinc anomalous scattering. The crystal of DinB2 belongs to C2 space group with unit cell dimensions of a = 108362 Å, β = 50.572 Å and c = 55.830 Å, β = 89.96°. The final structure of DinB2 was refined at a resolution of 2.06 Å with R factor of 0.23 and R free of 0.26. DinB2 contains a canonical four-helix bundle but no conserved histidine triad for nickel-binding among DinB-like proteins.

Chungnam National University

Lkh1, the fission yeast LAMMER kinase, phosphorylates Thr110 on Rum1, cyclin dependent kinase (CDK) inhibitor, which regulates G1/S cell cycle progression. The lkh1 deletion mutant has similar phenotype with rum1 deletion mutant and enters S phase earlier than wild type. These results suggest a positive effect of phosphorylation on Rum1 such as stimulation of the CKI activity of Rum1. Therefore, we performed CKI inhibitor assay to confirm the effect of Lkh1-dependent phosphorylation on Rum1 activity. Phospho-mimic forms of Rum1 exhibited increased inhibitor activity than wild type. Therefore Lkh1-mediated phosphorylation is crucial for the CDK inhibitor activity of Rum1. Next, we carried out flow cytometry analysis to figure out effect of Lkh1-dependent phosphorylation on Rum1. Moreover, microarray analysis using Lkh1 deletion mutant revealed that transcriptional regulator MBF (MluI cell cycle binding factor) related gene expression was increase. In vitro kinase assay and peptide mass fingerprinting implied that Thr40 and Thr41 residues on Yox1, a negative regulator of MBF, were phosphorylated by Lkh1. Our results indicated that Lkh1 positively modulates Yox1 activity for tight regulation of G1/S progression. In this study, in vitro kinase assay was performed to confirm the Lkh1-dependent phosphorylation of Thr40 and 41 on Yox1. We then performed cycloheximide chase assay using phospho-defect forms of Yox1 to confirm the effect of Lkh1 in Yox1 stability.

Songju Shin, Soo Jeong Kwon, and Hee-Moon Park*

Expression Analysis of Lignin Peroxidase Encoding Genes from Phanerochaete chrysosporium (ATCC20696) Exposed to Synthetic Lignin

Min-Kyu Kim, Jing Zhang, Ho Seong Seo, Jong-Hyun Jung, and Sangyong Lim*
Research Division for Biotechnology, Korea Atomic Energy Research Institute (KAERI)

Phanerochaete chrysosporium is a polyextremophilic bacteria that is capable of degrading synthetic biopolymers such as synthetic lignin, its relatives, and natural lignins. In this study, we demonstrate that HPr is dephosphorylated and interacts with Rsd when a favorable carbon source, such as glucose, is available. In this study, we demonstrate that only dephosphorylated form of HPr, but not phosphorylated HPr, can be used as a substrate for Rsd in vitro kinase assay.

Poster
**E017**

**N-Acetylglucosamine Transporter Enzyme IIBC Recruits the Global Repressor Mlc in Vibrio vulnificus**

JiHee Yoon1, So-Young Park2, Young-Ha Park1, and Yeong-Jae Seok1,2*

1Dept. of Biophysics and Chemical Biology, Seoul National University, 2Dept. of Biological Sciences and Institute of Microbiology, Seoul National University

The bacterial phosphoenolpyruvate: sugar phosphotransferase system (PTS) regulates a variety of physiological actions in addition to sugar uptake. Various regulatory roles have been known for the PTS components, especially in Escherichia coli. Although primary structures of the PTS are well conserved in both E. coli and Vibrio vulnificus, their regulatory roles are quite different. Chitin, a homopolysaccharide of N-acetylglucosamine (Nag), is the most abundant nutrient in the ocean. V. vulnificus has an N-acetylglucosamine-specific PTS component, EIIBC\textsuperscript{Vv}, the membrane bound transport protein. In this study, we identified a homolog of the global repressor Mlc (DNA-binding transcriptional repressor) in E. coli as a binding partner of EIIBC\textsuperscript{Vv} in V. vulnificus. In the presence of N-acetylglucosamine, the cytosolic domain of EIIBC\textsuperscript{Vv} transfers phosphate to the incoming sugar. The dephospho-form of EIIBC\textsuperscript{Vv} interacts directly with Mlc and induces the transcription of Mlc-regulated genes by displacing Mlc from its target sequences. Therefore, the N-acetylglucosamine induction of Mlc-regulated genes is caused by the dephosphorylation of the membrane bound transporter enzyme IIBC\textsuperscript{Vv}, which directly recruits Mlc to derepress its regulon. This novel finding indicates similar but different carbohydrate-dependent regulation mechanisms compared to what has already been discovered in E. coli. These observations may shed light on the unique physiology of marine bacteria.

**E018**

**Effect of the RNA Pyrophosphohydrolase RppH on Envelope Integrity in Escherichia coli**

Umji Choi and Chang-Ro Lee*

Department of Biological Sciences, Myongji University

The bacterial pyrophosphohydrolase RppH initiates mRNA decay by converting 5'-terminal triphosphate of mRNA to monophosphate. Bacillus subtilis RppH has strict specificity of substrate recognition, whereas *Escherichia coli* RppH is relatively promiscuous. Although the phenotypic analysis of the rppH mutant is required to accurately understand the physiological role of RppH in E. coli, the phenotype of the rppH mutant has remained largely unknown. In this study, we report various phenotypes of the rppH mutant involved in envelope permeability. Phenotype microarray analysis showed that the rppH mutant is sensitive to a variety of chemicals. The rppH mutant showed severe sensitivity to several antibiotics, such as rifampicin and colistin, and was also significantly sensitive to envelope stresses, such as osmotic, ethanol, and SDS stresses. All phenotypes of the rppH mutant were caused by loss of its enzymatic activity. The rppH mutant showed increased envelope permeability than the wild-type cells. Additionally, an increased activity of RppH resulted in significant growth retardation under low temperature conditions. Together, various phenotypes of the rppH mutant suggest that RppH is involved in regulation of envelope integrity.

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**E019**

**Direct Effects of Pafl Complex Components on H3K4 Methylation in Saccharomyces cerevisiae**

Jun-Soo Oh1,2 and Jung-Shin Lee1,2*

1Department of Molecular Bioscience, College of Biomedical Science, Kangwon National University, 2Critical Zone Frontier Research Laboratory(CFRL), Kangwon National University

Histone modifications regulate chromatin structure dynamics and consequently influence on the transcriptional output. Among them, H3K4 trimethylation is an well-known mark of transcription activation and dependent on another histone modification, H2BK123 monoubiquitination. Interaction of different two histone modifications is termed histone crosstalk. In Saccharomyces cerevisiae, Pafl complex consists of five proteins and functions as a platform for recruiting many types of transcription factors to elongating RNA polymerase II. Components Pafl and Rtf1 of Pafl complex contribute to H2B ubiquitination and indirectly influences on H3K4 di- and tri-methylation by histone crosstalk. But the specific effects of each component in Pafl complex on this histone crosstalk largely remained to be identified. In this study, we constructed the deletion mutants of Pafl complex components and observed their effects on H3K4 mono-, di- and tri-methylation as well as H2BK123L2 monoubiquitination. As a result, in each Δ-pafl, Δ-rtf1 and Δ ctr9 strain, we observed dramatic defects in H3K4 monomethylation, which are independent of H2B ubiquitination. This suggests that Ctr9 as well as Pafl and Rtf1 directly influences on H3K4 methylation by directly regulating the activity of Set1 or by contributing to the stability of COMPASS (Complex of Proteins Associated with Set1).

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**E020**

**Balance of Rps3p Mono-ubiquitination by Ubc4p, Hel2p and Ubp3p Regulates Protein Quality Control**

Youjin Jung, Se Woong Kim, Young Kwang Park, and Joon Kim*

Laboratory of Biochemistry, Division of Life Sciences, Korea University

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 Hel2p E3 ligase and Ubp3p de-ubiquitinase for protein quality control. First, we corroborate that Rps3p is a substrate for ubiquitination by a specific E3 ligase Hel2p in vivo and in vitro. Through in vitro ubiquitination assays and gene knockout experiments, we found that Hel2p was a major E3 ligase targeting Rps3p on ribosome complex. We also found that Ubp3p was a de-ubiquitinase (DUB; ubiquitin specific protease) which could modulate Hel2p mediated Rps3p mono-ubiquitination. In addition, we found that Hel2p and Ubp3p appeared to be differently localized in ribosome complex after UV irradiation. However, rapamycin induced Rps3p mono-ubiquitination was found to be caused by Ubp3p sequestration into the autophagosome. Together, our results support a model in which coordinated ubiquitination and de-ubiquitination activities can finely balance the level of Rps3p mono-ubiquitination in ribosome associated quality control (RQC) and autophagy processes.

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**E021**

Unique Features of Sulfur Regulatory Network, Mediated by a Short Met4p Homolog Hansenula polymorpha

Su Jin Yoo, Min Jeong Sohn, and Hyun Ah Kang*

Lab. of Molecular Systems Biology, Dept. of Life Science, Chung-Ang University

The thermotolerant methylotrophic yeast Hansenula polymorpha, has uniquely evolved its cysteine-centered sulfur metabolism. To elucidate the sulfur regulatory network in H. polymorpha, we investigated a bZIP transcription factor, encoded by the HpMET4 gene. Positioned at the boundary between the large and short Met4p subfamilies in ascomycetes, HpMet4p displays structural features with combined characteristics of yeast and filamentous fungal Met4 homologs. Its function was shown to be important not only for sulfur metabolism, but also for temperature, cadmium, and oxidative stress responses in H. polymorpha. Comparative transcriptome analysis further supported the role of HpMet4p as a master regulator for cell homeostasis under sulfur limitation. Despite its truncated IR domain, which is involved in ubiquitin (Ub)-dependent repression of Met4p activity in Saccharomyces cerevisiae, HpMet4p was subjected to Ub-mediated proteolysis in the presence of cysteine. The analysis of domain swapping, along with deletion analysis of the regulatory domains and the genes encoding cofactors Met28p and Met32p, revealed that the bZIP domain of HpMet4p is sufficient for the induced expression of sulfur genes in H. polymorpha, like those of other filamentous fungi. Our results showed novel features of HpMet4p as a representative of the short Met4p subfamily, widening our understanding of the evolution of the sulfur regulatory networks among eukaryotic organisms.

[Supported by grants from NRF and KHID]

**E022**

Structural Analysis of FliD Oligomerization and Its Implication in the Assembly of the Flagellar Filament

Wan Seok Song, So Yeon Cho, Ho Jeong Hong, Sun Cheol Park, and Sung-il Yoon*

Division of Biomedical Convergence, College of Biomedical Science, Kangwon National University

Bacteria migrate in the liquid phase using the flagellum, which plays critical roles in chemotaxis, colonization, and infection. Expression and assembly of each part of the flagellum is elaborately regulated by various proteins. The flagellar filament that extends out of the cell surface are constructed by assembling flagellin proteins through a FliD oligomer. To provide the molecular mechanism of FliD-mediated filament formation, we have determined FliD structures at atomic resolution. *Escherichia coli* (ecFliD) is composed of the D1, D2, and D3 domains. Six ecFliD chains assemble into a hexagonal cap plate using the D2 and D3 domains. In contrast, FliD from *Salmonella enterica* serovar Typhimurium petamerizes into a pentagonal plate through the self-oligomerization interface similar to ecFliD. The cap plates exhibited interdomain and intersubunit flexibility. The D1 domain of ecFliD forms a leg under the cap plate and is structurally homologous to the D1 domain of bacterial flagellin despite low sequence conservation. Based on these structural findings, we propose a structural model for FliD-mediated filament assembly.

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**E023**

Antioxidant Effects of Humulus japonicus Extracts

Jung Woo Chae1, Hui Seon Jo1, Jin Young Lee2, Seung Jae Lee3, and Sung Hyun Joo1*

1Gyeonggi Gido Forest Environment Research Center, 2Department of Herbal Cosmetics Science, Hoseo University, 3Aroma Newtech Co., 4Department of Forestry, Kyungpook University

*Humulus japonicus* (HJ) is an annual plant and a vine plant. It is distributed mainly in Korea and East Asia, and it is mainly used for skin diseases in Korean folk remedies. HJ are killed by winding other plants with vines and have strong fertility. Therefore, it is classified as a harmful plant which is disturbing the ecosystem. To make the utilization, one of the methods for removing this plant, the antioxidant capacity of HJ was verified. Experiment sample was collected in Mulhyanggi arboretum in June and august 2016, and extracted by 70% ethanol and hot water. After that, electron donating ability, ABTS radical scavenging activity and SOD-like activity was determined. In all the experiments, if extract concentration increased, it increased activities. In electron donating experiment, electron donating ability of ethanol extracts is higher than hot water extracts, and June-harvested extract is higher than August-harvested extract. In ABTS experiment, ABTS radical scavenging activity of June-harvested ethanol extract is higher than August-harvested ethanol extract. The scavenging activity of June-harvested and August-harvested hot-water extracts were 67.16% and 82.1% at 1000 μg/ml concentration. As a result of SOD-like experiments, June-harvested ethanol extract was more active than August-harvested ethanol extract at high concentration than 100 μg/ml. The hot-water extracts showed no activity.

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**E024**

Difference in Cellular Environments Affects the *in vivo* Sensitivity of PerR to H$_2$O$_2$

Jung-Hoon Kim, Chang-Jun Ji, Yoon-Mo Yang, Su-Hyun Ryu, and Jin-Won Lee*

Department of Life Science and Research Institute for Natural Sciences, Hanyang University

In many Gram positive bacteria, the metal-dependent transcriptional repressor PerR senses intracellular H$_2$O$_2$ and controls the genes involved in H$_2$O$_2$ resistance. *Bacillus licheniformis*, a close relative to the well-studied model organism *Bacillus subtilis*, contains PerR$_{BL}$. As demonstrated by B. subtilis PerR (PerR$_{BS}$), PerR$_{BS}$ uses either Fe$^{3+}$ or Mn$^{2+}$ as a co-repressor and only the Fe$^{3+}$-bound form of PerR$_{BS}$ senses low levels of H$_2$O$_2$ by iron-mediated histidine oxidation. Interestingly, despite the similar H$_2$O$_2$ sensitivity between PerR$_{BL}$ and PerR$_{BS}$, B. licheniformis expressing PerR$_{BL}$ or PerR$_{BS}$ could sense lower levels of H$_2$O$_2$, and was more sensitive to H$_2$O$_2$ than B. subtilis expressing PerR$_{BS}$ or PerR$_{BL}$. This result suggests that the differences in cellular milieu between B. licheniformis and B. subtilis, rather than the intrinsic differences in PerR$_{BL}$ and PerR$_{BS}$, per se, affect the H$_2$O$_2$ sensing ability of PerR inside the cell and the H$_2$O$_2$-resistance of cell. In contrast, B. licheniformis and B. subtilis expressing *Staphylococcus aureus* PerR (PerR$_{SA}$), which has higher intrinsic H$_2$O$_2$ sensitivity than PerR$_{BL}$ and PerR$_{BS}$, exhibited increased resistance to H$_2$O$_2$ than those expressing either PerR$_{BL}$ or PerR$_{BS}$. This result indicates that the sufficient difference in H$_2$O$_2$ susceptibility of PerR proteins can override the difference in cellular environment and affect the H$_2$O$_2$ resistance of cell.

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E025

Expression, Purification, Crystallization, and X-ray Diffraction Studies of FliD, the Flagellar Cap Protein, from Escherichia coli

So Yeon Cho, Wan Seok Song, Ho Jeong Hong, Sun Cheol Park, and Sung-il Yoon*

Division of Biomedical Convergence, College of Biomedical Science, Kangwon National University

The growth of the bacterial flagellar filament occurs at its distal end by polymerizing flagellin proteins. The flagellar capping protein, FliD, remains stably attached while permitting the flagellin insertion, and is essential for the protection of the flagellar filament and its growth. As a first step to reveal the structural mechanism for the flagellar growth, we have carried out expression, purification, crystallization, and X-ray diffraction studies on FliD from *Escherichia coli* (ecFliD). Full-length ecFliD protein was successfully produced and crystallized. However, the X-ray diffraction capacity of ecFliD was highly limited. To improve X-ray diffraction, we removed flexible N-terminal and C-terminal regions of ecFliD to generate ecFliD<sub>mut</sub> and chemically modified ecFliD<sub>mut</sub> by reductive methylation. An ecFliD<sub>mut</sub> crystal belonged to space group P2<sub>1</sub>, with unit-cell parameters a = 80.98, b = 181.32, c = 110.33 Å, and diffracted to a resolution of 3.00 Å. We have determined the ecFliD<sub>mut</sub> structure by SAD phasing using a selenomethionine-incorporated ecFliD<sub>mut</sub> crystal. The atomic resolution structure of ecFliD<sub>mut</sub> allowed us to design a shorter construct of ecFliD for a higher resolution structure and also to perform structural studies on FliD from *Salmonella Typhimurium*.

E026

Key Residues of Entolimod in TLR5 Activation

Jee-Hyeon Kim, Wan-Seok Song, and Sung-il Yoon*

Division of Biomedical Convergence, Kangwon National University

Acute radiation syndrome (ARS) is the clinical manifestation of pathologies that develops after exposure to toxic doses of whole or partial-body ionizing radiation. High-dose radiation causes severe hematopoietic, gastrointestinal, and cerebrovascular injuries, leading to the increased risk of sepsis due to immunosuppression and even causing death by raising the risk of bleeding. Entolimod is a recombinant protein derived from *Salmonella* flagellin and functions as a Toll-like receptor 5 agonist that reduces radiation toxicity. Entolimod directly binds TLR5 and activates NF-κB signaling. As a result, entolimod inhibits massive apoptosis-mediated cell loss, the main cause of ARS. To investigate the key residues of entolimod for TLR5 activation, we mutated TLR5-binding residues in entolimod and performed a TLR5-reporter cell assay. Among the mutants, Arg90Ala and Glu114Ala exhibited the lowest TLR5 activity. Interestingly, in the entolimod-TLR5 structure model, Arg90 and Glu114 are inserted into a cavity generated by a TLR5 loop. Our mutational study would provide valuable insights into designing second-generation drugs for ARS treatment.

E027

Identification of Antifungal Metabolites Produced by B. velezensis GH1-13 and Its Plasmid-cured Strain

Jiyoung Nam<sup>1</sup>, Jae-yeong Song<sup>2</sup>, Pyoung Il Kim<sup>3</sup>, Yong-Hak Kim<sup>1</sup>, and Chul Won Lee<sup>1,2</sup>*

<sup>1</sup>Department of Chemistry, Chonnam National University, <sup>2</sup>Agricultural Microbiology Division, National Institute of Agricultural Sciences, RDA, <sup>3</sup>Bio Control Research Institute, JBF; *Department of Microbiology, Catholic University of Daegu School of Medicine

The strain GH1-13, an endospore-forming Gram-positive bacterium, was isolated from a reclaimed paddy field and identified as a *Bacillus velezensis* using 16S rRNA and gyrB gene analysis. It has been known that the GH1-13 produces indole acetic acid (IAA) which is promoting the growth of rice root, and shows an antifungal effect against diverse pathogens for rice and main crops. However, the substances exhibiting antifungal activity are not known yet. Therefore, we used LC-MS and the antifungal assay to identify the antifungal substances produced by GH1-13. In addition, a plasmid-cured strain (GH1-13-CP) was constructed to examine the antifungal substance expressed by the plasmid. GH1-13 and GH1-13-CP strain showed inhibitory activity against *F. oxysporum* and *C. gloeosporioides*. The antifungal substances were extracted from the culture media using chloroform, ethyl acetate, and hexane. The extract exhibited the effective antifungal and antibacterial activity. Especially, the GH1-13-CP extract showed the higher inhibitory activity against Gram-negative bacteria. The crude metabolites in the extracts were analyzed by RP-HPLC and LC-MS. From the initial analysis, we identified that the extract contains different types of lipopeptides including fengycin, iturin, and surfactin family. This study suggests that GH1-13 and GH1-13-CP strain could be used as multifunctional agents for plant-promoting and biocontrol purposes. [Supported by grants from RDA (PJ012467).]

E028

LAMMER Kinase is Upstream Regulator for Gas2-mediated Flocculation in Fission Yeast

Soo Jeong Kwon, Won-Hwa Kang, Yoon-Dong Park, and Hee-Moon Park*

Department of Microbiology & Molecular Biology, College of Bioscience & Biotechnology, Chonnam National University

Our previous study reported that LAMMER kinase of *Schizosaccharomyces pombe* regulates cation-dependent, galactose-specific flocculation. We have isolated Gas2 (1,3-beta-glucanosyltransferase) protein from the EDTA-extracted cell-surface proteins (ESP) of the *lkh1<sup>−</sup>* null mutant. While disruption of the gas2<sup>−</sup> gene was not lethal and reduced the flocculation activity of the *lkh1<sup>−</sup>* mutant, the expression of a secreted form of Gas2, from which the GPI anchor addition sequences had been removed, conferred the ability to flocculate upon the wild-type cells. The Gas2-mediated flocculation was inhibited strongly by galactose but not by glucose. Immunostaining analysis showed that the cell surface localization of Gas2 was pivotal for flocculation of fission yeast. In addition, it is well known that gas2<sup>−</sup> is upregulated in transcription factor *mbx2*<sup>−</sup> overexpression mutant. We identified the regulation of *mbx2* expression by *lkh1<sup>−</sup>* using qRT-PCR. Taken together, these results indicate that Lkh1 induces asexual flocculation by not only regulating Gas2 localization but also controlling transcription of gas2<sup>−</sup> through Mbx2.
**E029**

**Elucidation of Role of Escherichia coli YrdC via Temperature Sensitive Mutant Analysis**

Eunsil Choi and Jihwan Hwang*

Department of Microbiology, Pusan National University

The YrdC superfamily is one of the proteins that are highly conserved in almost all organisms sequenced so far. YrdC was suggested to be an essential gene in *Escherichia coli*. Peculiarly, the first 12 nucleotide-deletion of *yrdC* was isolated as a suppressor of temperature-sensitive Release Factor 1. In this study, in order to unambiguously demonstrate that the *yrdC* gene is essential in *E. coli*, we constructed the two *yrdC* mutant strains of *E. coli*, and examined their phenotypes. The *yrdC* mutants did not grow under non-permissive condition and appeared to accumulate 16S ribosomal RNA precursors without significant accumulations of 30S ribosomal subunits. We also cloned human and yeast homologs and demonstrated that they complement the *E. coli* *yrdC*-deletion strain. By mutational study, we showed that the concave surface in the middle of YrdC protein plays an important role in human, yeast, and *E. coli* proteins. Moreover, we showed that the deletion of *yrdC* increased the translational readthrough activity in temperature-sensitive Release Factor 1 strain. We unambiguously demonstrate that the *yrdC* gene is essential in *Escherichia coli*, and human and yeast homologs can complement the *E. coli* *yrdC*-deletion strain. Furthermore, in order to clarify the null phenotype of *yrdC*, we have isolated temperature-sensitive mutant of *YrdC* protein.

**E031**

**Iron-dependent Regulation of Isocitrate Lyase Expression in Pseudomonas aeruginosa**

Sunhee Ha and Woojun Park*

Korea University

Glyoxylate shunt (GS) is a bypass of TCA cycle and composed of two specific enzymes, isocitrate lyase (*aceA*) and malate synthase (*glcB*) in *Pseudomonas aeruginosa*. Unlike *Escherichia coli*, the *aceA* and *glcB* genes are not in an operon in many bacteria, which led us to demonstrated that two genes are differentially regulated under our tested conditions: redox cycling compounds (RACs), iron, and H₂O₂. Addition of exogenous RACs increased the expression of *glcB*, however, the expression of *aceA* was upregulated under H₂O₂ or 2,2′-dipyridyl. Deletion of *aceA* made the strain more sensitive to H₂O₂ and iron-limiting stress. Interestingly, this growth defect of *aceA* mutant was recovered by Mn addition. More severe growth inhibition of the *aceA* mutant was observed under both H₂O₂ and iron limitation, indicating the *aceA* mutant underwent more radical-mediated killing. Measurement of intracellular iron contents, amounts of poverdine, and reduced chrome-azurol-S indicated that the *aceA* mutant has more iron inside cells and needs less iron demand compared to wild type. Biofilm formation of the *aceA* mutant was dramatically more increased under iron-deficient condition than wild type cells. Our data indicated that the *aceA* gene product is involved in metabolic strategy under H₂O₂ and iron limitation in *P. aeruginosa*. DNA-afinity chromatography will be used to identify transcriptional regulators that control the expression of the *aceA* under either H₂O₂ and iron-limited condition.

**E030**

**Molecular Characterization of the Medium-Chain-Length Polyhydroxyalkanoate Depolymerase from Variovorax sp. DSH1**

Hyun Wook Kim¹, Jiwon Lee², Do Young Kim¹, and Young Ha Rhee¹*²

¹Department of Microbiology and Molecular Biology, Chungnam National University; ²Industrial Bio-materials Research Center, KRIBB

An extracellular medium-chain-length polyhydroxyalkanoate (MCL-PHA) depolymerase gene (*phaZc1*) was cloned from the genomic DNA of *Variovorax* sp. DSH1 which was isolated from a soil sample. The *phaZc1* gene consisted of an 837 bp ORF encoding a protein of 278 amino acid with a deduced Mr of 30,692 Da. The deduced amino acid sequence had at least 68% homology to the known MCL-PHA depolymerases from *Pseudomonas* strains and consist of three domains in the sequential order; signal peptide, an N-terminal substrate binding domain, and a catalytic domain, indicating that *phaZc1* belongs to the type IV depolymerases family. The *phaZc1* gene was expressed in *E. coli* under the control of lac promoter and the gene product was purified and biochemically characterized. The enzyme consisted of a monomeric subunit having a Mr of 27.9 kDa as determined by SDS-PAGE. The maximum activity of the enzyme was observed at pH 8.5 and 45°C. Its hydrolyzing activity was significantly sensitive to phenylmethylsulfonyl fluoride, EDTA, thiorosucinimide, and non-ionic detergents, suggesting that serine residues, reduced thiol groups, and essential disulfide bonds are involved in the active site. The highly significant homology of the deduced amino acid sequence of *PhaZc1* with those of the known *Pseudomonas* MCL-PHA depolymerases and several characteristics that are common among these enzymes strongly suggest the possibility of horizontal transfer of the MCL-PHA depolymerase gene in bacterial strains.

**E032**

**Formation of Glucose Starvation-dependent Gdh1 Foci in Saccharomyces cerevisiae**

Woo Hyeon Lee, Ju Young Oh, and Pil Jae Maeng*

Department of Microbiology & Molecular Biology, Chungnam National University

In the yeast *Saccharomyces cerevisiae* glutamate is necessary not only for protein synthesis supporting growth of cells, but also for biosynthesis of glutathione (GSH) which required for ROS scavenging by GSH peroxidase. Glutamate is mainly synthesized from α-ketoglutarate and NH₄⁺ by glutamate dehydrogenase (GHDH). *S. cerevisiae* has two isoforms of NAD⁺-dependent GDH, Gdh1 and Gdh3. We have also uncovered that the differential contribution of the two isozymes to the stress resistance is due to the stationary-phase-specific expression of GDH3 and concurrent degradation of Gdh1. In the present study, we found that Gdh1, but not Gdh3, forms foci at the late stages of cell growth. Furthermore, we revealed that addition of glucose leads to rapid dissolution of the foci and redistribution of Gdh1 in the cytoplasm, indicating that glucose starvation is a key triggering factor for the foci formation of Gdh1. These findings provide broad implications for understanding the differential roles of the two isofunctional GDHs, Gdh1 and Gdh3.
A Novel Mitochondrial Serine O-Acetyltransferase, Encoded by SAT1, Plays a Critical Role in Sulfur Metabolism in the Thermotolerant Methylotrophic Yeast Hansenula polymorpha

Ji Yoon Yeon1, Su Jin Yoo1, Hiroshi Takagi2, and Hyun Ah Kang1*

1Dept. of Life Science, Chung-Ang University, 2Graduate School of Biological Sciences, Nara Institute of Science and Technology

Cysteine is synthesized only via O-acetylserine (OAS) pathway in the thermotolerant methylotrophic yeast Hansenula polymorpha. Here, we carried out the functional analysis of H. polymorpha SAT1 encoding a serine O-acetyltransferase (SATase), which catalyzes the addition of acetyl group to serine, generating a substrate for cysteine synthase. HpSat1p shows a high sequence identity to SATases of other yeast and filamentous fungal species but a low identity to those of bacteria and plants. In the in vitro activity assay, HpSat1p displays much lower enzymatic activity compared to Escherichia coli CysE, a bacterial homolog of SATase. Noticeably, HpSat1p was shown to be subjected to cysteine inhibition with much less degree than CysE. Along with its low expression at the transcription level, the weak enzyme activity of HpSat1p implies that de novo cysteine biosynthesis via OAS pathway might be a rate-limiting step in providing sulfur-compounds in H. polymorpha. As predicted from the presence of mitochondrial targeting sequence (MTS), HpSat1p is observed to localize solely in mitochondria. Interestingly, the expression of a mutant form of HpSat1p without MTS was not able to recover the cysteine auxotrophy of the Hpsat1Δ null mutant strain. Altogether, our results indicate that the mitochondrial localization of HpSat1p is critical in the de novo synthesis of cysteine, which is a key sulfur compound in modulating the cysteine-centered sulfur metabolism in H. polymorpha.

E035

Physiological, Genomic and Transcriptomic Analyses for Understanding of Feather-degradation by Fervidobacterium islandicum AW-1

Ji-Yoon Kim, Yong-Ilk Lee, Leesun Kim, Hyeon-Su Jin, and Dong-Woo Lee*

School of Applied Biosciences, Kyungpook National University

Fervidobacterium islandicum AW-1 is known to degrade native poultry feathers within 48 h at 70°C. Although several keratinases were found to be involved in feather degradation, its degradation mechanism still remains unclear. Herein we investigated physiological properties of the extremophilic anaerobe F. islandicum AW-1 and performed its transcriptomic analyses. It was observed that nutrient-dependent changes in the fatty acid composition of the outer membrane structures of F. islandicum AW-1 were significant, and that cellular attachment to native feathers and their degradation occurred in a nutrient-dependent and growth phase-specific manner. Subsequently, transcriptomic analysis by RNA-Seq with F. islandicum AW-1 cells grown on various nutrients demonstrated that general-stress response proteins and specific sets of metabolic pathways involved in cofactor and vitamin biosynthesis, membrane biosynthesis, chemotaxis, and motility were highly up-regulated. Notably, some hypothetical proteins up-regulated on feathers exhibited high levels of sequence similarity with DOC protein family and capsule biosynthesis protein, which are known as growth-dependent expression proteins. Taken together, it is strongly suggested that feather-degradation by F. islandicum AW-1 could be as a consequence of stringent-response, resulting in the acceleration of its degradation capability, concomitant with overexpression of fis family, chemotaxis protein and other secretory protein.

E034

Morphological Changes of Saccharomyces cerevisiae KCTC 7296 under Sub-MIC with Polymyxin-B Antibiotics

Hyungang Kim, Saemi Kwon, Hosik Shin, Sungjin Park, and Kuyjong Kim*

Department of Biology, Gangneung-Wonju National University

Polymyxin-B has a bactericidal action against almost all Gram-negative bacteria except a few genera. It is reported that this antibiotic bind to the cell membrane and alter its structure, making it more permeable. We determined minimum inhibitory Concentration (MIC) against Polymyxin-B for Saccharomyces cerevisiae, which is eukarotic. MIC was 15.62 μg/ml against Polymyxin-B. We investigated the morphological changes and division patterns of S. cerevisiae yeast under sub-MIC by using light microscope and scanning electron microscope. Much more budding cells were observed under sub-MIC than controlled state of culture at 5–7 days of culture without antibiotics. After 9–12 days of broth culture, The culture under sub-MIC was coaggregated with each other seen on the light microscope. In stead, the culture under normal state of condition was seen regularly orderline of culture.

E036

Interactions of Glyphosate Herbicide with Antibiotics

Ji-Young Pan, JungAe Kim, and Jae-Gu Pan*

Superbacteria Research Group, Infectious Disease Research Center, KRIIBB

Glyphosate is one of the most widely used herbicides. Apart from being known as an effective “once in a century herbicide”, glyphosate is an antimicrobial agent that blocks the shikimate pathway, essential for the production of aromatic amino acids and folates in plants and bacteria. Following the introduction of the genetically engineered (GE) crops and increased use of glyphosate in agriculture, we are all exposed to a far higher levels of glyphosate compared to the past. Therefore, it is essential to study interactions between glyphosate and commonly used antibiotics. In this study, we have carried out checkerboard assay to investigate interactions between different classes of antibiotics and glyphosate. Two different strains of each Gram-positive and Gram-negative bacteria were used in combination with different classes of antibiotics. The results show that the formulated glyphosates produced by Monsanto or Dong-bu Chem were far more toxic to bacteria with higher levels of antimicrobial activities than pure glyphosate. Interactions between antibiotics and glyphosate can be classified into non-interactive, synergistic or antagonistic. Different types of interactions were observed depending on the strains of bacteria, classes of antibiotics and glyphosate samples used.
Characterization of a Chemoreceptor, PctC, on the Growth and Chemotactic Responses to Triton X-100 in Pseudomonas nitroreducens TX1

Po-Chun Tsai and Shir-Ly Huang*
Institute of Microbiology and Immunology, National Yang Ming University, Taiwan

Triton X-100 (Octylphenol polyethoxylates) is a non-ionic surfactant used in many industrial and agricultural applications. It is often discharged into the environment, which leads to being finally degraded into octylphenol, which regrading as an endocrine disrupter to wild lives and humans. Pseudomonas nitroreducens TX1 is capable to use high concentrations of Triton X-100 as sole carbon source. In our previous study, identification of essential genes involved in the growth in this surfactant was conducted by transposon mutagenesis. Over 30,000 mutants were screened, and 42 genes have been identified from 93 non-growers. One mutant was disrupted in a chemotactic gene, which is highly similar to PctC, which was reported to be responsible for the chemotaxis to amino acids in P. aeruginosa. In addition, some specific chemotaxis genes were found to facilitate the degradation of aromatic toxic compounds in P. putida F1. We are investigating the role of PctC in the growth of P. nitroreducens TX1 in Triton X-100 and its chemotaxis responses to amino acids or a series of ethoxylated surfactants. This study is the first to investigate the bacterial chemotaxis responses toward surfactants.

Regulation of Anaerobic Nitrate and Nitrite Respiratory by the Iron Nitrosyl Complexes in FNR Transcriptional Regulator

Mu-Cheng Hung*1,2 and Steve S.-F. Yu1
1Institute of Chemistry, Academia Sinica, Taipei 11529, Taiwan, 2Chemical Biology and Molecular Biophysics Program, Taiwan International Graduate Program (TIGP), Academia Sinica, Taipei 11529, Taiwan

FNR, the Fumerate and Nitrate Reduction Regulator, is a widely conserved transcription factor across bacteria that contains 4Fe-4S cluster sensitive to the presence of dioxygen molecules. This system governs the physiological status from aerobic respiration to anaerobic nitrate respiration, i.e., the fermented growth of E. coli. When E. coli BL21DE (PLyS) grown with transformed plasmid pET22b containing fnr gene insert in anaerobic condition by the presence of the nitrate salts in LB buffer, significant amounts of recombinant FNR are accumulated from the SDS-Page analysis. The recombinant FNR with poly-histidine can be easily purified through Ni-NTA column chromatography. These purified FNR is subjected for EPR measurement. We observed a strong paramagnetic signal appeared at $g_v = 2.03$ indicating the formation of iron dinitrosylated complexes within the proteins. The iron contents of unit recombinant FNR monomer isolated from the anaerobic growth with and without the nitrate salts are 2.42 and 1.83, respectively. We ensure that there is a formation of Roussin’s Red ester (RRE) after the nitrosylation of FNR protein in vivo with the further reduction mediated by dithionites for the observation of EPR characteristic ($g_\perp = 2.005$, $g_\parallel = 1.97$) of anionic Roussin’s Red ester. The gene regulation of FNR in E. coli have further indicated that nitrosylated FNR in E. coli under anaerobic respiratory are auto-regulated.
Multiple Modes of Signaling by Redox-active Compounds as Monitored by Iron-based and Thiol-based Sensor-regulator Systems

Kang-Lok Lee, Ji-Sun Yoo, Gyeong-Seok Oh, Atul Singh, and Jung-Hye Roe*
Seoul National University

Bacteria in natural habitats are exposed to myriad redox-active compounds (RACs), which include producers of reactive oxygen species and reactive electrophile species (RES) that alkylate or oxidize thiols. RACs can induce oxidative stress in cells and activate response pathways by modulating the activity of sensitive regulators. However, the effect of a certain compound on the cell has been investigated primarily with respect to a specific regulatory pathway. Since a single compound can exert multiple chemical effects in the cell, its effect can be better understood by time-course monitoring of multiple sensitive regulatory pathways that the compound induces. We investigated the effect of representative RACs by monitoring the activity of three sensor-regulators in the model actinobacterium Streptomyces coelicolor; SoxR that senses reactive compounds directly through oxidation of its [2Fe-2S] cluster, CatR/PerR that senses peroxides through bound iron, and an anti-sigma factor RsrA that senses RES via disulfide formation. The course and magnitude of induction of their target transcripts were monitored to predict the chemical activities of each compound in S. coelicolor. This study showed that multiple chemical activities of a reactive compound can be conveniently monitored in vivo by examining the temporal response of multiple sensitive regulators in the cell to reveal novel activities of the chemicals.

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Analysis of Nitric Oxide Production and Its Role during Fungal Differentiation

Mayura Veerana1, Anchalee Pengkit1, and Gyungsoon Park1,2,4*
1Plasma Bioscience Research Center, Kwangwoon University, 2Department of Electrical and Biological Physics, Kwangwoon University

We analyzed production of intracellular NO and its possible roles during development of Neurospora crassa, a model filamentous fungus. Intracellular nitric oxide was detected using a fluorescent indicator DAF-FM DA. Our results show that nitric oxide is produced during conidiation and hyphal development in Vogel’s minimal liquid media. When intracellular NO was removed, hyphal branching was inhibited and conidiation was delayed. The exogeneous nitric oxide seemed to promote hyphal extension and conidia formation. NO scavenging reduced transcription of con-10 and con-13, genes preferentially expressed during conidiation. This result suggests that intracellular nitric oxide may be associated to the regulation of circadian rhythm and conidiation in N. crassa. This hypothesis is now being tested.

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Indole-induced Activities of β-Lactamase and Efflux Pump Confer Ampicillin Resistance in Pseudomonas putida

Jisun Kim, Bora Shin, Chulwoo Park, and Woojun Park*
Laboratory of Molecular Environmental Microbiology, Department of Environmental Sciences and Ecological Engineering, Korea University

Indole, which is widespread in microbial communities, has received attention because of its effects on bacterial physiology. Pseudomonas putida and Pseudomonas aeruginosa can acquire ampicillin (Amp) resistance during growth on indole-Amp agar. Transcriptome, mutant, and inhibitor studies have suggested that Amp resistance induced by indole can be attributed to increased gene expression of ttgAB encoding two genes of RND-type multidrug efflux operons and an ampC encoding β-lactamase. Expression, enzyme activities, and mutational analyses indicated that AmpC β-lactamase is important for acquiring Amp resistance of P. putida in the presence of indole. Here, we show, for the first time, that volatile indole increased Amp-resistant cells. Consistent with results of the volatile indole assay, a low concentration of indole in liquid culture promoted growth initially, but led to mutagenesis after indole was depleted, which could not be observed at high indole concentrations. Interestingly, ttgAB and ampC gene expression levels correlate with the concentration of indole, which might explain the low number of Amp-mutated cells in high indole concentrations. The expression levels of genes involved in mutagenesis, namely rpsO, recA, and mutS, were also modulated by indole. Our data indicates that indole reduces Amp-induced heterogeneity by promoting expression of TtgABC or MexAB-OprM efflux pumps and the indole-induced β-lactamase in P. putida and P. aeruginosa.

Enterococcus faecium-Lactamase and Efflux Pump

Jongbin Park1, Gwi-Deuk Jin1, Suk Jung Choi1, Inhwan You1, Jihyun Won2,4,5, and Eun Bae Kim1,2,4*
1Department of Animal Life Science, College of Animal Life Science, Kangwon National University, 2Institute of Animal Resources, College of Animal Life Science, Kangwon National University, 3Division of Applied Animal Science, College of Animal Life Science, Kangwon National University

Enterococcus faecium is a Gram-positive bacteria found in the gastrointestinal tracts (GIT) and frequently found in the soybean and chicken gut. To characterize E. faecium genomes, we isolated and identified 12 strains from chicken feces and the others from soybean paste (Cheonggukjang). We sequenced the genomes of 12 E. faecium strains. All genome sequences were assembled by using SPAdes 3.10.1 and genomes for annotated by RAST (Rapid Annotation using Subsystem Technology). We summarized their subsystems, two groups showed no significant difference in genome size (2,740,290 ± 96,539 bp). Any subsystems groups were not significantly different in the number of member genes. We will further analyze difference in SNPs and presence of specific genes.

Keywords: Comparative Genomics, Enterococcus faecium

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Aconitase, a highly conserved protein found in prokaryotes and eukaryotes, is required for converting citrate to isocitrate in TCA cycle. Additionally, it has been reported that aconitase functions as a nucleic acids binding protein. For example, in mammals, the cytosolic aconitase acts as an iron regulatory protein (IRP), binding to RNA hairpin structures known as IREs (Iron-responsive elements) within 5’ or 3’ UTR of specific RNAs. In the fission yeast S. pombe, two genes encode aconitases; aco1+, aco2+. Unlike Aco1, Aco2 is a fusion protein between aconitase and mitochondrial ribosomal protein L21, containing both mitochondrial targeting sequence (MTS) and a nuclear localization signal (NLS). Fluorescence microscopy experiments revealed that Aco2-GFP resides not only in mitochondria but also in the cytosol and the nucleus. To determine the role of nuclear Aco2, we constructed a NLS-deleted Aco2 mutant (aco2∆NLS), and found several interesting phenotypes of aco2∆NLS mutant. In the absence of Aco2 in nucleus, some iron homeostasis-related mRNAs were accumulated in aco2∆NLS, suggesting the role of nuclear Aco2 in regulating gene expression related with iron homeostasis. Evidences supporting these ideas will be presented.

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Epidemiological Characterization of Influenza Viruses Isolated in Busan, 2015-2016 Season

Su Jeong Hwang, Dong Ju Park, Hee Soo Koo, Ho Cheol Yun, Pyeung Tae Gu, and Mi Ok Lee*

Busan Metropolitan City Institute of Health & Environment

Influenza viruses cause acute respiratory tract infections and is often associated with increased rates of hospitalization and death. This study was carried out to investigate the epidemiological characterization for Influenza viruses in Busan, from September 2015 to August 2016. Total of 639 specimens (throat swabs) were collected from influenza-like illness patients and patients with acute respiratory tract infection. Multiplex real-time RT-PCR(RT-PCR) was performed to detect six influenza virus (A, B, A(H1N1), A(H3N2), A(H5N1), A(H1N1)pdm09) and detected 34(5.3%) cases of influenza viruses. Among 80 positive specimens, 17(2.7%) cases were Influenza type B, 13(2.0%) cases of A(H1N1)pdm09, and 4(0.6%) cases of type A(H3N2). The virus detection rate was the highest (32.4%) in 0-6 year-old group and was the lowest (8.8%) in 50-64 year-old group. Influenza A(H1N1)pdm09, A(H3N2) and B viruses were all sensitive to NA inhibitors, Oseltamivir (Tamiflu), Zanamivir (Relenza) and Peramivir. But both A(H1N1)pdm09 and A(H3N2) were all resistant against M2 inhibitor (Amantadine). To enhance and improve the influenza laboratory surveillance system, continuous cooperation with participating institutes is necessary and development of methods on the detection and analysis is also important.

Genomic Differences among Groups of Lactobacillus plantarum Strains Including Animal and Plant Origins

Suk Jung Choi1 and Eun Bae Kim1,2,∗

1Department of Animal Life Science, College of Animal Life Science, Kangwon National University, 2Division of Applied Animal Science, College of Animal Life Science, Kangwon National University

Lactobacillus plantarum is a species of probiotics that helps animal intestinal health and is a lactic acid bacterium (LAB) that found in a wide variety of habitats. As such adaptations to various environmental conditions are well known, we investigated to see if there are genetic differences between different origins of L. plantarum strains using pan-genomic methods. For the comparative genomic study, we used genomes of 105 L. plantarum strains that are available from the NCBI GenBank. On the basis of the 142 core genes that are shared by all strains, 105 strains were clustered into five major groups. However, such group-specific genes were not clearly associated with habitats, especially between the animal and plant origins. Our findings showed that there are critical differences among genetically clustered groups but such genetic differences might not be influenced by the living environments. We were able to observe distinct characteristics of each group, and why each group has these features and what are the factors that affect each group should be studied in the future.

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Investigation of Genetic Determinants Responsible for Temperature-dependent Exopolysaccharide Production in Ralstonia solanacearum

Seung Yeup Lee, Geun Ju Son, Shabir Ahmad, Kihyuck Choi, and Seon-Woo Lee*

Applied Bioscience of Dong-A university

Ralstonia solanacearum a soil borne plant pathogenic bacteria, is the causative agent of bacterial wilt which is one of the major diseases of tomato plant. When R. solanacearum colonize xylem of the tomato plant, it produces exopolysaccharide [EPS], a major virulence factor for disease development. Interestingly, EPS is not produced by R. solanacearum at 37°C while it is normally produced at 30°C. Previously, 3,900 transposon Tn5-insertion mutants were generated using R. solanacearum strain SL341. Among these, a mutant, thirty seven EPS positive (TSEP), produced the regular amount of EPS at both 37°C and 30°C. Sequence analysis revealed that Tn5 was inserted in the upstream regulatory sequences of rpoE1 gene which encodes an alternative sigma factor of RNA polymerase, involved in transcription initiation. However, rpoE1 gene has not been characterized in R. solanacearum in terms of EPS production. In order to characterize the role of rpoE1 and its upstream regulatory sequences for EPS production in a temperature-dependent manner, the complementation vectors were constructed. The complementation construct, carrying the upstream regulatory DNA of rpoE1 gene with its downstream two orfs successfully restored EPS production. This result suggests that regulated expression of rpoE1 and its downstream two orfs are necessary to produce EPS in a temperature-dependent manner in R. solanacearum.

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**F009**

Replication of *Vibrio cholerae* Classical CTX Phage

Eun Jin Kim1,2, Hyun Jin Yu1,2, Da Seu Ri Cha1,2, Dong-Hoon Shin1,2, Jae Hyun Lee1,2, and Dong Wook Kim1,2,*

1College of Pharmacy, Hanyang University, 2Institute of Pharmacological Research, Hanyang University

Experimental evidence for the replication of CTX-cla phage of classical biotype strains of *Vibrio cholerae* O1 serogroup and transduction of El Tor biotype strains by CTX phages in laboratory conditions have thus far remained undemonstrated. A plasmid-based CTX phage replication system that can support the replication of CTX-cla, CTX-cla, CTX-2, and CTX-O139 has been established. The replication of CTX-2 from the tandem repeat of lysogenic CTX-2 in Wave 2 El Tor strains has also been presented. El Tor strains can be transduced by CTX phages in vitro by introducing a point mutation in toxT, the transcription activator of tcp gene cluster and cholera toxin gene. The same change in toxT also increases the expression of cholera toxin of El Tor strains in a single phase culture. The results in this report provide experimental evidence of genetic mechanism of evolution of *V. cholerae*.

**F011**

Comparative Genomics on of *Lactobacillus plantarum* from Pigs and Kimchi with Antimicrobial Activities

Gwi-Deuk Jin1, Jongbin Park1, Jihyun Won1, Sook Jung Choi1, and Eun Bae Kim1,*

1Department of Animal Life Science, College of Animal Life Science, Kangwon National University, 2Institute of Animal Resources, College of Animal Life Science, Kangwon National University, 3Division of Applied Animal Science, College of Animal Life Science, Kangwon National University

*Lactobacillus plantarum* is a member of *Lactobacillus* spp. and found in the human or animal gut. Antimicrobial activities of *L. plantarum* have effect on Gram positive bacteria or Gram negative bacteria. For this reason, it is useful as probiotics in the pig industry South Korea. While biological functions of probiotics are well known, their genetic functions are still unknown. In this Study, we performed comparative genomic analysis on *L. plantarum* from pigs and kimchi with high/low antimicrobial activity. We sequenced genomes of 10 *L. plantarum* strains with high activity and 9 *L. plantarum* strains with low activity. Draft genomes were obtained by using a genome assembler, SPAdes (3.8.1). The draft genomes were annotated by RAST (RAPid Annotation using Subsystem Technology), and we summarized their subsystems. There was no significant difference in genome size between groups of *L. plantarum* that are higher or lower in microbial activity. In several gene groups, the number of genes were different between the two groups. Here, we found that there are several differences. We will analyze the genomes more in detail.

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**F010**

Whole-genome Comparison of *Erwinia amylovora* Strains that Caused a Fire Blight Outbreak in Korea

Ju Yeon Song1, Yeo Hong Yun2, Gi-Don Kim3, Seong Hwan Kim3, Sung Jin Lee4, Keum-Hee Lee2, Moon Nam5, and Jihyun F. Kim1,2,*

1Department of Systems Biology and Division of Life Sciences, Yonsei University, 2Department of Microbiology, Dankook University, 3Animal and Plant Quarantine Agency, 4Xenotype inc., 5Strategic Initiative for Microbiomes in Agriculture and Food, Yonsei University

*Erwinia amylovora* is a plant pathogenic bacterium which causes fire blight in Rosaceae plants. Since the disease is highly contagious and results in serious losses, it has been regulated as a quarantine disease. Recently, fire blight emerged in Korea, and strains of *E. amylovora* were isolated from lesions of infected trees. Five strains of those were selected and subject to whole-genome shotgun sequencing. Each of the five strains had two circular replicons: a 3.8-Mb chromosome and a 28-kb plasmid. The genome sequences were used to compare with those of previously published *E. amylovora* strains, which have been isolated from different hosts or geographical origins. Genome synteny was analyzed and sequence variations in terms of nucleotide substitution, inversion, insertion, and deletion were detected. A phylogenomic analysis was performed to infer the evolutionary relationships among *E. amylovora* strains, and it indicated that the Korean isolates are closely related to a lineage of North American strains. We are in the process of analyzing additional genomes, and the results may provide useful information to understand the genome dynamics of *E. amylovora* strains in Korea and to develop genetic makers for surveillance of the pathogen.

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**F012**

Complete Genome Sequence of *Lactobacillus helveticus* LHS, a Korean Probiotic Isolate

Yusook Chung2, Min-Jung Kwak3, Hong-man Kim3, Myung Jun Chung2, and Jihyun F. Kim1,*

1Department of Systems Biology and Division of Life Sciences, Yonsei University, 2R&D Center, Cell Biotech Co., Ltd.

*Lactobacillus helveticus* is a member of lactic acid bacteria and is widely used as a starter culture in the manufacture of fermented dairy products, because of its high metabolic function to produce lactic acid and nutty flavor. Studies on the species revealed that it has an ability to adhere to epithelial cells, and suggested that it may prevent gastrointestinal tract infection and modulate the immune system. Here we report the genome sequence of *Lactobacillus helveticus* LHS, which was isolated from a healthy adult Korean. DNA sequencing was performed by the PacBio platform for genome assembly and by Illumina miseq for sequence accuracy. The complete genome consists of a circular chromosome of 727,711,759 bp and two small plasmids. Genome annotation indicated that it has 2,311 coding sequences, 64 tRNAs, and 12 tRNAs operons. The genome sequence of *Lactobacillus helveticus* LHS was compared with those of nine other strains in the species, along with those of *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus*, and the results will be presented.
**F013**

Sequential Modulation of RNase III and RNase G Expression in Response to Host Environment Conditions Promotes Pathogenicity through the Control of hns mRNA Abundance in Salmonella Typhimurium

Minho Lee1, Minkyung Ryu2, Hong-Man Kim3, Yong-Hak Kim3, and Kangsaeok Lee*1

*1Department of Life Science, Chung-Ang University, 2Department of Microbiology, School of Medicine, Catholic University of Daegu

Bacterial ribonucleases regulate gene expression through RNA processing and decay. Among them, RNase G (Rng) is an endoribonuclease, which is involved in RNA processing and degradation of a subset of mRNAs in Escherichia coli. However, its physiological role remains largely uncharacterized. Here, we report that RNase G controls expression levels of histone-like nucleoid structuring protein (H-NS) encoded by hns, which are strongly associated with the pathogenicity of S. Typhimurium cells in both epithelial cells and mice. In fact, RNase G expression in S. Typhimurium cells were induced when they were exposed to high-salt condition and infected into epithelial cells, which coincided with decreased expression levels of hns. 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. In addition, we show that the induced expression of RNase G in host environments is attributable to reduced RNase III (Rnc) cleavage activity on rgg mRNA. In conclusion, 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 Salmonella in host cell. [This work was supported by National Research Foundation of Korea (NRF-2015R1A1A1A01054585 to M. L. and NRF-2014R1A2A2A09052791 to K. L.)]

**F015**

Complete Genome Sequence of Salmonella enterica Serovar Virchow FORC_038 Isolated from Raw Chicken Meat in South Korea

Jin Su Song1,2, Han Young Chung1,2, Eun Jung Na1,2, Han Hyeok Lim1,2, Anna Cho1,2, Duhyun Ko1,2, and Sang Ho Choi1,2*

1Department of Agricultural Biotechnology, Center for Food Safety and Toxicology, Seoul National University, 2Food-borne Pathogen Omics Research Center (FORC), Seoul National University

Salmonella enterica serovar Virchow has been identified as an important cause of poultry meat outbreaks, showing multiple resistance to antibiotics. The genome of S. Virchow FORC_038 isolated from raw chicken meat was completely sequenced using Illumina MiSeq and PacBio RS II platform. The genome consists of a circular chromosome of 4,938,076 bp with a GC content of 51.92%. BLAST analysis against VFD (Virulence Factor Database) identified that the genome of FORC_038 contains the genes encoding SPI-1 (Salmonella pathogenic island 1) and SPI-2 effectors. Afterwards, the invasion assay towards the HEla cells revealed that the invasion activity of FORC_038 was higher than the positive control such as virulent strain SL1344. Additional genomic analysis revealed that genes related to resistance such as β-lactams were widely disseminated on the chromosome of FORC_038. In silico prediction was phenotypically confirmed by the Kirby-Bauer Disk Susceptibility Test, indicating that FORC_038 is resistant to the antibiotics bona fide. Taken together, these results indicated that the genome of FORC_038 contains the genes encoding effector proteins and related to resistance to many antibiotics. Providing new insight on S. Virchow, this report will support further research in prevention and epidemiological investigation of Salmonella outbreaks in South Korea. [This research was supported by a grant (14162MFDS972) from Ministry of Food and Drug Safety in 2017.]

**F014**

Genome and Transcriptome Analyses of Vibrio vulnificus FORC_036, a Food-borne Pathogen Isolated from a Surf Clam

Han Young Chung1,2, Se Jong Gil1,2, Eun Jung Na1,2, Jin Su Song1,2, Garam Choi1,2, and Sang Ho Choi1,2*

1Department of Agricultural Biotechnology, Center for Food Safety and Toxicology, Seoul National University, 2Food-borne Pathogen Omics Research Center (FORC), Seoul National University

Vibrio vulnificus is a Gram-negative marine pathogen that causes gastroenteritis in humans. In order to characterize the pathogenesis of V. vulnificus at the genomic level, V. vulnificus FORC_036 was isolated from a surf clam and then it was completely sequenced. The genome of the FORC_036 strain is composed of two circular chromosomes, and a plasmid. Among the complete genome sequences of V. vulnificus strains, the highest average nucleotide identity (ANI) value was obtained between genomes of the FORC_036 strain and CMCP6, which was isolated from a patient. Comparative genome analysis of FORC_036 and CMCP6 revealed that genome of the FORC_036 strain has an additional virulence factor including thermostable hemolysin delta-VPH. RNA sequencing of the FORC_036 strain exposed to small octopus indicated that many genes, probably involved in adherence and N-acetylgalactosamine (GalNAc), were up-regulated. Interestingly, the up-regulated genes include the genes related to iron uptake and oxidative stress resistance, suggesting that the FORC_036 strain neutralizes the host defense system to utilize the animal as a reservoir for survival. The genomic and transcriptomic analyses of the FORC_036 strain provide new insights for understanding the molecular mechanisms by which V. vulnificus survives in small octopus. [This research was supported by a grant (14162MFDS972) from Ministry of Food and Drug Safety in 2017.]
Genome and Transcriptome Analyses of Vibrion vulnificus FORC_037, a Food-borne Pathogen Isolated from Soft-shell Clam (Mya arenaria oonogai)

Eun Jung Na,1 Kyung Ku Jang,1 Garam Choi,1 and Sang Ho Choi1,2,4
1Department of Agricultural Biotechnology, Center for Food Safety and Toxicology, Seoul National University, 2Food-borne pathogen Omics Research Center (FORC), Seoul National University.

To study Vibrion vulnificus, an opportunistic human pathogen, FORC_037 was isolated from a soft-shell clam. Its whole genome consists of two chromosomes and a plasmid, and altogether contains 4,506 protein coding genes, 118 tRNA and 34 rRNA genes. Genes encoding several hemolysins and iron uptake-related proteins were found. Average nucleotide identity analysis with nine other whole genomes of V. vulnificus showed that FORC_037 is most closely related to FORC_017 and CMCP6, a clinical isolate. Comparative genome analysis with CMCP6 revealed that FORC_037 has additional virulence factors such as accessory cholera enterotoxin and zonula occludens toxin. This may explain why FORC_037, an environmental isolate, exhibits a high level of cytotoxicity in lactose dehydrogenase release assay. FORC_037’s transcriptome was sequenced to determine differentially expressed genes upon contact with small octopus, often consumed raw in South Korea. The results hinted that V. vulnificus may use the octopus as a reservoir, as genes related to adhesion, galactose utilization, oxidative stress resistance and iron uptake were upregulated and motility-related genes were downregulated. A number of putative virulence factors, such as type II secretion system proteins, were upregulated. More research is imperative to further our understanding of this important pathogen and to prevent future outbreaks.

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The C2H2 Zinc Finger Protein AslA is a Novel Transcriptional Regulator of Development and Secondary Metabolism in Aspergillus nidulans

Yong Jin Kim, Yeong Man Yu, and Pil Jae Maeng*
Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University.

In Aspergillus nidulans, multiple regulatory factors are involved in coordinated regulation of development and secondary metabolism. Here, we report functional features of the putative C2H2 zinc finger transcription factor, AslA, in relation to asexual differentiation and secondary metabolism. Deletion of aslA led to significantly reduced conidia formation and expression of the conidiation-specific genes, brlA, abaA and wetA. On the other hand, ΔaslA mutant showed increased sterigmatocystin biosynthesis and enhanced expression of the relevant genes, aflR and stcU. aslA deletion also caused increased expression of the genes required for terrauquinone biosynthesis, tdA and tdbB. Overexpression of aslA caused quite the opposite effect on both the phenotypes and gene expression related to conidiation and secondary metabolite production in comparison to aslA deletion. AslA was localized in the nuclei of hyphae, conidiophores, but not in those of conidia. The C-terminal glutamine-rich domain (230-260) of AslA was found to function as a transcriptional activation domain. Either of the two aslA orthologues, AfaslA and AfaslA from Aspergillus fumigatus and Aspergillus flavus, respectively, complemented the phenotypes of ΔaslA mutation related to conidiation and secondary metabolism. It thus seems that the function of aslA is highly conserved among the aspergilli closely related to A. nidulans.

Unraveling the Role of Pseudouridylation in a Fungal Pathogen Cryptococcus neoformans

Seung-Heon Lee, Jin-Young Kim, and Yong-Sun Bahn*
Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University.

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 S. cerevisiae, 8 proteins exist as stand-alone pseudouridylases, and each protein has specific pseudouridylation sites and roles. To discover the characteristics of pseudouridylase synthases, we aim to identify 6 putative pseudouridylases in C. neoformans. We sorted out putative pseudouridylases based on the annotation 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, A. fumigatus, C. albicans, and N. crassa. To characterize the function of pseudouridylases, we constructed 8 mutant strains representing 2 putative pseudouridylase synthases so far. Currently, we are in the process of constructing deletion mutants of the remaining 4 genes, and examining their phenotypic traits under various conditions. By using pseudouridylation RNA sequencing, we will identify pseudouridylated RNA transcripts and characterize their role in pathogenicity of C. neoformans.

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Functional Characterization of EKC/KEOPS Complex in the Fungal Sterol Regulation

Eunji Jeong, Dong-Gi Lee, and Yong-Sun Bahn*
Department of Biotechnology, Yonsei University

EKC/KEOPS complex, consisting of Kae1, Pcc1, Cgi121 and Bud32, is known to be essential for several cellular functions. Bud32 was shown to be indispensable for the pathogenicity of diverse fungal pathogens. Bud32 is evolutionarily conserved in eukaryotes; however, homology between fungal Bud32 and human Bud32 is significantly low. In addition, the human EKC/KEOPS complex appears to have functions distinct from those of fungi. For this reason, Bud32 can be a novel antifungal drug target. Here, we identified and functionally characterized the EKC/KEOPS complex with relation to Bud32 in a global fungal meningitis pathogen Cryptococcus neoformans. The components of the EKC/KEOPS complex are conserved in C.neoformans. In this study, the \textit{bud32}$\Delta$, \textit{kae1}$\Delta$, and \textit{pcc1}$\Delta$ mutants showed severe growth defects, indicating that each component of the EKC/KEOPS complex is critical for its function in \textit{C. neoformans}. Previously, it was shown that the \textit{bud32}$\Delta$ mutant is resistant to fluconazole treatment. Here, we found that Bud32 regulates expression of a number of ergosterol biosynthesis genes. In addition, most of Bud32 related phenotypes were also found in the KEOPS complex mutant. Therefore, this study suggests that each component of the KEOPS complex could be effective antifungal drug targets.

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Systematic Functional Analysis of Phosphatases in the Fungal Pathogen Cryptococcus neoformans

Jae-Hyung Jin¹, Dong-Gi Lee¹, Kyung-Tae Lee¹, Yee-Seul So¹, Kwang-Woo Jung¹, Yeonseon Lee¹, Eunji Jeong¹, Dongpil Lee¹, Seung-Heon Lee¹, Jin-Young Kim¹, Eun-Ha Jang¹, Jaeyoung Choi¹, Anna F. Averette³, Joseph Heitman³, Yong-Hwan Lee³, and Yong-Sun Bahn¹*¹
1 Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, ²The Samuel Roberts Noble Foundation, Ardmore, Oklahoma 73401, USA, ³Department of Molecular Genetics and Microbiology, Medicine, and Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, USA, ⁴Department of Agricultural Biotechnology, Seoul National University

Cryptococcus neoformans causes fatal cryptococcal meningoencephalitis in immunocompromised patients as well as immunocompetent people. Despite its clinical importance, the signaling networks governing its virulence remains elusive and therapeutic options for treatment of 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 fungal pathogens. We selected putative phosphatases based on annotation in the C. neoformans var. grubii genome database provided by the Broad Institute and NCBI and performed a BLAST search with their protein sequences to identify any 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 constructed 182 signature-tagged gene-deletion strains representing 94 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.

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The Complete Genome Sequence of *Bacillus velezensis* Strain GH1-13 Reveals Agriculturally Beneficial Properties and a Unique Plasmid

Sang Yoon Kim, Hajin Song, Mee Kyung Sang, Hang-Yeon Weon, and Jaekyeong Song*

Agricultural Microbiology Division, National Institute of Agricultural Sciences (NIAS), Rural Development Administration (RODA)

Members of the genus *Bacillus* are widely used in agriculture due to their ability to promote plant growth and/or suppress phytopathogens. The bacterial strain *Bacillus velezensis* GH1-13, isolated from rice paddy soil in Korea, has been shown to promote plant growth and have strong antagonistic activities against plant pathogenic microbes. Here, we report the complete genome sequence of GH1-13, revealing that it possesses a single 4,071,980-bp circular chromosome with 46.2% GC-content. The chromosome encodes 3,930 genes, and we have also identified a unique plasmid in the strain that encodes a further 104 genes (71,628 bp and 31.7% GC-content). The genome was found to contain various enzyme-encoding operons, including indole-3-acetic acid (IAA) biosynthesis proteins, 2,3-butanediol dehydrogenase, various non-ribosomal peptide synthetases, and several polyketide synthases. These properties are responsible for the promotion of plant growth and the biosynthesis of secondary metabolites. They therefore have multiple beneficial effects that could be applied to agriculture. The complete genome sequence of *B. velezensis* GH1-3 contributes to our understanding of this beneficial strain and will encourage research into its development for agricultural or biotechnological applications, enhancing productivity and crop quality.

Exploiting the DNA Damage Response Pathway to Unravel Radiation Resistance Regulatory Networks in the *C. neoformans*

Kwang-Woo Jung1, Dong-Ho Kim1, Sangyong Lim1,*, and Yong-Sun Bahn1,*

1Research Division for Biotechnology, Korea Atomic Energy Research Institute, 2Department of Biotechnology, Yonsei University

The living organisms are constantly exposed to DNA damage caused by endogenous events and exogenous stress. To counteract DNA damage stress, eukaryotic cells harbor evolutionarily conserved surveillance mechanisms, such as DNA damage checkpoint system. Our previous study revealed that a unique transcription factor, Bdr1, whose expression is regulated by DNA damage response protein kinase Rad53, governs DNA damage responses and gamma radiation resistance by controlling expression of DNA repair genes. However, the DNA damage signal cascade mediated by Rad53 kinase is not well understood in the basidiomycetous fungus, *C. neoformans*. Here, we performed genome-wide transcriptome analysis using RNAseq to identify genes regulated by DNA damage signal pathway and functionally characterized DNA damage signal pathway by reverse genetics approaches. We found that expression levels of genes involved in DNA repair, DNA replication, and DNA recombination were controlled by Rad53 in response to gamma radiation. Taken together, the current transcriptome and functional analyses could shed light on understanding the DNA damage response mechanism of *C. neoformans*.

Mutations of the TATA-binding Protein Gene Improves Tolerance to Acetic Acid

Hyun A Cho1, Ji Eun An2, Hye ji Kwon2, Eun Jung Kim3, Young Mi Lee1, Hyeok Jin Ko1, Hong Jae Park4, In Geol Choi5, Soo Ah Kim6, Kyoung Heon Kim7, Wan Kee Kim6, and Won Ja Cho6,8,*

1Interdisciplinary Program of EcoCreative, Ewha Womans University, 2Division of Life and Pharmaceutical Sciences, Ewha Womans University, 3Department of Pharmacology, School of Medicine, Ajou University, 4School of Life Sciences and Biotechnology, Korea University, 5Division of Life and Pharmaceutical Sciences, Ewha Womans University, 6Microbial Resources Research Center, Ewha Womans University

Screening a library of overexpressing mutant alleles of the TATA-binding gene SPT15 yielded two *Saccharomyces cerevisiae* strains (MRRC 3252 and 3253) with enhanced tolerance to acetic acid. They were also tolerant to propionic acid and hydrogen peroxide. Transcriptome profile analysis identified 58 upregulated genes and 106 downregulated genes in MRRC 3252. Stress- and protein synthesis-related transcription factors were predominantly enriched in the upregulated and downregulated genes respectively. Eight deletion mutants for some of the highly downregulated genes were acetic acid-tolerant. The level of intracellular reactive oxygen species was considerably lessened in MRRC 3252 and 3253 upon exposure to acetic acid. Metabolome profile analysis revealed that intracellular concentrations of 5 and 102 metabolites were increased and decreased, respectively, in MRRC 3252, featuring a large increase of urea and a significant decrease of amino acids. The *dur1/2* mutant, in which the urea degradation gene *DUR1/2* is deleted, displayed enhanced tolerance to acetic acid. Enhanced tolerance to acetic acid was also observed on the medium containing a low concentration of amino acids. Taken together, this study identified two SPT15 alleles, nine gene deletions and low concentration of amino acids in the medium that confer enhanced tolerance to acetic acid.
Overexpression of PMA1 Enhances Tolerance to Variable Environmental Conditions and Constitutively Activates the MAPK Pathways in Saccharomyces cerevisiae

Ye Ji Lee1, Oliviai Nasution2, Young Mi Lee3, Eun Jung Kim4, Won Ja Choi5, and Wan Kee Kim6*

1Interdisciplinary Program of EcoCreative, College of Natural Sciences, Ewha Womans University, 2Department of Life Sciences College of Natural Sciences, Ewha Womans University, 3Department of Pharmacology, School of Medicine, Ajou University

PMX1 encodes a transmembrane polypeptide that functions to pump protons out of the cell. Ectopic PMX1 overexpression in Saccharomyces cerevisiae enhances tolerance to weak acids, reactive oxygen species (ROS) and ethanol, and changes the following physiological properties: better proton efflux, lower membrane permeability, and lessened internal hydrogen peroxide production. The enhanced stress tolerance was dependent on the mitogen-activated protein kinase (MAPK) Hog1 of the high osmolality glycerol (HOG) pathway, but not the MAPK Sti2 of the cell wall integrity (CWI) pathway; however, a PMX1 overexpression constitutively activated both Hog1 and Sti2. The constitutive Hog1 activation required the MAPK kinase (MAPKK) Ssk2 of the HOG pathway, but not Ste11 and Ssk22, two other MAP3Ks of the same pathway. The constitutive Sti2 activation did not require Rom2 and the membrane sensors of the CWI pathway, whereas Bck1 was indispensable. The PMX1 overexpression activated the stress response element but not the cyclic AMP response element and the Rlm1 transcription factor. PMX1 overexpression may facilitate the construction of industrial strains with simultaneous tolerance to weak acids, ROS, and ethanol. [Supported by grants from BK21]

Systematic Analysis of Signaling Pathways Associated with Melanin Production of Cryptococcus neoformans

Dongpil Lee and Yong-Sun Bahn*
Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

Cryptococcus neoformans is an opportunistic fungal pathogen. Among its virulence factors, melanin is important to protect against host environment. Melanin production is known to be regulated by several signaling pathways, including the cAMP and HOG pathway. However, the comprehensive overview of signaling networks regulating melanin production still remains elusive. To systematically 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 in Niger seed media, confirmed by our 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 media. Based on the above experiment, we focused on the 10 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 VPS15, BZIP, GSK3, KIC1, CBK1, MECl, KET3, MPS1, PRO1 and PKH202; which are all expected as positive regulators of melanin production. Our study will provide insights into the regulatory mechanism of melanin-regulating signaling pathways in C. neoformans. [Supported by grants from MSIP]

Characterization of Plasmid-cured Strain of Bacillus velezensis GH1-13

Hajin Song, Sang Yoon Kim, Mee Kyung Sang, Hang-Young Weon, and Jaekyung Song*
Agricultural Microbiology Division, National Institute of Agricultural Sciences (NAR), Rural Development Administration (RDA)

Bacillus velezensis GH1-13 is a multifunctional bacterium which has agriculturally beneficial characterization such as plant growth promotion, biocontrol of pathogenic microbes and drought tolerance of plant. Genome analysis showed GH1-13 has 4.14 Mbp sequences with a chromosome and a unique large plasmid (pBV71, 71.6 kb). The plasmid-cured strain (GH1-13cp) of GH1-13 was made by serial incubation at 45°C and confirmed by PCR with specific primers. The morphology, growth, and diverse biological activity of GH1-13cp were compared with GH1-13. It was confirmed by using microscopy that polysaccharide morphology on cell wall of GH1-13cp was changed. It was found that GH1-13cp grew up more rapidly than GH1-13. It was disclosed that GH1-13cp produced higher quantity of Indole acetic acid (IAA) than GH1-13. The antagonistic activities of GH1-13cp against some fungi were decreased in comparison with GH1-13. These results demonstrated that the plasmid curing of GH1-13 affects growth rate, and production of IAA, exopolysaccharides, and antifungal activity, expecting the plasmid plays crucial roles in the physiology of GH1-13.
Unveiling of Complex Signaling Networks Involved in the Developmental Process of Cryptococcus neoformans

Jin-Young Kim, Yeonsoon Lee, and Yong-Sun Bahn*
Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

The fungal pathogen Cryptococcus neoformans causes cryptococcosis by the inhalation of infectious spores generated by unicellular or bisexual reproduction. To understand complex signaling networks modulating the developmental process, an 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.

Cross-talk between TOR and cAMP-PKA/CK2 Signaling to Control Sir2 Activity for Lifespan Extension in Saccharomyces cerevisiae

Mayur Devare1, Woo Kyu Kang1,2,3, Yeong Hyeock Kim2, and Jeong-Yoon Kim1,4*
1Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, 2Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, 3Department of Neurobiology, University of Massachusetts Medical School, Worcester, USA

Target of rapamycin 1 (Tor1), a protein kinase of the phosphoinositide 3-kinase family, is a component of TOR complex 1. TOR signaling connects nutrient availability and stress conditions to metabolic activity to regulate cell growth. Inhibition of TOR signaling increases lifespan from yeast to higher eukaryotes. Sir2, a NAD+-dependent protein deacetylase, is known to be regulated by cAMP-PKA and CK2 signaling and extend lifespan by repressing the transcription of longevity genes, such as PMA1 and ribosomal protein genes. In this study, we investigated whether TOR signaling is associated with the role of Sir2 for lifespan extension through the PKA/CK2 signaling. We found that expression of PMA1 was lower in tor1Δ mutant than in wild type, but deletion of SIR2 increased the PMA1 expression level in tor1Δ mutant up to that in sir2Δ mutant. We also showed that the effect of TOR signaling on PMA1 expression is linked with Sir2 phosphorylation at the Serine 473 residue. Additionally, we demonstrated that expression of the phospho-mimetic Sir2 S473E, but not the phospho-deficient Sir2S473A, dramatically decreased the replicative lifespan (RLS) of tor1Δ mutant, suggesting that the decrease in the RLS of tor1Δ mutant is achieved through the Sir2 phosphorylation by cAMP-PKA and CK2 signaling. Collectively, this study suggests that TOR signaling cross-talks with cAMP-PKA and CK2 signaling to control Sir2 activity for lifespan extension in yeast.
The Zinc Finger Transcription Factor CasS Represses Genes for Hyphal Growth under Yeast Growth Condition in Candida albicans

Jong-Myeong Kim1, Hye Yun Moon2, Hyun Ah Kang2, and Jeong-Yoon Kim1**

1Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, 2Department of Life Science, College of Natural Science, Chung-Ang University

Candida albicans, an opportunistic pathogen of humans, exists as yeast, hyphal, or pseudohyphal form, depending on pH, nutrient and temperature. The morphological transition from yeast to hypha, which is important to virulence, is controlled by many transcription factors that activate or repress hypha-specific genes (HSGs), including HGC1, ALS3, ECE1 and HWP1. The putative zinc finger transcription factor CasS is known to activate expression of many cell wall integrity genes, but it remains unknown whether CasS affects hyphal growth in C. albicans. Interestingly, we found that casSΔ/Δ mutant cells could not maintain yeast form under non-hyphal inducing condition. Analysis of CasS expression revealed that CasS transcription is significantly downregulated upon hyphal initiation in wild type, which suggests that CasS is a kind of transcription factor repressing genes required for hyphal growth. Consistently with the traits, the casSΔ/Δ mutant highly expressed hypha-specific ALS3, ECE1 and HWP1 genes under non-hyphal inducing condition. In addition, the casSΔ/Δ mutant showed decreased transcription of several genes involved in ergosterol biosynthesis pathway. Collectively, this study suggests that CasS represses transcription of genes responsible for hyphae formation during yeast-form growth in C. albicans.

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In vitro and in vivo Characterization of Antifungal Drug Efficacy of FK506 Analogs

Yeonsoon Lee1, Kyung-Tae Lee1, Soo jung Lee1, Ji Yoon Beom2, Jin A Jung2, Myung-Chong Song2, Joseph Heitman2, Yeo Joon Yoon2, Eenji Cheong3, and Yong-Sun Bahn1**

1Department of Biotechnology, Yonsei University, 2Department of Chemistry and Nanoscience, Ewha Womans University, 3Departments of Molecular Genetics and Microbiology, Medicine, and Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, USA

FK506 (tacrolimus) is an FDA-approved immunosuppressant used to prevent allograft rejections for patients who get organ transplants. It inhibits the calcineurin-NFAT pathway and early T cell activation to prevent the T cell proliferation by forming a complex with its binding protein, FKBP12. FK506 also possesses antifungal activity by inhibiting calcineurin, which is essential for virulence of several human pathogenic fungi. However, it cannot be used due to its immunosuppressive action. In this study, we analyzed the antifungal activity of 4 FK506 derivatives, 9-deoxo-FK506, 9-deoxo-prolyl-FK506, 9-deoxo-31-O-demethyl FK506 and 31-O-demethyFK506 that have lower immunosuppressive activity than FK506. We performed various drug efficacy tests when treating derivatives to Candida albicans and Cryptococcus neoformans. To correlate the antifungal activities with immunosuppression activity in the 4 FK506 analogs, we also measured the level of T cell proliferation and elucidated the minimum drug concentration that could maximize the drug effects in safety-guaranteed condition. Considering the antifungal efficacy and immunosuppressive level, we selected the 9-deoxo-31-O-demethyl-FK506 as a final candidate. In future studies, we will further analyze the in vivo efficacy of the selected FK506 analog with or without co-treating commercial drugs against a wide variety of human fungal pathogens.

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Molecular and Functional Characterization of Two Pyruvate Decarboxylase Genes PDC1 and PDC5 in the Thermotolerant Yeast Kluyveromyces marxianus

Chang Pyo Han, Jin Ho Choo, Dong Wook Lee, and Hyun Ah Kang*

Department of Life Science, College of Natural Science, Chung-Ang University

In this study, we identified two genes in the thermotolerant yeast Kluyveromyces marxianus, KmPDC1 and KmPDC5, encoding pyruvate decarboxylase (Pdc), an important enzyme at the branch point of the respiratory and fermentative pathways. Despite the conservation of important Pdc domains in both KmPdc1 and KmPdc5, a few amino acid sequences essential for enzymatic activity are not conserved in KmPdc5p. The single deletion of KmPDC1 diminished most of Pdc activity, preventing the growth of KmPdc1ΔΔ under anaerobic condition. In contrast, the single deletion of KmPDC5 did not affect Pdc activity and growth patterns. The expression of KmPDC1 gene was shown to be induced by glucose but weekly repressed by ethanol, whereas the expression level of KmPDC5 was quite low without any detectable change under various conditions. Moreover, the overexpression of KmPDC5 could not complement the growth defect of KmPdc1ΔΔ in the presence of antymycin A. The KmPdc1ΔΔ single and KmPdc1ΔΔ:pdc5::S. double deletion strains were able to grow not only on glucose but also on fermentable carbon sources under aerobic condition, producing significantly less amount of glycerol, acetate, and ethanol compared to the wild-type strain. Our data indicate that the single deletion of KmPDC1 alone, which governs most of Pdc activity in K. marxianus, is sufficient to generate a good starting host strain to be engineered for the production of high-value biomaterials derived from pyruvate without byproduct formation.
Unravelling of the Polysaccharide Capsule Regulatory Signaling Pathways in the Human Fungal Pathogen Cryptococcus neoformans

Eunha Jang and Yong-Sun Bahn*
Department of Biotechnology, Yonsei University

Cryptococcus neoformans is an opportunistic pathogen that causes fungal meningitis. 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. Our previous studies revealed that 50 transcription factors and 55 kinases are implicated in capsule formation. Here we aim to elucidate the complex signaling pathways involved in the formation of capsules by using genomic and molecular biology studies and multiple omics approaches to identify their regulatory mechanisms. To identify core signaling components in capsule production, we examine the capability of the 50 TFs and 55 kinase mutants to produce capsule in major capsule inducing media, such as dulbecco’s modified eagle’s/dME, fetal bovine serum/rBS, and Littman’s media and quantitatively analyze the transcriptional levels of CAP10, CAP59, CAP60 and CAP64, which are key regulators for capsule production, in the mutants. We observe that the transcriptional levels of the CAP genes were highly induced in the Littman’s medium after 4 hours in the wild-type strain. Next, we have a plan to check the expression level changes of each 50 TFs and 55 Kinase gene in the capsule-inducing media to identify another regulators of capsule production. This study will allow us to reveal the capsule production related mechanisms.

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Functional Characterization of Transcription Factors, Hob1 and Sre1, Regulating Sterol-biosynthesis in Pathogenic Cryptococcus Species Complex

Dong-Gi Lee, Suyeon Cha, and Yong-Sun Bahn*
Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

Sterol lipid is essential for cell membrane structure in eukaryotic cells. In mammalian cells, sterol regulatory element binding proteins (SREBPs) act as principal regulators of cellular cholesterol which is essential for proper cell membrane fluidity and structure. SREBP and sterol regulation are related to levels of cellular oxygen because it is a major substrate for sterol synthesis. In our previous study, we found that sre1 and hob1 is involved in sterol regulating and resistance under general environmental stresses such as cell membrane, oxidative, osmotic and genotoxic stresses. In this study, we observed phenotypes in other strains of Cryptococcus species by constructing hob1Δ and sre1Δ mutants to confirm whether the functions of both genes are conserved in most serotypes. As a result, hob1Δ showed no noticeable phenotype under treatment of antifungal drugs and most environmental stresses in R265 (C. gattii) and XL280 (C. neoformans), suggesting that Hob1 is related to sterol regulation only in H99 (serotype A). On the other hand, the function of Sre1 was found to be conserved in most serotypes. In conclusion, HOB1 and SRE1 play crucial role in regulating sterol-homeostasis in C. neoformans, moreover, Hob1 is specific gene in Cryptococcus neoformans. It suggests that Hob1 is considered as potent factor-targeted new safety antifungal drug.

Elicitation of the Hypersensitive Response-like Cell Death on Nicotiana benthamiana by a Marine Bacterium Haella chejuensis

Soon-Yeong Kwon1, Jeong-im Lee2, Choong-Min Ryu1, and Ji hyun F. Kim*
1Department of Systems Biology and Division of Life Sciences, Yonsei University, 2Super Bacteria Research Center, Korea Research Institute of Bioscience and Biotechnology

Haella chejuensis contains two type III secretion system (TTSS) gene clusters that are similar to those in the virulence plasmids of the mammalian pathogens Yersinia spp. Transcriptional expression of the two copies each of four TTSS representative genes of H. chejuensis, hctC, hctN, hctO, and hctV that are homologous with yscC, yscX, yscO, and yscV of Yersinia spp., respectively, was assessed by RT-PCR at different growth stages. Signals of hctC, hctO, and hctV from TTSS-I were stronger than those of TTSS-II. We were curious whether these H. chejuensis TTSSs of an animal pathogen type could elicit a defense response to plants and the plants recognize this bacterium as a potential invader. Infiltration of the bacterial suspension of H. chejuensis induced necrosis similar to that of a typical hypersensitive reaction (HR) on the leaf of Nicotiana benthamiana, which was chosen as a model plant. It depended on the bacterial growth stage; necrosis appeared when bacterial suspension at early stationary phase was infiltrated. A previous study reported that AvrPto1, a type III effector protein in pseudomonads known for its ability to interact with the resistance protein in tomato, suppressed the HR on the incompatible host plant, N. benthamiana. H. chejuensis containing avrPto1 indeed suppressed elicitation of the HR in N. benthamiana. Taken together, the TTSSs of H. chejuensis are functional in planta and responsible for induction of the HR-like necrosis in N. benthamiana.

Evolutionary Genetic Traits for Thermal Adaptation Using Pan-genomic Approach in Bacilliae

Min-Kyu Park1, Yong-Jik Lee1, Leesun Kim2, Sang-Jae Lee2, Sang Jun Lee1, and Dong-Woo Lee*
1School of Applied Biosciences, Kyungpook National University, 2Major in Food Biotechnology, Silla University, 3Department of Systems Biotechnology, Chung-Ang University

The order Bacillales, one of the most thriving and diverse microorganisms, has actively evolved under various extreme environments. Hence many taxonomic and physiological studies on individual strains in the order Bacillales have been performed, but their genetic traits for thermal adaptation still remain unclear. Herein, 75 thermophiles and mesophiles in the order Bacillales, comprised of ten genera based on 16S rRNA gene, were selected for comparative genomics to understand molecular evolution for thermal adaptation. Firstly, comparative phylogenetic analyses yielded a tree with five major clades. Subsequently, a multiple pan-genome analysis combined with 16S rRNA genes revealed 102 core genes that 75 strains possess. Core gene sets, obtained from pan-genomic analysis, were analyzed genetically and functionally. Comparative analyses of G + C contents in genome, 16S tRNA, and core genes revealed that thermophiles have higher G + C ratio than mesophiles. Secondly, amino acid contents and codon usage frequencies in the core genes were also analyzed. Increase in hydrophobic amino acids in the core genes of thermophiles implicated their thermostability. Furthermore, the codon usage of thermophiles in weak hydrophobic amino acids suggested that thermophiles selected codon usage especially containing G and C for increasing stability at elevated temperatures. Therefore, amino acid substitutions between thermophiles and mesophiles might be associated with thermal adaptation in Bacillales.
A Putative Transcription Factor, MoAf01, is Involved in Sensing during Appressorium Formation in the Rice Blast Fungus

Jaehyuk Choi
Division of Life Sciences, College of Life Sciences and Bioengineering, Incheon National University

Host signal sensing is one of the most important steps for successful disease establishment of fungal pathogens. The rice blast fungus, *Magnaporthe oryzae*, is one of the best known models showing plant-microbe interactions. The fungus has been known to recognize some of host signals from the leaf surface and to initiate development of infection structure, called appressorium. Here, we found that a putative transcription factor MoAf01 is involved in sensing multiple host signals such as hydrophobicity from artificial surfaces, cutin monomers, and long chain primary alcohols. The ΔMoAf01 mutant, however, formed an appressorium on the rice leaf, suggesting that other host signals can be detected by it. The mutant led to defects in appressorium morphology, appressorium penetration, invasive growth, and pathogenicity. Exogenous cAMP and reintroduction of the deleted gene restored the phenotypic defects of the mutant. Thus, a host signal sensor or mediator, MoAf01 plays multiple roles in the disease cycle of the rice blast fungus. These findings will broaden our understanding on the role of signal sensing in the plant disease.

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Helicobacter pylori Outer Membrane Protein, HomC, Shows Polymorphism and Its Association with *bab* Family in American and South Korean Populations

Aeryun Kim1,2, Stephanie L. Servetas3, Yun-Jung Yoo1,2, D. Scott Merrell4*, and Jeong-Heon Cha1,2,4*

1Department of Oral Biology, Oral Science Research Center, BK21 Plus Project, Yonsei University College of Dentistry, 2Department of Applied Life Science, The Graduate School, Yonsei University, 3Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., Bethesda, MD 20814, USA, 4Microbiology and Molecular Biology Laboratory, Key Laboratory of Oral Medicine, Guangzhou Institute of Oral Disease, Stomatological Hospital of Guangzhou Medical University, P. R. China

*Helicobacter pylori* is a Gram-negative bacterium, which causes persistent infections colonizing gastric mucosa of human stomach. Interestingly, *H. pylori* shows highly genetic diversity and *H. pylori* infection prevalence shows large geographical variation. The array of outer membrane proteins (OMPs) found in *H. pylori* provides a crucial component for persistent colonization within the gastric niche. Many of the more commonly studied OMPs are polymorphic and are associated with variable disease outcomes. Previous work from our group described OMP differences among the Bab family (*babA, babB*, and *babC*) and Hom family (*homA* and *homB*) from 80 American *H. pylori* clinical isolates (AH) and 80 South Korean *H. pylori* clinical isolates (KH). In the current study, we expanded our investigation to include the less well characterized Hom family member, HomC. Overall, we identified and genotyped three *homC* variants: *homC* S, *homC* L, and *homC* M, in both populations. Similar to other polymorphic genes, the KH group showed less overall diversity, with 97.5% of strains harboring *homC* L. Since 97.5% KH had *homC* L, whereas AH carried almost equally *homC* S and *homC* L, it was identified for the significant association of distributions of *homC* polymorphism between two populations. Our results provided that the *homC* L polymorphism predominated in South Korean population in which reported high levels with *H. pylori* infection and gastric cancer.

Further analysis of the AH group identified associations between *homC* polymorphism and *bab* family genotype; in AH strains, there was a significant association between *homC* and carriage of *babA* at locus A. Since *babA* is an important virulence factor for the development of severe gastric disease, these data may suggest that *homC* polymorphism plays a role in *H. pylori* pathogenesis.
**F043**

A Novel Mechanism by Which *Helicobacter pylori* Regulates Expression of CagA

Hanfu Su, Sungil Jang, and Jeong-Heon Cha*

Department of Oral Biology, Applied Life Science, the Graduate School, BK21 Plus Project, Yonsei University College of Dentistry

*Helicobacter pylori* is a human pathogen that colonizes on gastric mucosa and is a major risk factor for development of gastric disease, including gastric cancer. Severity of *H. pylori*-associated disease is directly associated with carriage of the CagA toxin. This study describes a new molecular mechanism by which cagA gene number dynamically expands and contracts in *H. pylori*. Analysis of strain PMSS1 revealed a heterogeneous population in terms of cagA copies; strains carried from zero to four copies of cagA that were arranged as direct repeats within the chromosome. Strains with more cagA repeats exhibited higher levels of CagA expression and increased levels of delivery and phosphorylation of CagA within host cells. This concomitantly resulted in higher virulence phenotypes as measured by cell elongation and IL-8 induction. Sequence analysis of the repeat region revealed three cagA homologous areas (CHAs) within the cagA repeats. Analysis of a large panel of clinical isolates showed that 7.5% of United State *H. pylori* strains harbored multiple cagA repeats, while none of the tested Korean isolates carried more than one copy of cagA. The multi-cagA-containing strains were shown to belong to hpEurope but not any other *H. pylori* population, suggesting that some strains in hpEurope are more virulent than others. Taking together, this study demonstrates a novel mechanism by which *H. pylori* dynamically modulates CagA expression and thus may affect development of disease.

**F044**

How *H. pylori* Changes cagA Gene Number

Kavinda Tissera, Sungil Jang, Hanfu Su, and Jeong-Heon Cha*

Department of Oral Biology, Oral Science Research Center, Department of Applied Life Science, the Graduate School, BK21 Plus Project, Yonsei University College of Dentistry

*Helicobacter pylori* is one of the most genetically diverse bacteria causing various gastric diseases due to its different virulence factors. Of these, CagA is known to be the major component associated with gastric carcinoma and it has been recently found that some of the *H. pylori* strains consist of a heterogeneous population in terms of cagA copies enabling them to express more 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 behind of this change in copy number has yet to be revealed. Hence, this study is conducted with the objective of generating isogenic mutants with the CHA-ud sequence to identify its involvement on this numerical gene variation. Initially, a mutant was generated consisting a kan-sacB cassette and CHA-ud with contrast to its parental strain (G27) which was reported of having a single cagA gene. After testing and gaining favorable evidence showing its capability of changing cagA copy number, it was decided to make a similar but marker-less mutant with CHA-ud flanking the both ends of cagA gene. This mutant will be further employed to study the intimacy of CHA-ud behind this strain specific variation in terms of cagA copy number.

**F045**

The Putative C_{2}H_{2} Transcription Factor DsdA Is a Novel Regulator of Differentiation in *Aspergillus nidulans*

Da Hye Kim, Yong Jin Kim, and Pil Jae Maeng*

Department of Microbiology & Molecular Biology, College of Biological Sciences and Biotechnology, Chungnam National University

*Aspergillus nidulans* research has advanced the study of eukaryotic cellular physiology, contributing to metabolic regulation, development, cell cycle control, morphogenesis and human genetic diseases. Also, *A. nidulans* is a homothallic ascomycetes and has a central role as a model organism. During asexual development, several morphological changes generate to form conidiophores and asexual spores called conidia. In addition, mycelial mass are formed from which Hülle cells and cleistothecia are developed for sexual development. Several genes involved in asexual development have been genetically characterized and interactions between them have been investigated. In contrast, the ‘sex-related’ genes have not been well dissected.

We have performed transcriptome analysis of *A. nidulans* throughout the whole life cycle from vegetative growth to asexual differentiation, and selected fifty-one genes of putative transcription factors (TFs) exhibiting significant stage-dependent variation in their transcription levels. In this study, we characterize the novel regulator DsdA with C_{2}H_{2} domain regulating asexual and sexual development in *A. nidulans*. The dsdA (defective sexual development) mRNA specifically accumulates during the late phase of asexual development and the early to middle phase of sexual development. The deletion of *dsdA* leads to increased number of conidia and delayed production of sexual fruiting bodies (cleistothecia). In the *dsdA* deletion mutant, mRNA levels of the *brlA, abaA, wetA* genes that regulate sequential activation of asexual sporulation increase. Overexpression of *dsdA* causes reduced condiation and increased forming sexual structures. These results suggest that DsdA functions as a negative regulator of asexual development and a positive regulator of sexual development. Further studies should be devoted to investigate the gene network for regulation of asexual and sexual development in relation to the function of *dsdA* in *A. nidulans*.
Isolation and Fermentation of Novel Bacillus spp. for Stereospecific Production of (R,R)-2,3-BD

Chan Woo Song, Jong Myoung Park, Chelladurai Rathnasingh, Julia Lee, and Hyohak Song*
Research and Development Center, GS Caltex Corporation

2,3-Butanediol (2,3-BD) has great potentials in the diverse industries including chemical, cosmetics, agricultural, and medical areas. Among three types of isomers (R,R), (R,S), and (S,S), it is reported that (R,R)-2,3-BD has great potential uses as biopesticide, growth stimulator, drought resistance material, etc. Generally, it is known that strains of the genus Klebsiella and Enterobacter mainly produce (S,S)-2,3-BD and (R,S)-2,3-BD, while members of the genus Bacillus normally generate (R,R)-2,3-BD. In this study, isolation of novel Bacillus spp. from soil samples was carried out to find efficient producer of (R,R)-2,3-BD without accumulation of other isomers. Next, fermentation conditions were developed and optimized for enhanced production of (R,R)-2,3-BD. Finally, newly isolated strain Bacillus spp. 2198 produced more than 30 g/L of pure (R,R)-2,3-BD by fed-batch fermentation.

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Application of 2,3-Butanediol for Cosmetics, Personal Cares, and Home Cares and Characterization of Klebsiella oxytoca Strain

Jong Myoung Park, Chan Woo Song, Duk-Ki Kim, and Hyohak Song*
Research and Development Center, GS Caltex Corporation

The isolated K. oxytoca strain was characterized based on genomic comparison with K. pneumoniae strain. Most K. pneumoniae strains have been reported to synthesize large amounts of capsular polysaccharides (CPS) on their surface and exhibit virulence in humans. On the other hand, less such materials were detected on the cell surface of the isolated K. oxytoca strain, so it is recognized as a GRAS (generally regarded as safe) microorganism. The U.S. National Institutes of Health (NIH) Guidelines have noted that K. oxytoca belongs to RG 1, indicating that it is safe to handle (http://www.absa.org/). K. oxytoca strain is a promising microorganism for 2,3-BD production. Recently, we found out that 2,3-BD, especially (2R,3S)-2,3-BD, has excellent antiseptic, antimicrobial, and moisturizing properties. This implies that (2R,3S)-BD can be used for cosmetics, personal cares, and home cares. In the present study, we carried out a number of tests, such as patch response test and MIC test, by using (2R,3S) rich-BD to determine its potential applicability. These results strongly suggest that (2R,3S) rich-BD produced by microbial fermentation can be used for cosmetics, personal cares, and home cares.

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Metabolic Engineering of Escherichia coli for Efficient Production of 1,3-Diaminopropane Using in silico Flux Analysis

Jiha Kim1, Tong Un Chae2, Won Jun Kim1, Sol Choi1,2, Si Jae Park1, and Sang Yup Lee1,4,*
1Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 Plus program), KAIST, 2Research Institute of Biotechnology, Cj Blossom Park, Cj Cheiljedang, 3Department of Environmental Engineering and Energy, Myongji University, 4Bioinformatics Research Center, KAIST, 5BioProcess Engineering Research Center, KAIST

Bio-based production of chemicals is important for sustainable chemical industry. Here, Escherichia coli is metabolically engineered to produce 1,3-diaminopropane (1,3-DAP), a monomer for polyamide. Comparison of heterologous C4 and C5 pathways for 1,3-DAP production by in silico flux analysis revealed that the C4 pathway employing Acinetobacter baumannii dat and ddc genes, encoding 2-ketoglutarate 4-aminotransferase and L-2,4-diaminobutanoate decarboxylase, respectively, was more efficient. In a strain having feedback resistant aspartokinases, the ppc and aspc genes were overexpressed to increase flux towards 1,3-DAP synthesis. Also, knocking out pfgA was found to increase 1,3-DAP production by applying 128 synthetic small RNAs. Overexpression of the ppc and aspc genes in the pfgA deleted strain resulted in even higher production of 1,3-DAP. Fed-batch fermentation of the final engineered E. coli strain allowed production of 13 g/L of 1,3-DAP in a glucose minimal medium.

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**Production of 1,5-Diaminopentane by Metabolically Engineered Corynebacterium glutamicum**

Cindy Pricilla Surya Prabowo, Seok Hyun Park, and Sang Yup Lee

The production of 1,5-diaminopentane (cadaverine) from renewable feedstock is a sustainable and promising alternative to petroleum-based chemical synthesis. In this project, *Corynebacterium glutamicum* is metabolically engineered to produce 1,5-diaminopentane. L-lysine decarboxylase gene encoded by *cadA*, which converts L-lysine directly to 1,5-diaminopentane, was amplified in plasmid-based overexpression under the tac promoter in an industrial L-lysine producer *C. glutamicum* (U2 strain). However, the 1,5-diaminopentane was not produced in the recombinant *C. glutamicum* (U2/pCEcadA) and L-lysine was detected in the culture medium. Thus, modification of the *cadA* gene was done using a codon adaptation program. Using the redesigned *cadA* gene, 1,5-diaminopentane was successfully produced with the titer of 31.94 g/L without exogenous feeding of L-lysine by fed-batch fermentation of *C. glutamicum* (U2/pCEcadA).

*This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557).*

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**Metabolic Engineering of Mannheimia succiniciproducens for the Production of L-Malic Acid**

Kyeong Rok Choi, Jung Ho Ahn, and Sang Yup Lee

L-Malic acid is a commonly used building block chemical for synthesis of valuable chemicals in industry. A gram-negative facultative capnophilic rumen bacterium *Mannheimia succiniciproducens* produces succinic acid as its major metabolite using anaerobic pathway under anaerobic condition rich in CO₂. Although wild-type *M. succiniciproducens* strain does not naturally produce malate, use of *M. succiniciproducens* to produce fumaric and malic acids is advantageous due to its strong anaerobic pathway under CO₂ conditions. Based on a high succinic acid production strain by genome engineering, previously reported by our group, the malic acid production strain was constructed. Especially, deletion of *fumC* gene encoding fumarase, which converts malate to fumaric acid, resulted in a strain producing mostly malic acid instead of succinic acid. These results can be applied to optimize metabolic fluxes of strains with a strong anaerobic pathway to produce malic acid in higher titer by rational metabolic engineering.

*This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea.*

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**Production of L-Ornithine by Metabolically Engineered Corynebacterium glutamicum**

Cindy Pricilla Surya Prabowo, Seok Hyun Park, and Sang Yup Lee

L-Ornithine is a non-essential amino acid which is used for various applications in food industry. Here, we report high-titer production of L-ornithine by *Corynebacterium glutamicum* ATCC 13032 through metabolic engineering. Firstly, proA and proG genes were deleted to optimize the metabolic pathway. Next, argF gene encoding the regulatory repressor of the L-arginine operon was also deleted to enhance the flux toward ornithine. Flask cultivation was done, then this base strain was further engineered by plasmid-based overexpression of argC/D/B genes. The start codons of the *pgi* and *zwf* genes were changed and the native promoter of the *tkt* operon was replaced with the strong *sd* promoter to enrich NADPH pool. Fed-batch cultivation of the final strain YW06 (pSY223) resulted in the final titer of 51.5 g/L of L-ornithine in 40 h with productivity of 1.29 g/L/h. The results demonstrates how engineered *C. glutamicum* can produce L-ornithine.

*This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557).*

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**Enhanced Production of Fumaric Acid in Escherichia coli by Combining Metabolic Engineering and Flux Optimization Strategies**

Jiha Kim, Chan Woo Song, and Sang Yup Lee

Fumaric acid, a four-carbon dicarboxylic acid, has been widely used in chemical, food, and pharmaceutical industries. For the enhanced production of fumaric acid, *Escherichia coli* was further developed by rational metabolic engineering together with flux optimization. The engineered strain, CW4F4N overexpressing phosphoenolpyruvate carboxylase (PPC), produced 5.30 g/L of fumaric acid. 24 types of synthetic PPC expression vectors were developed to optimize PPC flux which led to increase the titer up to 5.72 g/L with a yield of 0.432 g/g glucose. Overexpression of the succinate dehydrogenase complex (sdhCDAB) also increased the carbon yield up to 0.493 g/g glucose. Based on this strain, citrate synthase (CS) was combinatorially overexpressed and balanced with PPC using 48 types of synthetic expression vectors. As a result, 6.24 g/L of fumaric acid was produced with a yield of 0.500 g/g glucose. Fed-batch culture of the final strain allowed production of 25.5 g/L of fumaric acid with a yield of 0.366 g/g glucose. Deletion of *aspA* gene and supplementation of aspartic acid further increased the fumaric acid titer to 35.1 g/L with a yield of 0.490 g/g glucose.

*This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557).*
Unraveling Structure and Function of the N-terminal Domain of Ralstonia eutropha Polyhydroxyalkanoate Synthase, and Proposing Structure and Mechanisms of the Whole Enzyme

In Jin Cho1, Yeo Jin Kim2, So Young Choi1, Jieun Kim1, Kyung Jin Kim1, Kyung Jin Kim1, and Sang Yup Lee*

1Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 plus Program), KAIST; 2School of Life Sciences, KNU Creative BioResearch Group, Kyungpook National University; 3Pohang Accelerator Laboratory, Pohang University of Science and Technology; 4School of Life Sciences, KNU Creative BioResearch Group, Kyungpook National University

The polyhydroxyalkanoates (PHAs) are bacterial polyesters and have attracted much attention as alternatives of petroleum-based plastics. In PHA biosynthesis, PHA synthase (PhaC) is a key polymerase enzyme. In this study, the authors first demonstrate the 3D reconstructed models of PHA synthase from Ralstonia eutropha and the complex with PhaM, a PHA granule associated protein by small angle X-ray scattering (SAXS) analysis. The catalytic C-terminal domain of RePhaC1 dimer is located at the center, and the N-terminal domain of RePhaC1 is located opposite the dimerization subdomain of C-terminal domain, indicating that N-terminal domain is not directly involved in the enzyme catalysis. These studies newly found that N-terminal domain plays important roles on positioning the enzyme to the PHA granules and stabilizing the growing PHA polymer beside the active site. The serial truncation study on N-terminal domain implied that the predicted five α-helices (N-α3 to N-α7) are essential for proper folding and granule binding function of N-domain. This work provides in-depth research into PHA biosynthesis and basis of enzyme engineering for tailor-made bio-plastic production.

Methods for Improving Human Gut Microbiome Analysis by Quantitative Real-Time PCR

Kyeong-soon Kim, Jung Shin, Suli Yeon, and Moon Gyu Chung*

Korea Research Institute of Bio-medical Science

Gut microbiome analysis is used to understand many diseases like inflammatory bowel disease, obesity, and diabetes. In this study, quantitative analysis of intestinal microorganisms in gastrointestinal tract was performed using Real-Time PCR method. This quantitative analysis is known as the most numerically superior and most effective method for the analysis of microbes affecting various genes of human body. Thus, several beneficial intestinal microorganisms to the human body, such as Bifidobacterium spp, Lactobacillus spp, and Clostridium spp, were quantified using Real-Time PCR method.

Using the developed protocol, patients with poor intestinal health and normal people were compared. Analyses of the feces of patients with poor intestine showed that the amount of Bifidobacterium spp. and Lactobacillus spp. were appeared relatively low, while the amount Clostridium spp. was relatively high. However, in the case of healthy people, the opposite results were obtained.

Therefore, it is clear that the possibility of using these results as an evaluation method for intestinal health was confirmed.

In addition, further detailed analysis on the more various microorganisms with higher number of various patients would be valuable.

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Construction of Wild Type EBV Reverse Genetic System from B95-8 Strain of EBV

Seok Won Jung1, So Ra Huh1, Su Jin Choi1, Hysoun Cho2, and Hyojeong Kang*

1College of Pharmacy, Research Institute of Pharmaceutical Sciences and Institute for Microorganisms, Kyungpook National University; 2College of Pharmacy, Duksum Women’s University

Epstein-barr virus (EBV) is the first human tumor virus to be discovered and the most common and persistent virus infection in human, with approximately 95% of the worldwide. This virus infection is highly associated with gastric, breast cancers and several lymphoproliferative disorders, including Hodgkin’s disease, Burkitt’s lymphoma, and nasopharyngeal carcinoma (NPC). B95-8 strain of EBV was derived from EBV-infected leukocytes of marmoset monkey. This strain has a 12-kb deletion region where a number of BART-miRNAs are encoded. Lambda (λ) red recombination is well-known technique based on homologous recombination system to generate genetic changes such as insertion, deletion, and point mutation on chromosomal, plasmid, or BAC. Endonuclease I-SceI is a key component of the two-step λ-red recombination system. Herein, we used the λ-red recombination system to generate B95-8 strain of EBV with fully restored BART region. Firstly, we generate GS1783 E.coli strain with B95-8 EBV genome and transform selectable marker cassette (kanamycin resistance region) by first red recombination. Next, we performed second red recombination to replace from the selective marker gene to the BART region via homologous recombination. So, we established the EBV genome fully restored BART region in B95-8 EBV.
**Enhanced Production of Recombinant Protein with Leuconostoc citreum by Engineering of Shine-Dalgarno Sequence**

Seung Hoon Jang and Ki Jun Jeong*

Department of Chemical and Biomolecular Engineering, KAIST

Leuconostoc strains are hetero-fermentative lactic acid bacteria and their importance has been widely recognized in the dairy industry. They play important roles in production and preservation of fermented foods such as kimchi and yogurt. In addition, they also have a broad spectrum of products such as lactic acid, alcohol, aromatic compound, and antimicrobial peptides. However, despite their importance in food and biotechnology industries, there has been little effort to develop genetic tools for engineering of the bacteria. In this study, we tried to engineer the Shine-Dalgarno (SD) sequence for increase of gene expression in *L. citreum*. For this purpose, we introduced bicistronic design (BCD) expression system into *L. citreum* and observed whether it was working. After the verification of the expression system in *L. citreum*, the SD2 library was constructed by using super-folder green fluorescent protein (sfGFP) as a reporter. Highly fluorescent clones were screened from the library by fluorescent activated cell sorting (FACS) and one strong SD2 (eSD2) was successfully isolated. From the sequence analysis, changes of four bases in eSD2 were found and we confirmed that the mutations were responsible for the higher performance of eSD2. The usefulness of the eSD2 for the overexpression of recombinant proteins was successfully demonstrated with two protein models Glutathione-S-transferase (GST) and Human Papillomavirus (HPV) type 16 L1. [Supported by grants from MSIP]

**Development of High MK-7 (Menaquinone-7) Cheonggukjang by Bacillus subtilis SRCM100757 Isolated from Meju**

Gwang-Su Ha, Seung-Wha Jo, Sung-Ho Cho, and Do-Youn Jeong*

Microbial Institute for Fermentation Industry

Vitamin K2 (menaquinone-7; MK-7) has been proved to play an important role in bone metabolism and blood coagulation. Many studies indicate that MK-7 is necessary to maintain health in liver, bone, arterial. The objective of this study was to develop a cheonggukjang with high content of MK-7. Bacterial strain for cheonggukjang production was SRCM100757 isolated from Meju and our results showed that the strain was identified as *Bacillus subtilis*. Type strain *B. subtilis* KCCM32835 was used as a strain for comparison of characteristics. To compare enzyme activity of *B. subtilis* SRCM100757 with *B. subtilis* KCCM32835, agar-plate methods were conducted. HPLC was performed to measure MK-7 concentration of each of cheonggukjangs. At the comparing of the MK-7 quantity, the results showed that MK-7 synthesis level of SRCM100757 is about two-fold higher than KCCM32835. Optimization of fermentation conditions for MK-7 production from SRCM100757 in cheonggukjang were carried out. Changes of MK-7 content during cheonggukjang fermentation is increased remarkably from the initial amount of 5.78 ppm (24 h) to 25.38 ppm at the end of fermentation (72 h). Fermentation at 40°C was the best temperature for highest MK-7 production in cheonggukjang, and concentration of inoculum did not have significant effect on MK-7 content.
**G015**

Exogenous Inducer-free Expression of a Genetically Encoded Anticancer Drug by a Quorum-sensing System in vivo

Kwangsoo Kim1, Jae-Ho Jeong1, Hyun-ju Kim2, Daejin Lim2, Kyong-Hwan Byeon1,2, Shinan Li1,2, Geun-jung Kim2, and Hyon E. Choy1,2*

1Department of Microbiology, Chonnam National University Medical School, 2Molecular Medicine, BK21 plus Chonnam National University Graduate School, 3Department of Biological Sciences, College of Natural Sciences, Chonnam National University

Bacterial cancer therapy relies on the characteristics of certain bacteria that are capable of targeting and proliferating in solid malignancies. The efficacy of such approaches could be greatly improved if the bacteria were loaded with antitumor proteins. However, because most antitumor proteins are also toxic to normal tissue, they must be expressed exclusively by bacteria that specifically target and exclusively localize to tumor tissue. As a strategy for treating solid malignancies, we recently evaluated the potential of L-asparaginase (L-ASNase) delivered by tumor-targeted Salmonella. In this system, L-ASNase was under the control of the araBAD promoter (P araBAD) of the E. coli arabinoase operon, which is inducible by injection of L-arabinose. In this study, we further improved the performance of recombinant Salmonella in cancer therapy by exploiting the quorum-sensing (QS) system using cell mass-dependent auto-induction logic. This approach obviates the requirements for monitoring intratumoral bacterial status and inducing cargo protein expression with an exogenous compound. The recombinant Salmonella localized within tumors expressed and secreted large amounts of active ASNase in a cell mass-dependent manner, resulting in significant anticancer activity. These results suggested that expression of a therapeutic protein under the control of the QS system represents a promising engineering platform for the production of recombinant proteins in vivo.

**G016**

Concanavalin A and Its Interaction with Viral Proteins for the Early Detecion

Min Young Song and Seung Jae Lee*

Department of Chemistry and Research Center for Physics and Chemistry

Rapid methods for the detection and clinical treatment of human norovirus (HuNoV) are needed to control foodborne disease outbreaks, but reliable techniques that are fast and sensitive enough to detect small amounts of HuNoV in food and aquatic environments are not yet available. We explore the interactions between HuNoV and concanavalin A (Con A), which could facilitate the development of a sensitive detection tool for HuNoV. Biophysical studies including hydrogen/deuterium exchange (HDX) mass spectrometry and surface plasmon resonance (SPR) revealed that when the metal coordinated region of Con A, which spans Asp16 to His24, is converted to nine alanine residues (mCon A region is responsible for the observed virus-protein interaction. The mutated carbohydrate binding region of Con A (mCon A) does not affect binding affinity significantly, indicating that MCR of Con A is a major region of interaction to HuNoV (GII.4). The results further contribute to the development of a HuNoV concentration tool, Con A-immobilized polycrylate beads (Con A-PAB), for rapid detection of genotypes from genogroups I and II [G1 and GII].

**G017**

Purification and Characterization of Regulatory and Reductase Component from Type II Methanotrophs

Min Young Song and Seung Jae Lee*

Department of Chemistry and Research Center for Physics and Chemistry, Chonbuk National University

Methane hydroxylation through methane monoxygenases (MMOs) is a key aspect due to their control of the carbon cycle in the ecosystem and recent applications of methane gas in the field of bioenergy and bioremediation. Methanotrophic bacteria perform a specific microbial conversion from methane, one of the most stable carbon compounds, to methanol through elaborate mechanisms. MMOs express particular methane monoxygenase (pMMO) in most strains and soluble methane monoxygenase (sMMO) under copper-limited conditions. The mechanisms of MMO have been widely studied from sMMO included in the bacterial multicompontenent monoxygenase (BMMO) superfamily. Mechanism studies of sMMO have been intensively supported by the supports of advanced biophysics, especially in the Methylococcus capsulatus (Bath) and Methylosinus trichosporium OB3b strains. Structural studies of three components of sMMO, a hydroxylase (MMOH), a regulatory component (MMOB), and a reductase (MMOR), have provided crucial information about their catalytic activities. In this study, we report successful growing and expression from type II methanotrophs, Methylosinus sporium and Methylcystis sp. Strain M.

**G018**

Cloning and Expression of a Putative Chitinase-encoding Gene from a Thermophilic Marine Bacterium Rhodothermaceae sp. MEBiC09517

Mi Jeong Park1,2, Kyeong Won Lee1, Young Jun An1, Ji Hye Oh1, Byung Kwon Kim1, and Kae Kyung Kwon1,2,3*

1Korea Institute of Ocean Science and Technology, 2Korea University of Science and Technology, 3Chonnam National University Medical School, 4Korea Institute of Ocean Science and Technology

Rhodothermaceae sp. MEBiC09517, a thermophilic marine bacteria with considerable evolutionary distance from previously reported, was isolated from a sediment near an wood processing company in Incheon City. Based on the completed genome data, a gene (ROTo835) among glycoside hydrolases affiliated with variety of GH families was selected. The ROTo835 has 1,140 nucleotides encoding 379 amino acids, and molecular weight was predicted to be 42,354 Da. The amino acid sequence showed identity with chitinase from Salinibacter ruber (56%) and glycoside hydrolase from Rhodothermus marinus (54%). ROTo835 was cloned and expressed through E.coli system using T-easy vector and pET-24a vector. An expressing protein was purified by affinity chromatography & size exclusion, and optimal condition for enzyme activity was estimated. [Supported by MOF (PIT200620) & KOIST (P999514)]
Artificial ncRNAs Leading to Resistance to Cinnamaldehyde in Escherichia coli

Wonkyong Kim and Younghoon Lee*
KAIST

Interaction of noncoding RNA (ncRNA) with its target mRNA usually causes gene repression by inhibiting translation or degrading mRNA. Recently, most of ncRNAs identified in Escherichia coli repress translation by base-pairing with their target mRNAs through their antisense RNA sequences, leading to gene silencing. Although this RNA-RNA interaction concept can allow us to design artificial noncoding RNAs that could silence specific target genes, designing them appears not easy due to difficulty in predicting effective target regions. Practically they could lie on all over the entire mRNA sequence such as 5' UTR, 3' UTR, or the coding region. To overcome this problem, a genome-originated artificial ncRNA expression library (GOAL) was constructed, from which any RNA segments derived from the whole genomic sequence could be generated. The GOAL library was screened for selecting artificial ncRNAs that could confer resistance to cinnamaldehyde in E. coli. We found that one of the selected ncRNAs represses expression of decR gene encoding a regulator with an important role in cysteine detoxification, suggesting that decR repression is related to the acquisition of resistance to cinnamaldehyde resistance in E. coli.

Maximal Production of Antifungal Fusaricidins from Paenibacillus kribbensis CU01 through Fed-batch Fermentation

Jaewon Ryu¹ and Si Wouk Kim¹,²*
¹Department of Energy Conversion, Chosun University, ²Department of Environmental Engineering, Chosun University

In our previous studies, a bacterial strain showing strong antifungal activity was isolated from yellow lichen and was identified as Paenibacillus kribbensis CU01. After cell cultivation, extraction and structural analysis, the purified antifungal substances were identified as fusaricidin A and B. Their productions significantly increased by the addition of glucose, Fe²⁺ and Mn²⁺ to M9 medium. Maximal production concentrations of fusaricidins A and B at flask-scale comprised of 460 and 118 mg/L, respectively, which were the highest production concentrations yet reported in the literature. The fed-batch fermentation was performed in a 2 liters of culture with batches of 500 ml each of M9 medium, which contained 20 g/L of glucose, at 4 hour intervals starting 24 hours after the start of the batch cultivation. Under this condition, the maximal productivities of fusaricidin A and B were found to be 96.7 and 51.2 mg/L/h, respectively. This result demonstrates that P. kribbensis CU01 has enormous commercial potential for the mass production of fusaricidin.

Characterization of Transcripts Encoding Isolectins Based on RNAseq from an Edible Mushroom Hericium erinaceus

Seonghun Kim¹,²
¹Jeonbuk Branch Institute, Korea Research Institute of Bioscience and Biotechnology, ²Biosystems and Bioengineering Program, University of Science and Technology (UST)

Mushrooms are potential resources to find glycan-specific binding proteins, lectins. Many lectins binding to N-linked or O-linked glycan structures present in animal glycoconjugates have been reported over the past several decades. However mushroom species are still favorable source to identify novel lectins. In this study, we characterized different transcripts encoding isolectins based on RNAseq data from an edible mushroom Hericium erinaceus. Despite low overall identities, they share conserved carbohydrate binding module as well as peptide motifs that are hallmarks for lectin identification. To identify the carbohydrate binding specificity of the selected mushroom lectins, the lectin-coding genes within codon usage optimization were expressed, purified and characterized. The recombinant lectins were characterized by using Tricine-PAGE, IEF and MALDI-TOF mass spectrometry. Hemagglutination assay displayed the agglutination activities of the lectins contained the unique carbohydrate binding activities toward animal red blood cells. Glycan binding analysis also showed that the recombinant lectins interacts with a model glycoprotein containing both N-linked and O-linked glycoconjugates. These mushroom isolectins could be useful to detect linkage-specific glycan in glycoconjugates as novel carbohydrate-recognizing probes. [This work was supported by NRF (2013R1A1A06E3657).]
Introduction of a Stem-loop at the 5' Untranslated Region Stabilizes and Enhances Gene Expression in Clostridium acetobutylicum

Kyeong Rok Choi¹, Joungmin Lee¹, Yu-Sin Jang¹, Eleftherios T. Papoutsakis², and Sang Yup Lee¹,3*,

¹Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering, KAIST; ²Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE, USA; ³BioProcess Engineering Research Center, BioInformatics Research Center, KAIST

Overexpression of genes is a frequently used strategy in metabolic engineering. However, factors involved in gene expression level determination have been poorly studied in Clostridium spp. In this study, we found that presence of a short single-stranded 5’ untranslated region (UTR) sequence on mRNA reduces gene expression level in Clostridium acetobutylicum. An in vitro enzyme assay and reverse transcription-quantitative PCR further revealed that addition of a small stem-loop at the 5’ end of mRNA increases mRNA levels and thereby protein expression levels up to 4.6-fold, possibly protecting mRNA from exonuclease attack. Expression levels of the modified genes were unaffected by the stability of the introduced stem-loop, inferring the existence of a stem-loop itself is more important factor for the mRNA stability. Based on these findings, efficient expression cassettes can be designed by modulating 5’ UTR on the target gene, in addition to the engineering of promoter and ribosome binding sites. These findings will be applied to develop a more reliable gene expression system for metabolic engineering of Clostridium strains.

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**H003**

**Biosynthesis of Polylactic Acid and Other 2-Hydroxyacid Containing Copolymers by Metabolically Engineered *Escherichia coli***

In Jin Cho, So Young Choi, Si Je Park, Won Jun Kim, Jung Eun Yang, Hyeuk Lee, Jihoon Shin, and Sang Yup Lee

1. Metabolic and Biomolecular Engineering, National Research Laboratory, Department of Chemical and Biomolecular Engineering, KAIST
2. Division of Drug Discovery Research, Korea Research Institute of Chemical Technology
3. Center for Bio-based Chemistry, Green Chemistry & Engineering Division, Korea Research Institute of Chemical Technology

Lactate containing polymers such as poly(lactate-co-glycolate) (PLGA) are widely used biodegradable and biocompatible polymers. Here we report one-step fermentative production of polylactic acid and other 2-hydroxyacid containing copolymers in engineered *Escherichia coli*. This recombinant strain harbors an evolved polyhydroxalkanoate (PHA) synthase that polymerizes d-lactyl-CoA, 2-hydroxyalkanoyl-CoA and glycolyl-CoA into synthetic polymers with various combinations. For producing PLGA, introducing Dahms pathway enables production of glycolate from xylose with deleting *ldhA*. Moreover, an evolved propionyl-CoA transferase converts d-lactate and 2-hydroxyalkanoyl-CoA into d-lactyl-CoA, 2-hydroxyalkanoyl-CoA and glycolyl-CoA, respectively. We also demonstrate modulation of the monomer fractions in lactate containing polymers. In case of PLGA, monomer fractions are modified with increasing the modulation of the monomer fractions in lactate containing polymers. In one-step fermentative production of polylactic acid and other 2-hydroxyacid containing copolymers in engineered *Escherichia coli*. This recombinant strain harbors an evolved polyhydroxalkanoate (PHA) synthase that polymerizes d-lactyl-CoA, 2-hydroxyalkanoyl-CoA and glycolyl-CoA into synthetic polymers with various combinations. For producing PLGA, introducing Dahms pathway enables production of glycolate from xylose with deleting *ldhA*. Moreover, an evolved propionyl-CoA transferase converts d-lactate and 2-hydroxyalkanoyl-CoA into d-lactyl-CoA, 2-hydroxyalkanoyl-CoA and glycolyl-CoA, respectively. We also demonstrate modulation of the monomer fractions in lactate containing polymers. In case of PLGA, monomer fractions are modified with increasing the proportion of d-lactate by overexpressing *ldhA* and deleting *ddl*, or increasing the proportion of glycolate by deleting *aceB* and *gltDEFGB*. Production of 2-hydroxybutyrate integrated polymers can be controlled by either deleting *ldhA* or feeding strains with l-isoleucine.

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

**H004**

**Introduction of the Process of Human Gut Microbiome Analysis System**

Jung Shin, Kyeong-soon Kim, Suki Yeon, Ji Soo Sim, and Moon Gyu Chung

Korea Research Institute of Bio-medical Science

The composition and function of the developing gut microbiome of the human has huge ramifications for the health and well-being of the human and thorough-out life. Gut Microbiome Analysis analyzes various bacterial species present in the intestine through genetic analysis, which is a molecular biological method of fecal samples. The ratio of the beneficial bacteria, the harmful bacteria and the intermediate bacteria is analyzed, and the change of the intestinal environment is monitored by analysis method. Korea Research Institute of Bio-medical Science (KRIBS) has established a method for measuring bacteria present in the intestinal environment through the above method. Therefore we have constructed an gut microbiome analysis system for *Bifidobacterium* spp., *Lactobacillus* spp. and *Clostridium* spp.

Human Gut microbiome analysis system process is as follows.
1. MOU of KRIBS and medical institutions.
2. The client or patient applies for Gut microbiome analysis after consultation with a medical doctor.
3. KRIBS or medical institutions will send you a sample collection kit.
4. Collect your samples and parcel service the sample kit back to KRIBS.
5. KRIBS will analyze the samples and send the result report to E-mail of client and medical institutions.
6. The client or patient will consult the doctor again.

[Supported by grants from Small and Medium Business Administration]

**H005**

**Antioxidative Activity and Chemical Characteristics of *Annona muricata* (Graviola) Leaf Extracts**

Young Wan Kim, Hee Young Ahn, Tae Hoon Kim, So Yeon Sim, Seung Hwan Shin, and Young Su Cho

Department of Biotechnology, Dong-A University

*Annona muricata* belongs to the Annonaceae family and is an evergreen, erect tree reaching 5-8 m in height, with large, dark green leaves. The contents of polyphenolic compounds, flavonoids, Minerals and the antioxidative activities of DPPH (α,α'-diphenyl-β-picrylhydrazyl) free radical scavenging activity, Fe/Cu reducing power, peroxidation of rat hepatocyte microsome, β-Carotene bleaching assay were tested by *in vitro* experimental models using water, ethanol and methanol extracts of *Annona muricata* leaf (AML). Water extract of AML showed the highest extraction yield (1.76%). The total polyphenol compound concentration was the highest in the methanol extract of AML. However flavonoids concentration was the highest in the ethanol extract of AML. AML’s major minerals were Ca, K, and Mg. In DPPH radical scavenging activity, this contents exhibited strong scavenging effect on ethanol and methanol extracts of AML. In addition, Fe/Cu reducing power were strong in ethanol and methanol extracts of AML. Autoxidation of rat hepatic microsomes membrane, antioxidative activities were strong in ethanol extracts of AML. β-Carotene bleaching also were highest in the ethanol extract of AML. These results may provide the basic data to understand the chemical characteristics and antioxidative activities of *Annona muricata* (graviola) leaf extract for development of functional foods.
**H006**

**Quasispecies of Chrysanthemum chlorotic mottle viroid in Different Chrysanthemum Plants**

Yeonhwa Jo, Hoseong Choi, and Won Kyong Cho*
Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University

Virions are known as the smallest plant pathogens infecting plant species. They are composed of a circular, single-stranded RNA without an ability to encode any protein. Chrysanthemum species are susceptible to two known viroids such as *Chrysanthemum chlorotic mottle viroid* (CChMVd) and *Chrysanthemum stunt viroid* (CSVd) in the family Paspiviroidae. Here, we studied quasispecies of CChMVd in different chrysanthemum plants. Fifty chrysanthemum plants were randomly collected in the flower market in Korea. Infection of CChMVd in 50 chrysanthemum plants were examined by RT-PCR using CChMVd specific primers. Thirteen out of 50 chrysanthemum plants were infected by CChMVd. We conducted RT-PCR to obtain full length genome sequences of CChMVd from CChMVd infected plants. After that, the obtained PCR products derived from each cultivar were cloned and sequenced. As a result, we obtained 98 variants from a total of 116 clones. This result indicates strong genetic diversity of CChMVd. The splitstree based on 98 variants demonstrated four groups of CChMVd variants. In addition, we generated a reference CChMVd sequence by averaging all 98 variants. Comparative sequence analysis revealed that several regions showing high level of sequence variations. In summary, our study is a comprehensive analysis of CChMVd genomes providing quasi-species of CChMVd in different chrysanthemum plants.

**H007**

**Metagenome Analysis of Perilla Leaf**

Yeon-Cheol Yu, Su Jin Yum, Da Young Jeon, and Hee-Gon Jeong*
Department of Food Science and Technology, Chungnam National University

In South Korea, the consumption of perilla leaf (*Perilla frutescens*) is rising along with the meat intake. Perilla leaf’s microbiota may affect the food-safety because people usually eat the perilla leaf without cooking. To observe the microbiota of perilla leaf, 3 bundles of perilla leaf were collected from 5 different sites at 2 seasons (April and July). Extracted microbial DNA of 16S ribosomal RNA gene of V5-V6 region was amplified using specific primers and amplicon was sequenced using Illumina MiSeq. At the phylum level, the mean relative abundances of Proteobacteria that was dominant in both seasons were 85.30% in April and 66.03% in July. Firmicutes was the next dominant phylum following Proteobacteria except C site in July. Unclassified Enterobacteriaceae genus was the most abundant genus in both seasons. In April, *Sphingomonas* that can cause the wound infections was the second dominant genus, whereas *Bacillus* that including pathogenic species was the next dominant genus in July. In all samples, *Acinetobacter* that was one of the top 5 dominant genera has the tendency that is more abundant in June. PCoA results showed the different separation according to the seasons. April groups were separated two clusters, however, July groups were formed one cluster. Further research is required to confirm the affection of the seasonal condition on perilla leaf’s microbiota. [This research was supported by a grant [14162MFD0972] from Ministry of Food and Drug Safety in 2017.]

**H008**

**Biological Activities and Chemical Characteristics of Extracts from *Kaempferia parviflora***

Tae Hoon Kim, Hee Young Ahn, Young Wan Kim, So Yeon Sim, Seung Hwan Shin, and Young Su Cho*
Department of Biotechnology, Dong-A University

*Kaempferia parviflora* as known as Thai black ginger, Thai ginseng of krachai dum is a herbaceous plant in the family Zingiberaceae, native to Thailand. It has some historical and medicinal use for treating metabolic ailments and improving viability in Thailand and surrounding regions. This study was worked out to investigate the biological activities and the chemical characteristics of extracts from *Kaempferia parviflora*. The contents of bioactive materials (polyphenolic compound, flavonoids, minerals) and antioxidative activities (DPPH·, α-tocopherol (β-picyrylhydrazyl) free radical scavenging activity, Cu/Fe reducing power, peroxidation of rat hepatocyte microsome) with water, ethanol and methanol extracts from *Kaempferia parviflora* were investigated. Methanol extract from KP showed the highest extraction yield (6.73%). The total polyphenolic compounds concentration was the highest in the water extracts from KP. And Flavonoids concentration was the highest in the ethanol extracts from KP. DPPH radical scavenging activity was stronger in the ethanol extracts from KP. However, these all extraction samples exhibited a relatively low activity compared with butylated hydroxytoluene (BHT). Cu reducing power was the highest in the ethanol extracts from KP. However, Fe reducing power was the highest in the water extracts from KP. Autooxidation of rat hepatic microsomes membrane was the highest in the water extracts from KP.

**H009**

**Protein Crystal Structure of a Unique Light-driven Chloride Pump Rhodopsin, CIR**

Kuglae Kim, Jeong Seok Cha, Ho Young Kim, and Hyun-Soo Cho*
Yonsei University

Recently, light-driven sodium pump rhodopsin (NaR/KR2/NDQ rhodopsin) and chloride pump rhodopsin (CIR/NTQ rhodopsin) from marine flavobacteria were identified by metagenomics study. One of them, light-driven sodium pump rhodopsin (NaR) structure was determined. The other one we have solved the first crystal structure of a unique class light-driven chloride pump (CIR) from Nonlabens marinus S1-08, at resolutions of 1.57 Å. Like structured Halorhodopsin (HR), CIR can transfer chloride ion from extracellular to cytosol. Although both CIR and HR are same light-driven chloride pump rhodopsin, we found some evidences that CIR and HR are different in structure and mechanism. In this structure, we suggest that how chloride ion transfer from extracellular to cytosol, determine significant residues for controlling functions and confirm light-driven pump activity through mutagenesis and functional assay. Also, unlike rhodopsin family, we found that CIR has structural differences such as ECL1 and Helix 8. These results suggest that together with NaR, CIR is a unique and new molecules for optogenetic study.
Marine Fungal Resource Bank

Ji Eun Eom, Myung Soo Park, and Young Woon Lim*
School of Biological Sciences, Seoul National University

The Marine Fungal Resource Bank (MFRB), overseen by Dr. Young Woon Lim at Seoul National University, was designated as a marine bioresource bank of Korea by the Ministry of Oceans and Fisheries. The main goal of the MFRB is to establish a culture collection of marine fungi for educational, scientific, and industrial purposes. MFRB will undertake following tasks: 1) Survey marine environments across Korea to catalogue marine fungal diversity, 2) Establish a robust system of polyphasic species identification, 3) Evaluate the usefulness of the discovered fungi, 4) Create a secure preservation and loan system, 5) Provide web-based access to the database. With a global focus on utilizing natural resources, marine fungal resources provide excellent opportunities for educating the public on marine ecosystem, vitalizing marine research, and discovering novel substances for use as medicine and energy. [This work was supported by the Marine BioResource Bank Program of the Ministry of Ocean & Fisheries.]

Korea Mushroom Resource Bank

Young Ju Min, Nam Kyu Kim, and Young Woon Lim*
School of Biological Sciences, Seoul National University

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.

Synergistic Effects of Co-treatment with Quercetin and Ganoderma lucidum in EBV-associated Gastric Carcinoma

So Ra Huh, Su Jin Choi, Seok Won Jung, Hyosun Cho, and Hyojeong Kang*
1College of Pharmacy, Research Institute of Pharmaceutical Sciences and Institute for Microorganisms, Kyungpook National University, 2College of Pharmacy, Doksung Women’s University

Gastric carcinoma (GC) is the fourth most common cancer and the second leading cause of cancer death in worldwide. In several risk factors such as infection with helicobacter pylori (H. pylori) and Epstein - Barr virus, smoking and dietary factor, EBV infection is one of the risk factor and is detected about 10% of GC patients globally. EBV-associated gastric carcinoma (EBVaGC) has characteristic clinicopathological and molecular features, including predominance among males, a proximal location in the stomach, lymphoepithelioma-like histology, favorable prognosis and CpG island Methylator Phenotype. Quercetin (QST) is one of the natural flavonoid and well-known as anti-oxidant, anti-cancer, and anti-viral biological properties. Ganoderma lucidum has been evaluated for its anti-tumor effect particularly in China and other Asian countries. Although GST and Ganoderma lucidum reported several biological activities, synergistic effect for co-treatment with QST and Ganoderma lucidum study has not been fully elucidated. In this study, we investigated the biological effects for co-treatment with QST and Ganoderma lucidum in EBVaGC. The co-treatment with QST and Ganoderma lucidum showed significantly increased expression of apoptosis-related genes such as Bax, Cytochrome C, cleaved-Caspase3, and cleaved-PARP-1 in SNU-719(EBV+GC cells) but not MKN74(EBV-GC). Our studies found that co-treatment with QST and Ganoderma lucidum have synergistic apoptotic activities on EBVaGC.

Isolation and Characterization of Quorum Quenching Bacteria for Biofouling Control in MBR

So-Young Ham and Hee-Deung Park*
School of Civil, Environmental and Architectural Engineering, the Graduate School of Korea University

Quorum sensing (QS) is bacterial communication using chemical signal molecules in response to cell population density. In general, gram-negative bacteria produce and release N-Acyl-homoserine lactones (AHLS), resulting biofilm formation. Biofilms have impact on industrial processes, especially biofouling in membrane bioreactor (MBR). Biofouling is undesired development of microbial layers on surfaces and results in serious operational and economic problems in MBR. To control biofouling in MBR, the interference of quorum sensing, also known as quorum quenching (QQ), has been introduced. In this study, quorum quenching bacteria (Enterococcus sp. HEMM-1) was isolated from MBR using an enrichment culture with BHL as a sole carbon source. The morphology of HEMM-1 typically corresponded with that of the genus Enterococcus. And HEMM-1 could degrade various AHLS using extracellular lactonase enzyme. In addition, HEMM-1 inhibited P. aeruginosa PA14 biofilm formation in a concentration-dependent manner under batch and continuous conditions. Taken together, Enterococcus sp. HEMM-1 inhibited PA14 biofilm formation via degradation of AHLS by extracellular lactonase enzyme activity - its quorum quenching capacity can be promising application in MBR process for the control of biofouling. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2015R1D1A1A09057657)].
**Biofilm Inhibition Effects of the Ginger Compounds Oleic and Linoleic Acid**

Eunji Cha¹, Ted Inpyo Hong¹, Han-Shin Kim³, and Hee-Deung Park¹*¹

¹School of Civil, Environmental and Architectural Engineering, the Graduate School of Korea University, ²Department of Bioscience and Biotechnology, Chungnam National University, ³Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI48824, USA

Development of new antibiotics has been continuously required to treat a growing number of antibiotics resistance bacteria. And, there are risks to human body from some of those chemicals. Natural compounds have been reported as one of the new biofilm inhibition strategies because of their biofilm inhibition properties without affecting bacterial growth. In the previous study, 6-gingerol and raffinose, which are ginger extracts, showed biofilm inhibition activities in different mechanisms. The object of this study is to investigate the role of the other ginger compounds oleic/linoleic acid in pseudomonas aeruginosa PA14 biofilm. The two chemicals inhibit biofilm in a concentration-dependent manner in static/flow conditions without affecting bacterial growth. Also, the chemicals showed the biofilm cell dispersion. Biofilms were decreased while the released cells were increased by increasing the concentration of oleic/linoleic acid from 1 μM to 1,000 μM. Taken together, this study demonstrated that oleic/linoleic acid have the capacity to inhibit PA14 biofilm formation as well as disperse the biofilm cell. Therefore, these results suggest that oleic/linoleic acid have potential for application as a biofilm inducer in variety of fields such as dispersion of biofilm formed on membrane for water treatment.

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**Phylogenetic Analysis of Sinonovacula constricta Based on Mitochondrial Cytochrome Oxidase I Gene**

Mi Sun Kim, Ji Hee Lee, Joo Won Kang, Seon Choi, and Chi Nam Seong*

Department of Biology, College of Life Science and Natural Resources, Sunchon National University

Sinonovacula constricta is a benthic clam and one of the important economic shellfish. The mitochondrial cytochrome oxidase subunit 1 (CO I) gene has been widely used in genetic diversity and population genetic structure of marine species. In this study, we compared the partial sequences of the CO I gene of S. constricta. Samples were collected from 4 coastal sites in Korea and one coastal site in China. Forty-five haplotypes were identified out of 100 individuals. The most frequent haplotype S1 was found from 33 individuals including nine from Beolgyo, eight from Goheung, six from Seosan and each five from Garijin, Korea and Wenzhou, China.

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**Anti-Inflammatory Activity of Streptomyces sp. MJ12405E in LPS-stimulated RAW 264.7 Macrophages**

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

Natural Product Research Team, Gyeonggi Biocenter, Gyeonggi Business and Science Accelerator

As a result of our ongoing search for novel bioactive natural products from cultures Streptomyces sp., the methanol (MeOH) extract of the Streptomyces sp. MJ12405E was found to show significant anti-inflammatory activity in lipopolysaccharide (LPS)-treated RAW 264.7 cells. To investigate the anti-inflammatory properties of Streptomyces sp. MJ12405E Ext. and its main component, we performed their effects on the survival and immune status of RAW 264.7 murine macrophage cells. Cell viability was determined using an MT assay after treatment with various concentrations of the isolated constituent. Inhibition of NO production in cells treated with LPS was tested by reaction with Griess reagent.

**Genome Information of Dokdonella koreensis DS-123 and Comparative Analysis of Rhodanobacteraceae Genomes**

Hyeon Gwon Lee¹, Min-Jung Kwak¹, Jung-Hoon Yoon¹, Soon-Hyeong Kwon¹, and Jihyun F. Kim¹*²

¹Department of Systems Biology and Division of Life Sciences, Yonsei University, ²Department of Food Science and Biotechnology, Sungkyunkwan University, ³Strategic Initiative for Microbiomes in Agriculture and Food, Yonsei University

Dokdonella koreensis DS-123, isolated from the soil sample in the Dokdo island of Korea, is a Gram-negative, motile, non-spore-forming, and rod-shaped bacterium belonging to the family Rhodanobacteraceae. We determined the genomic sequence of D. koreensis DS-123, and performed a comparative analysis of the genome-sequenced strains in Rhodanobacteraceae. The complete genome of DS-123 consists of a single chromosome of 4,446,619 bp (70.3% G+C) in size, and contains 3,775 protein-coding sequences, 47 transfer RNAs, and two ribosomal RNA operons. A phylogenetic analysis based on the core gene set of Xanthomonadales clarified the phylogenetic relationship between Dokdonella and other genera in the family. As compared to other strains in Rhodanobacteraceae, DS-123 has less genes associated with carbohydrate metabolism. Instead, it has genes encoding methylotrophy-associated proteins. These results raise a possibility that DS-123 adopted the single-carbon metabolism to attain competitiveness under nutrient-limited conditions. The genome sequence of DS-123 is the first in the genus and would provide valuable information in understanding the metabolic features of Dokdonella species and elucidating their roles in natural environments.
The active nationwide surveillance network termed the KINRESS has been implemented since 2009 in Korea. The nationwide influenza laboratory surveillance was expanded into KINRESS to analyze patterns of influenza and respiratory viruses in Korea. During the 2016/2017 influenza surveillance season, 5,680 respiratory specimens were collected and tested by Real-time PCR to influenza viruses (A/H1N1pdm09, A/H3N2 and B-Yamagata/B-Victoria) with influenza-like illness or acute respiratory infection. As a result, 834 (14.7%) cases were identified as influenza positive. Among these, 827 (99.0%) cases were influenza A viruses and 7 (0.8%) cases were influenza B viruses. Influenza A (H3N2) viruses (B26, 99.2%) have been predominated and influenza viruses A (H1N1) pdm09 (1, 0.1%) and B (7, 0.7%) were detected to a lesser extent. Ten weeks apart from initial case, the A (H3N2) subtype is uniquely prevalent in the 2016/2017 season, unlike the last two seasons.

[supported by grants of the KCDC]
H023

Inhibition of Influenza Virus Infection by Poncirus trifoliata Rafin Seeds

Yoonki Heo1, Jongkwang Yoon1, Kwonsung Ju2, Kihoon Park1, Seongeun Bae2, Hee-jung Lee3, and Young Bong Kim1* 1Department of Bioindustrial technologies, Konkuk University, 2Department of Biomedical Science and Engineering, Konkuk University

The emergence of drug resistant variants of the influenza virus has led to a need to develop novel and effective antiviral drugs. Numerous studies have focused on developing antiviral drugs using natural resources such as traditional herbal medicines. Poncirus trifoliata Rafin. (Rutaceae), also known as trifoliate orange, is a close relative to the Citrus trees. Traditionally, trifoliate oranges (P. trifoliate) have been widely used in oriental medicine as a remedy for gastritis, dysentery, inflammation, digestive ulcers, etc. A scientific investigation into the health-maintaining properties of trifoliate orange fruit has revealed its anti-inflammatory, antibacterial and anti-anaphylactic activities. In this study, we investigated whether trifoliate orange seeds extract inhibits influenza virus during the early stage of the infectious cycle. An ethanol extract of the P. trifoliate seeds inhibited all kinds of influenza viruses in Madin-Darby canine kidney cells, including strains that are resistant to oseltamivir. We identified that P. trifoliate seeds effectively inhibit the viral attachment and penetration into the host cells. In conclusion, an ethanol extract from seeds of the trifoliate orange might be a promising source for the development of new antiviral drugs to fight influenza viruses pandemics.

H024

Development of the Chimeric ZIKA Virus Vaccine Using SA14-14-2, Attenuated Japanese Encephalitis Virus Vaccine Strain

Hanul Choi1, Hansam Cho1, Him Nath Jnawali2, Jungmin Chun1, Yuyeon Jang1, Seong Su Kim1, Hee-Jung Lee2, and Young Bong Kim1* 1Department of Bioindustrial Technologies, Konkuk University, 2Department of Biomedical Science and Engineering, Konkuk University

ZIKA virus is a widespread virus by mosquito with most serious after-effect, microcephaly. The study of the virus has been extremely active because of its association with fetal microcephaly. However, commercial vaccines and remedies have not yet been developed. So, a development of a safe and efficient vaccine is important for public health. In this study, we produced chimeric ZIKA viruses based on an attenuated flavivirus by using reverse genetic system. From the SA14-14-2, a Japanese encephalitis virus live attenuated vaccine strain, we isolated the full-length RNA and made an infectious cDNA clone. Using full length infectious cDNA of Japanese encephalitis virus as a backbone, we constructed a chimeric ZIKA virus substituted with the preM and envelope genes of ZIKA virus. Using reverse genetic system, viral RNA from in vitro transcription was transfected into BHK cells and chimeric virus was identified by polymerase chain reaction (PCR). We are going to test the animal experiments on vaccine efficiency, and these findings may present a new avenue for developing ZIKA virus vaccine development.

H025

Development of a Middle Eastern Respiratory Syndrome Coronavirus Vaccine from Recombinant Spike Protein

Yeondong Cho1, Yoonki Heo1, Hee-Jung Lee2, Sehyun Kim1, Hanul Choi1, Sung-Tae Moon1, Kihoon Park1, Ha Youn Shin1, and Young Bong Kim1* 1Department of Bioindustrial Technologies, Konkuk University, 2Department of Biomedical Science and Engineering, Konkuk University

Middle Eastern respiratory syndrome (MERS) continues to spread throughout the pandemic spread around the globe. MERS was first discovered at the end of 2012 and has caused more than 1,800 infections and 650 deaths. Increased MERS cases and no licensed MERS vaccines highlight the need for safe and effective vaccine development for MERS. The MERS-CoV spike (S) protein is responsible for receptor binding and virion entry into the cell and is highly immunogenic and induces neutralizing antibodies. In this study, we have expressed the eS1-770 MERS-CoV Spike protein fused with human Fc4 (eS1-770-Fc4) using baculovirus system and purified. Different doses of the eS1-770-Fc4 vaccine candidate were intramuscular injected into mice, and blood samples were collected every 10 days after immunization. The eS1-770-Fc produced high titers of MERS-CoV antibodies and neutralizing antibody. Only 2 ug of eS1-770-Fc4 elicited enough immunogenicity without adjuvant. Next, we plan to use this eS1-770-Fc to perform the vaccine effect with the various adjuvants. [Supported by grants from KHIDI (No. H15C2842)]
Mannosylerythritol lipids (MEL) are glycolipids that are surface active compounds with variable biochemical functions that are produced from certain species of yeasts. MELs show structural variations depending on nutrient sources and yeast strains used in producing MEL. Their ability to self assemble into different number and position of acetyl group on mannose and erythritol, and saturation of fatty acid chains contributes to their diverse functions. We investigated the cell structures using scanning electron microscopy (SEM) and high-resolution transmission electron microscopy (HRTEM) and chemical molecular vibrations of MEL-A, -B and -C compounds.

The observed elongated, cylindrical, and ellipsoid cells with polar budding revealed that B1 and TS18 cells contain large lipid bodies (LBs) that might contain MELs. Raman spectra of MEL extracts show profound vibrations in the ranges of 800-1800 cm\(^{-1}\). Raman bands in the ranges of 840-940 cm\(^{-1}\) correspond to C-H banding, C-O and C-C stretching, and C-H vibrations in –C compounds. The NIR absorptions of MEL show significant absorption bands in the range of 1100-2600 nm. These vibrational bands –1 represent biochemical features that are produced from MEL-A, -B and -C by micro-Raman spectroscopy and near infrared (NIR) absorption bands in the range of 1100-2600 nm. These vibrational bands –1 mainly correspond to C-H banding, C-O and C-C stretching, and C-H vibrations in CH\(_2\) and CH\(_3\) groups. The NIR absorptions of MEL show significant absorption bands in the range of 1100-2600 nm. These vibrational bands that can be indicators to discriminate MEL-A, -B, and -C compounds.

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**Overlooked Gene Transfer Mediator between Organisms**

Hiroshi Xavier Chiura*, Yohei Kumagei, Susumu Yoshizawa, and Kazuhiro Kagure

**Technicolor Research Group, Centre for Earth Surface System Dynamics, Atmosphere and Ocean Research Institute, The University of Tokyo, Japan**

We have demonstrated the existence of “broad-host range vector particles” (VPS) in the natural virus-like particle assemblage, which is characterised by trafficking capability of host chromosomal and plasmid bearing genetic traits to the recipients of phylogenetically broad range [Archaea-Bacteria-Eukarya] (Chiura, 2004 Microbes Environ). Still more, VP displays the structural features of membrane vesicles, was again infectious to the recipient, producing daughter VPs with transduction capability by budding, i.e., a phenomenon termed serial transduction provides evidence of a new method of horizontal gene transfer via VPS (Chiura et al., 2009 Microbes Environ, 2011 FEMS Microb Ecol). Comparative genomic analysis of the transductant (PfEtrans) that acquired VP production, with the recipient [Escherichia coli AB1157], and the VP donor [Polaribacter filamentus ATCC700397], showed the genome sequence similarity virtually resembles with that of the recipient. VP would be prevalently distributed among cellular organisms to acquire environmental adaptation and biodiversity, since homology survey for budding essential membrane protein exhibited that not viral but cellular origin.

**The Isolation of the Symbiotic Bacteria from the Accessory Nidamental Glands and Eggs of the Pharaoh Cuttlefish, Sepia pharaonis (Cephalopoda: Sepiidae)**

Mong-Fong Lee\(^1\), Jing-Duan Huang\(^2\), Shin-Yu Lee\(^3\), Jiann-Horng Leu\(^4\), and Shiu-Mei Liu\(^2\)

**Department of Aquaculture, National Penghu University of Science and Technology, \(^1\)Institute of Marine Biology, National Taiwan Ocean University, \(^2\)Institute of Biomedical Engineering, National Taiwan University**

The nidamental glands (NGs) and accessory nidamental glands (ANGs) are two accessory reproductive organs in the female pharaoh cuttlefish, *Sepia pharaonis* and play both an important role in providing protective functions for the embryonic development without parent care. The NGs secret gelatinous substances to form the eggshells which surround the fertilized eggs. The ANGs consist of many tubules which harbor symbiotic bacteria. The characteristics of these bacterial consortia in the ANGs and eggs of the *S. pharaonis* remain an open question. In the present study, three symbiotic bacteria from the ANG were isolated by serial transfers cultured with marine broth (Difco). Stained with PTA and observed by TEM, these bacteria were either rod- or coccoid-shaped. According to the alignment results for 16S rDNA sequences, we defined that one isolate was Shimia marina with 99% identity, another one was highly similar to some *Pseudoalteromonas* species and the other one was *Vibrio* sp. *Phaeobacter* sp. was also identified from the perivitelline fluid of the developing eggs. Antimicrobial effects of the extracts from the bacteria in the ANG and eggs are now under investigations. [This study was supported by grants to M.F. Lee from the Ministry of Science and Technology in Taiwan (MOST 103-2313-B-346-002-MY3).]
**H029**

**RtxA1 Toxin Binds Filamin A to Regulate Pak 1- and MAPK-dependent Cytoskeleton Reorganization and Cell Death**

Ju Young Lim, BoA Kim, Ji Hye Kim, Joon Haeng Rhee, and Young Ran Kim

1College of Pharmacy and Research Institute of Drug Development, Chonnam National University, 2Clinical Vaccine R&D Center and Department of Microbiology, Chonnam National University Medical School

Cytoskeletal rearrangement and acute cytotoxicity are observed in *Vibrio vulnificus* RtxA1 intoxication. Amino acids 1491–1971 of *V. vulnificus* 29307 RtxA1 toxin exhibit approximately 25% identity to other ezrin, radixin, moesin (ERM) family proteins, which function as linkers between the plasma membrane and the actin cytoskeleton. HeLa cells expressing RtxA1 amino acids 1491–1971 (RtxA1ERM) fused to green fluorescence protein were rounded and the fusion protein colocalized with actin. Through a yeast two-hybrid screening and subsequent immunoprecipitation validation assay, we confirmed a specific binding of RtxA1ERM with host-cell filamin A, an actin-crosslinking scaffold protein. siRNA-mediated downregulation of filamin A decreased the cytotoxicity of RtxA1 to HeLa cells. Phosphorylation of JNK and p38 mitogen-activated protein kinases (MAPKs) was located downstream of RtxA1-filamin A binding during the RtxA1-mediated death of HeLa cells and filamin A-expressing A7 cells. However, phosphorylation of the two MAPKs was not caused by RtxA1 toxin in filamin A-deficient M2 cells. In addition, filamin A may be essential for Pak1-induced cytoskeletal reorganization and MAPKs activation. These results suggest that RtxA1 toxin binds filamin A to regulate Pak1- and MAPK-dependent cytoskeleton reorganization and cell death.

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**H030**

**An Ethyl Acetate Fraction from *Dendropanax morbifera* Leaves Increases EL-4 T Cell Growth by Upregulating NF-AT-mediated IL-2 Secretion**

Jung Up Park, Hwa-jeong Lee, Bok Yun Kang, and Young Ran Kim

1College of Pharmacy and Research Institute of Drug Development, Chonnam National University, 2Department of Biomedical Sciences, BK21 PLUS Center for Creative Biomedical Scientists at Chonnam National University, Research Institute of Medical Sciences, Chonnam National University Medical School

*Dendropanax morbifera* Leveille (Araliaceae) is an endemic species that grows in southwestern Korea and has been used as a folk medicine. Several studies have shown that the leaves of *D. morbifera* have diverse therapeutic potential. We found that the water extract of the leaves of *D. morbifera* increased the growth of EL-4 T cells and its ethyl acetate (W-EA) fraction showed a more significant effect than the other fractions on the growth of EL-4 T cells, splenocytes, and isolated murine CD4+ T cells. The W-EA fraction was evaluated on the regulation of interleukin-2 (IL-2), a potent T cell growth factor. The W-EA fraction significantly increased IL-2 secretion in EL-4 T cells activated with phorbol 12-myristate 13-acetate (PMA) plus ionomycin (Io) and also IFN-γ production in isolated splenocytes activated with ConA (1 μg/ml). The W-EA fraction increased significantly in PMA/Io-induced promoter activity of nuclear factor of activated T cells (NF-AT) in EL-4 T cells and slightly increased in activator protein 1 (AP-1), but did not show any significant effects on the promoters of NF-κB. These results suggest that an ethyl acetate fraction from *Dendropanax morbifera* leaves increases EL-4 T cell growth by upregulating NF-AT-mediated IL-2 secretion.