A001

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

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A novel strain designated YelD216^T was isolated from the tidal flat of Asan Bay in the Yellow Sea, South Korea. Strain YelD216^T 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 YelD216^T belongs to the family *Alteromonadacea.* Higher similarities of 16S rRNA gene sequences were found with *Bowmanella denitrificans* (93.2%), *Aestuariibacter salexigens* (93.1%), *Salimonas chungwhenis* (92.8%), and *Alteromonas macleodii* (92.7%). Strain YelD216^T contained Q-8 as a major isoprenoid quinone; polar lipid consisted of phosphatidylethanolamine, phosphatidylglycerol, and an unidentified amino lipid. The major fatty acids were $C_{16:0}$, $C_{17:0}$ 10-methyl, summed feature3 ($C_{16:1}\omega6c$ and /or $C_{16:1}\omega7c$), and summed feature8 ($C_{18:1}$ ω 6c and/or $C_{18:1}\omega7c$). 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 YelD216^T (= KCTC 32984^T = CGMCC 1.15039^T).

A002

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

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Dongguk University Biomedi Campus 32

A novel Gram-negative bacterium, designated strain S8^T, was isolated from a soil sample from Gyeonggi Province, South Korea. Cells of strain S8^T were endospore-forming, motile by means of peritrichous flagella, and rod-shaped. S8^T colonies were round, convex, wavy and white. Strain S8^T grew optimally at 37°C, pH 7–8, and 0–2.0% NaCl (w/v). On basis of 16S rRNA gene sequence similarity, strain S8^T were affiliated with genus *Paenibacillus* in the family *Paenibacillaceae*, *Paenibacillus yonginensis* KCTC 33428^T being its closest relative (98.5% sequence similarity). The DNA G+C content of the novel strain was 53.1±0.3 mol%. Strain S8^T contained four phospholipids, four aminophospholipids, an aminolipid and three unidentified lipids. The major fatty acid was found to be anteiso-branched $C_{15:0}$. The predominant quinone was MK-7 menaquinone. The DNA-DNA hybridization value of strain S8^T with *Paenibacillus yonginensis* KCTC 33428^T and *Paenibacillus physcomitrellae* DSM 29851^T were 44.10% and 32.02%. Based on DNA-DNA hybridization, biochemical analysis, phylogenetic and physiological tests, strain S8^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus mobilis* sp. nov. is proposed.

A003

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

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A novel Gram-negative bacterial strain, designated strain S10^T, was isolated from rice field soil collected in Goyang, South Korea. Cells of strain S10^T were strictly aerobic, motile and rod-shaped. Colonies were round, convex, smooth and white. Strain S10^T 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 S10^T revealed that this bacterium was related to the family *Comamonadaceae*, and it is related to members of the genus *Hydrogenophaga*, with *Hydrogenophaga caeni* KCTC 12613^T being its closest relative (97.9% sequence similarity). The DNA G+C content of strain S10^T was 68.17 ± 0.03 mol%. Strain S10^T contained a phosphatidylethanolamine, a diphosphatidylglycerol, and two unidentified aminophospholipids as the major polar lipids. The major fatty acids were $C_{16:0}$ and summed feature 3 ($C_{16:1}$ $\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The predominant respiratory quinone was ubiquinone Q-8. DNA-DNA hybridization values of strain S10^T with *Hydrogenophaga caeni* KCTC 12613^T, *Hydrogenophaga atypica* DSM 15342^T, and *Hydrogenophaga defluvii* DSM 15341^T 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 S10^T (=KCTC 52520^T=JCM 31711^T) represents a novel species of the genus *Hydrogenophaga*, for which the name *Hydrogenophaga soli* sp. nov. is proposed.

A004

Flavobacterium communis sp. nov., Isolated from Freshwater

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A Gram-negative, yellow-pigmented, facultative-anaerobic, rod-shaped, non-spore forming bacterium designated PK15^T was isolated from freshwater. Growth was observed at 4–40°C (optimum, 30°C), pH 6–9 (optimum, 8), and in the presence of 0–0.8% NaCl (optimum, 0.4%). Strain PK15^T exhibited both catalase and oxidase activities and was able to reduce nitrate. On the basis of 16S rRNA gene sequence similarity, strain PK15^T was shown to belong to the genus *Flavobacterium* with close similarities to *Flavobacterium palustre* S44^T (97.9%) and *Flavobacterium seoulense* EM1321^T (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-$C_{15:0}$ (17.3%), a summed feature comprising $C_{16:1\,\omega}7c$ and/or $C_{16:1}$ $\omega6c$ (15.1%) and iso-$C_{15:0}$ (10.0%). Chemotaxonomic data supported the affiliation of strain PK15^T to the genus *Flavobacterium.* The results of the physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain PK15^T from closely related species. It is therefore evident that PK15^T represents a new species, for which the name *Flavobacterium communis* sp. nov. is proposed with the type strain PK15^T (=KCTC 52562^T).

A005

Deinococcus gammatolerans sp. nov., a Novel Bacterium Isolated from Soil, South Korea

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A Gram-positive, catalase and oxidase positive short-rod-shaped bacterial strain designated as Ant2-2^T was isolated from soil in South Korea. Cells showed high gamma-ray and UVC radiation resistance. The 16S rRNA sequence of strain Ant2-2^T represents a novel subline within the genus *Deinococcus* in the family *Deinococcaceae*. The 16S rRNA gene sequences of the strain Ant2-2^T were 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 $C_{16:1}$ *ω7c* / $C_{16:1}$ *ω6c*), $C_{15:1}$*ω6c*, and $C_{16:0}$. The DNA G+C content of the strain Ant2-2^T is 57.3 mol%. The polar lipids profile included major amounts of phosphatidylglycerol, phosphatidylcholine, and unknown aminolipid. On the basis of its phenotypic and genotypic properties, and phylogenetic distinctiveness strain Ant2-2^T should be classified in a novel species in the genus *Deinococcus*, for which the name *Deinococcus gammatolerans* sp. nov. is proposed.

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A006

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

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Strain KSM4-11^T, 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 0–0.5% (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^T revealed that it belongs to the genes *Deinococcus* in the family *Deinococcaceae*. The highest degree of sequence similarities of 94.0% with *Deinococcus radioresistens* 8A^T. 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 C16:1 ω7c / C16:1 ω6c), C15:1ω6c, and C16:0. The DNA G+C content of the strain KSM4-11^T 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^T should be classified as the representative of a novel species the genus *Deinococcus*, for which the name *Deinococcus koreense* sp. nov.is proposed.

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A007

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

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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 *Xaj* 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^T and LMG747^T(100%).

[Supported by grants from National Institute of Forest Science]

A008

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

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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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A009

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

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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 gelatinilytica* 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-$C_{15:0}$ (27.4%), iso-$C_{15:1}$ G (9.6%), iso-$C_{17:0}$ 3OH (13.2%) and summed feature 3 (comprised of $C_{16:1}$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-glucosidase differentiate strain MEBiC09412^T from *M. gelatinilytica* 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 citrea* sp. nov. The type strain is MEBiC09412^T (=KCCM 43216^T =JCM 31588^T).
[Supported grants from KIOST & MBRB.]

A010

Complete Genome Sequence of the *Aneurinibacillus soli* CB4^T from Soil of Mountain

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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 KRIBB Research Initiative Program.]

A011

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

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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 hinnuleoarmillatus, Cruentomycena kedrovaya, Galerina sulciceps, Hebeloma arenosum, Hymenopellis chiangmaiae, Inocybe stellate, Leiotrametes lactinea, Parasola setulosa, Piptoporellus soloniensis, Pluteus longistriatus, Simocybe serrulata, Strobilomyces pteroreticulosporus*.

A012

Isolation of Probiotic *Leuconostoc gasicomitatum* BB7 from Cabbage Kimchi

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Isolation of *Leuconostoc* species from fermented kimchi was carried out in order to study *L.* species as probiotics. After CFU was tested, each 10^9 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, disaccharide 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±0.03, 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.

A014

Identification of Dextran Producing Lactic Acid Bacteria that Isolated from Kimchi

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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. 8.50±0.01 in 1% NaCl) than other 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, JA1-1 and JC2-3 revealed different pattern of fermentation comparing to JA2-3 and JB1-2. Same tendency of fermentation was found in trisaccharide. In case of complex sugar fermentation, all strains ferment very well with amygladin and salicin (O.D. 1.74±0.01~ 2.03±0.01 and 1.19±0.02~2.13±0.04). From 16S rRNA sequence analysis 2 strains were turned out to be *Weissella cibaria* (JA1-1 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 JA1-1, JA2-3 and JB1-2. Thus it is concluded that the JC2-3 is valuable LAB in order to study dextran production in relating with *Weissella cibaria*.

A015

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

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Extremely halophilic archaea, called haloarchaea, are adapted to hypersaline environments. In this study, a novel halophilic archaeon designated strain CBA1114^T was isolated from solar salt. Strain CBA1114^T, which is a coccoid 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 CBA1114^T showed a 91.7% similarity to that of *Haloterrigena thermotolerans* PR5^T. A phylogenetic tree generated from the results of 16S rRNA gene and MLSA of the five housekeeping genes showed that strain CBA1114^T was closely related to the species of the genus *Halorientalis* in the family *Halobacteriaceae*. The draft genome sequence of strain CBA1114^T 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 "Cofactors, Vitamins, Prosthetic Groups". According to the results of phylogenetic, phenotypic, chemotaxonomic and genomic analyses, we designate strain CBA1114^T 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 haloarchaeal researches and industries with extremozymes produced from the extremophiles.

A016

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

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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 *Soonwooa buanensis* HM0024^T of the family *Flavobacteriaceae*. Neighbour joining phylogenetic analysis using MEGA6 software package showed that strain I54 clustered with *Soonwooa buanensis* KCTC 22689^T 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-$C_{15:0}$, anteiso-$C_{15:0}$, Sum in feature 3 (comprising of $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and iso-$C_{17:0}$ 3-OH. The G+C content of the genomic DNA was 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^T is proposed as the representative of a novel species within the genus *Soonwooa*.

A017

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

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A Gram-negative, rod shaped, facultatively anaerobic, motile bacterium strain AR1^T 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 AR1^T is closely related to *Aeromonas sharmana* GPTSA-6^T with a sequence similarity of 96.83%, a value below the threshold for description of novel species. However, based on the a typical biochemical characters of *Aeromonas sharmana* GPTSA-6^T 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 MEGA6 software package showed that strain AR1^T formed a separate clade with *Aeromonas sharmana* GPTSA-6^T 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-6^T (=DSM 17445^T=MTCC 7090^T=CIP 109378^T=CCUG 54939^T) as the type species of the genus.

A018

Isolation and Characterization of Novel *Paucibacter* Species

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Four strains belonging to the genus *Paucibacter* were isolated from sediment of Nakdong River including Neodeol spring (Head of Nakdong River, Taebaek) and Changnyeong Haman 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 gene sequences, all isolates were closely related to *Paucibacter toxinivorans* 2C20^T 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 *Paucibcter*, 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*.

A019

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

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A Gram-stain-negative, rod shaped, non-motile, aerobic and yellow pigmented bacterium, designated strain ECD12^T, was isolated from a seaweed *Ecklonia cava* obtained from the South Sea (34° 02′ 58″ N, 127° 20′ 00″ E), Republic of Korea. *Spongiibacterium pacificum* sw169^T was the nearest neighbor of strain ECD12^T with 96.7% 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 ECD12^T 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 ECD12^T (=KCTC 52351^T).

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A020

Description of Novel Strain *Lysobacter* sp. 119BY6-57 Isolated from Marine Sponge

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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 6.0. 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^T, with a gene sequence similarity of 97.25%. The major fatty acids were iso-$C_{16:0}$ (31.8%), iso-$C_{15:0}$ (16.4%), summed feature 9 ($C_{17:1}$ iso ω9c/$C_{16:0}$ 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 data presented in this study, strain 119BY6-57 is considered to represent a novel species of the genus *Lysobacter*.

A021

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

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A Gram-stain-negative and non-motile bacterial strain that formed straight rods and reddish colonies, designated HMF3095^T, 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^T belonged to the genus *Hymenobacter* and was most closely related to *Hymenobacter seoulensis* 16F7G^T (96.7% sequence similarity), *Hymenobacter latericoloratus* YIM 77920^T (96.3%) and *Hymenobacter luteus* YIM 77921^T (96.3%). The major fatty acids were iso-$C_{15:0}$, $C_{16:1}$ ω5c, summed feature 4 (iso-$C_{17:1}$ I and/or anteiso B), summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) 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^T 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^T (=KCTC 52398^T =NBRC 112669^T).

A022

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

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Three strains designated MMS16-UL250^T, MMS16-UL253^T and MMS16-UL482^T were isolated from seawater near Ulleung Island, Korea. The isolates were Gram-negative, non-spore-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-UL250^T, MMS16-UL253^T, and MMS16-UL482^T 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 algicola* St-6^T. However, the 16S rRNA gene sequence similarity between the three isolates and *S. algicola* St-6^T 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, which was consistent with that of *Shewanella*. The major polar lipids of all strains were phosphatidylglycerol and phosphatidylethanolamine, and fatty acid a summed feature of 16:1 w7c/16:1 w6c, but 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. algicola*. 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-UL250^T), *Shewanella ulleungensis* sp. nov. (type strain, MMS16-UL253^T) and *Shewanella litoralis* sp. nov. (type strain, MMS16-UL482^T) are proposed.

A023

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

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Bacteriophage BK30P is a lytic bacteriophage that infects the geuns *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

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A non-motile, orange-pigmented bacterium, designated strain HME7103^T, was isolated from lagoon water in Republic of Korea. A phylogenetic tree based on 16S rRNA gene sequences showed that strain HME7103^T formed a lineage within the genus *Lacihabitans* and family *Cytophagaceae*. The strain HME7103^T was closely related to *Lacihabitans soyangensis* (95.7% sequence similarity). The major fatty acids of strain HME7103^T were iso-$C_{15:0}$ and summed feature 3 (comprising $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*). The major respiratory quinone was MK-7. The major polyamine was spermidine. The major polar lipids were phosphatidylethanolamine (PE), two unidentified aminolipids (AL), one unidentified aminophospholipid (APL) and three unidentified polar lipids (PL). The DNA G+C content of strain HME7103^T was 40.6 mol%. On the basis of the evidence presented in this study, strain HME7103^T represents a novel species within the genus *Lacihabitans*, for which the name *Lacihabitans jumunjinensis*, sp. nov. is proposed. The type strain is HME7103^T (=KCTC 23619^T =CECT 7956^T).

A025

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

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A Gram-negative, aerobic, rod-shaped (1.4±0.46 μm × 0.53±0.17 μm) and motile marine bacterium, designated as MEBiC11861^T 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^T showed high similarity with members of the genus *Nisaea* (97.8-98.0%). Growth was observed at 10-42^{O}C (optimum 26-29^{O}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 $C_{12:0}$ (5.6%), $C_{16:0}$ (29.0%), $C_{12:0}$ 3-OH (4.3%), summed feature 3 ($C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c; 9.9%), summed feature 8 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c; 31.2%), and $C_{19:0}$ cyclo ω8c (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 gluconate, malate, adipate, arabinose etc., DNA G+C ratio, composition of cellular fatty acids, and growth range of pH and salinity differentiate strain MEBiC11861^T from members of the genus *Nisaea*. On the basis of this polyphasic taxonomic data, strain MEBiC11861^T 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^T (=KCCM 43046^T =JCM 30369^T). Emended descriptions of the genus Nisaea Urios *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

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A Gram-staining-negative, aerobic, motile, nonflagellated rod-shaped bacterium, designated ST58-10^T, was isolated from an estuarine sediment in Korea. Growth of strain ST58-10^T was observed at 4–35°C (optimum, 20–25°C), pH 6.0–9.0 (optimum, pH 7.0–8.0) and 0–7% NaCl (optimum, 2–3%). Phylogenetic analyses based on 16S rRNA gene sequences showed that strain ST58-10^T formed a phyletic lineage within the genus *Marinobacterium* of the family *Oceanospirillaceae*. Strain ST58-10^T was most closely related to *Marinobacterium profumdum* PAMC 27536^T (99.5%) and *Marinobacterium rhizophilum* CL-YJ9^T (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 30.9 ± 2.8 with *M. profundum* PAMC 27536^T and 50.6 ± 7.4% with *M. rhizophilum* DSM18822^T, respectively. Major fatty acids of strain ST58-10^T were summed feature 3 (comprising $C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*) and summed feature 8 (18:1 *ω7c*) and $C_{16:0}$ 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 ST58-10^T represents a novel species of the genus *Marinobacterium*, for which the name *Marinobacterium aestuarii* sp. nov. is proposed. The type strain was ST58-10^T (=KCTC52193^T =NBRC112103^T).

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A027

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

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MicroRNAs (miRNAs) can regulate posttranscriptionally target gene expression. Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (Mtb). 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 compared with HCs. In addition, 180 miRNAs in GSE34608 showed increased expression levels in 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 Mtb in a multiplicity of infection (MOI)-dependent manner. These data indicate that *MIR144** expression is robustly increased in human MDMs following Mtb infection, and that *MIR144** levels are upregulated in PBMCs/tissues from TB patients compared with HCs.

A028

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

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An aerobic Gram-stain-negative, orange-pigmented, rod-shaped bacterium, designated ATAX6-5^T, was isolated from a marine red alga *Asparagopsis taxiformis* in South Korea. Cells were catalase- and oxidase-positive reactions. Growth of strain ATAX6-5^T was observed at 5–35°C (optimum, 30°C), at pH 6.0–9.5 (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 $C_{16:0}$, $C_{17:1}$ *ω6c* and $C_{18:1}$ *ω7c* were identified as the major cellular fatty acids. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and sphinoglycolipid were the major polar lipids. The G+C content of the genomic DNA was 60.4 mol%. Strain ATAX6-5^T was most closely related to *Parasphingopyxis lamellibrachiae* JAMH 0132^T with a 96.89% 16S rRNA gene sequence similarity. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain ATAX6-5^T formed a phylogenic lineage with *Parasphingopyxis lamellibrachiae* JAMH 0132^T within the family *Sphingomonadaceae*. On the basis of phenotypic, chemotaxonomic and molecular features, strain ATAX6-5^T clearly represents a novel species of the genus *Parasphingopyxis*, for which the name *Parasphingopyxis algicola* sp. nov. is proposed. The type strain is ATAX6-5^T (=KACC 18993^T = JCM 31719^T).

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A029

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

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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.]

A030

Albirhodobacter aestuarii sp. nov., Isolated from the Estuary Sediment

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A Gram-staining-negative and strictly aerobic, circular convex colony bacterium, designated strain S1-47^T, was isolated from estuary sediment in South Korea. Cells of strain S1-47^T were oxidase- and catalase-positive short rods. Growth was observed at 10–30°C (optimum, 25°C), at pH 5.0–10.0 (optimum, pH 7.0–8.5) and in the presence of 0.0–6.0% (w/v) NaCl (optimum, 2.0%). The respiratory quinone detected was only ubiquinone 10 (Q-10) and summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and summed feature 8 (comprising $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) were found as the major fatty acids (>10% of the total fatty acids). Phosphatidylethanolamine was identified as the major polar lipid and an unidentified aminolipid, an unidentified phospholipid and an unidentified lipid were also detected as minor polar lipids. The G+C content of the genomic DNA was 69.26 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain S1-47^T formed a tight phyletic lineage with *Albirhodobacter marinus* N9^T 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^T clearly represents a novel species of the genus *Albirhodobacter*, for which the name *Albirhodobacter aestuarii* sp. nov. is proposed. The type strain is S1-47^T (=KACC 18804^T =JCM^T 31536). An emended description of the genus *Albirhodobacter* is also proposed.

A031

Flavobacterium hwacheonense sp. nov., *Flavobacterium chuncheonense* sp. nov., and *Flavobacterium chungpyungense* sp. nov., Isolated from Freshwater Lakes

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Three Gram-negative, non-motile, rod-shaped bacterial strains were isolated from freshwater lakes in Korea. Strain IMCC25901^T was isolated from Lake Paro and strains IMCC26013^T and IMCC26026^T were from Lake Soyang. Phylogenetic analysis based on 16S rRNA gene sequences showed that three strains belonged to the genus *Flavobacterium* and strains IMCC25901^T, IMCC26013^T, and IMCC26026^T were most closely related to *Flavobacterium yonginense* (96.7% sequence similarity), *Flavobacterium psychrophilum* (96.5%), and *Flavobacterium myungsuense* (97.7%), respectively. Optimal growth conditions of these strains were observed at 20°C, at pH 7.0 and without NaCl. DNA G+C contents of three strains ranged from 33.7 to 37.8 mol%. DNA-DNA relatedness of strain IMCC26026^T with *F. myungsuense* was 56.4%, showing a novel species status of strain IMCC26026^T. Major fatty acid constituents of three strains were iso-$C_{15:1}$ G, iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{15:1}$ ω6c, $C_{17:1}$ ω6c, and summed feature 3 (comprised $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*). Respiratory quinone detected in the strains was MK-6. On the basis of these results, strains IMCC25901^T, IMCC26013^T, and IMCC26026^T were considered to represent novel species in the genus *Flavobacterium*, for which the names *Flavobacterium hwacheonense*, *Flavobacterium chuncheonense*, and *Flavobacterium chungpyungense* are proposed, respectively.

A032

Flavobacterium sediminis MEBiC7310 sp. nov., a Marine Bacterium Isolated from a Tidal Flat Sediment

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A Gram-negative, 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 haoraii* LQY-73^T (96.7%) and followed by *Flavobacterium indicum* GPTSA 100-9^T (95%) and *Flavobacterium cucumis* R2A45-3^T (92.5%). Growth was observed at 17–43°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-$C_{15:0}$ 3-OH (8.4%), iso-$C_{17:0}$ 3-OH (18.6%), summed feature 1 (13.2%, iso-C15:1 H and/or C13:03-OH) and summed feature 3 (16.9%, C16:1ω6c and/or C16:1ω7c). 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 MEBiC073104^T represents a novel species in the genus *Flavobacterium*, for which the name *Flavobacterium sediminis* MEBiC7310 sp. nov. is proposed.

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A033

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

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Strain A2^T, was isolated from the rumen of cow. Cells were catalase-positive and oxidase-weakly positive. Growth of strain A2^T was observed at 25–45°C (optimum, 37–40°C), pH 5.5– 9.5 (optimum, pH 7.5) and in the presence of 0–3.5% (w/v) NaCl (optimum, 1%). Strain A2^T contained iso-$C_{16:0}$ and anteiso-$C_{15:0}$ as the major cellular fatty acids. Menaquinone-11 was detected as the sole respiratory quinone. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain A2^T formed a distinct phyletic lineage within the genus *Leucobacter*. Strain A2^T was most closely related to '*Leucobacter margaritiformis*' A23 (97.7%) and *Leucobacter tardus* K 70/01^T (97.2%). The major polar lipids of strain A2^T consisted of diphosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid. Strain A2^T contained a B-type cross-linked peptidoglycan based on 2,4-diaminobutyric acid as diagnostic diamino acid with threonine, glycine, alanine and glutamic acid but lacking 4-aminobutyric acid. The G+C content of the genomic DNA was 67.0%. From the phenotypic, chemotaxonomic and molecular features, strain A2^T was considered to represent a novel species of the genus *Leucobacter*, for which the name *Leucobacter ruminantium* sp. nov. is proposed. The type strain is A2^T (=KACC 17571^T = JCM 19316^T).

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A034

Strain H0056^T, a Novel Member of the Genus *Flavobacterium*

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A Gram-staining-negative, aerobic, non-motile, rod-shaped, and yellow-pigmented bacterial strain, designated strain H0056^T, 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 *Flavabacterium*, a member of the family *Flavobacteriaceae.* The highest sequence similarity was observed with *F. arsenitoxidans* (97.6%), and followed by *F. ginsengisoli* (97.5%) and *F. defluvii* (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 *Flavabacterium.* The polyphasic study including whole genome sequencing is still underway.

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A035

A Novel Species Candidate belonging to the Genus Mucilaginibacter

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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 Mucilaginibacter, a member of the family Sphingobacteriaceae. The highest sequence similarity was observed with Mucilaginibacter oryzae (98.0%) showing the independence of the new isolate as a genospecies. Cells grew on R2A, NA, and TSA, but not on MacConkey agar or MA. Colonies on R2A were pale-pink, mucoid, convex, and round with entire margins. Optimum growth occurred at 30°C and in the presence of 1% NaCl. The general phenotypic properties of strain H0046T were similar to the members of the genus Mucilaginibacter, but several physiological features such as casein degradation and Tween 80 hydrolysis distinguished the isolate from the closely related species. Other genomic, physiological, biochemical, and chemotaxonomic properties of the isolate are under investigation to clearly demonstrate the taxonomic status of strain H0046T as a novel species within the genus Mucilaginibacter.

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A036

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

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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 DRC1506^T, 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 20241^T) shared lower ANI (<94.1%) and *in silico* DDH values (<57.0%) with other four *Leu. mesenteroides* lineage strains. Here, we report that strain DRC1506^T represent a novel subspecies within the species *Leuconostoc mesenteroides,* for which the name *Leu. mesenteroides* subsp. *jonggajibkimchii* subsp. nov. is proposed. The type strain is DRC1506^T (=KCCM 43249^T =JCM 31787^T). In addition, *Leu. mesenteroides* subsp. *suionicum* is also reclassified as *Leu. suionicum.* sp. nov., comb. nov. (type strain DSM 20241^T =ATCC 9135^T =LMG 8159^{T}CIMB 6992^T).

A037

A Novel *Tenacibaculum* sp. Isolated from a Squid

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A novel Gram-reaction-negative, aerobic, rod-shaped bacterium, designated strain LPB0136^T, 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^T 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. Phylogenic analysis based on its 16S rRNA gene sequence indicated strain LPB0136^T belongs to the genus *Tenacibaculum* and is most closely related to *T. aestuarii* SMK-4^T (95.9% 16S rRNA gene sequence similarity) and *T. caenipelagi* HJ-26M^T (95.9%). The respiratory quinone was menaquinone-6 and major fatty acids were iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{15:1}$ G, and iso-$C_{15:1}$ ω6*c*. The size of genome, chemotaxonomic features, and physiological characteristics supported the assignment of strain LPB0136^T 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 LPB0136^T 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.]

A038

Isolation of Two Novel Marine Bacteria belonging to the 4-Org1-14 and OCS116 Clades of *Alphaproteobacteria* and Their Genomic Characterization

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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 Program (PJT200620), funded by the MOF, Korea.]

A039

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

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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 *Nnibrrimonas geomnyongensis*.

A040

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

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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 25–30°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.*

A041

Cold Adaptation and Diversity of Bacteria Isolates from Chukchi Sea

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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 phylogentic analysis based on 16S rRNA gene sequence, psychrophilic bacteria consisted 22 taxa from 8 genera ((*Bacillus* (10), *Pseudoaltermonas* (3), *Paenisporosarcina* (3), *Stenotrophomonas* (2), *Brevibacterium* (1), *Altermonas* (1), *Sediminicola* (1), *Sulfitobacter* (1)) and psychrotolerant bacteria consisted of 15 taxa from 4 genera ((*Bacillus* (10), *Paenisporosarcina* (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.

A042

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

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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 (*Cladosorium* (23), *Penicilium* (16), *Aspergillus* (3), *Engyodontium* (2), *Epicoccum* (1), *Peniophora* (1), *Pseudocercosporella* (1), *Talaromyces* (1), *Acremonium* (1), *Acrodontium* (1), *Ustilago* (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.

A043

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

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Microbial communities were investigated with a metagenomic approach in four commercial salts: Ethiopian Afdera 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 *Halorubrum* 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

A044

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

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A Gram-staining-negative, aerobic, light brown pigment bacterium, designated strain CE80 was isolated from marine sponge *Callyspongia elegans* in Jeju. Strain CE80^T was e isolate grew optimally 25°C, at pH 6.0–10.0 (optimum 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^T belonged to the genus *Labrenzia* and were closely related to *Labrenzia suaedae* YC6927^T (97.01%), *Labrenzia aggregata* IAM 12614^T (95.90%) and *Labrenzia alexandrii* DFL-11^T (95.90%), *Labrenzia marina* mano18^T (95.72%) and *Labrenzia alba* CECT 5094^T (95.34%). The major fatty acids (>2%) of strain CE80^T were $C_{18:1\ w7c}$ (66.76%), $C_{16:1\ w7c}$ (10.26%), $C_{18:0}$ (4.71%), 11-methyl $C_{18:1\ w7c}$ (4.47%), $C_{20:1\ w7c}$ (2.63%), $C_{18:0}$ 3-OH (2.25%) and unknown 14.502 (2.05%). The major respiratory quinone was Q-10. The Polar lipid were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, two unknown aminolipids, and three unknown lipids. The DNA G+C content of strain CE80^T was 55.86 mol%. On the basis of, physiological and biochemical characterization, phylogenetic and chemotsxonomic analysis, it is proposed that isolated a new species, *Labrenzia* sp. nov.,. The type strain is CE80^T (=KCTC 42149^T =JCM30735^T).

A045

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

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National Institute of Agricultural Sciences

A novel actinomycete strain, designated KIS14-16^T, 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^T is a member of the genus *Arthrobacter*, exhibiting highest sequence similarity with *Arthrobacter livingstonensis* LI2^T (97.7%), *Arthrobacter cryoconiti* Cr6-08^T (97.6%), and *Arthrobacter stackebrandtii* CCM 2783^T (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^T from phylogenetically related type strains. The peptidoglycan type of strain KIS14-16^T was A3α, with an interpeptide bridge comprising L-Thr, Gly, and L-Ala_4. Strain KIS14-16^T contained a large amount of MK-9(H_2) with the relatively small amounts of MK-10(H_2) and MK-8(H_2). The main polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and one unidentified glycolipid. On the basis of these phenotypic, chemotaxonomic and phylogenetic data, strain KIS14-16^T 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^T (= KACC 17303^T = DSM 27180^T = NBRC 109660^T).
[Supported by grant from RDA]

A046

Description of *Parapedobacter lycopersici* sp. nov., Isolated from Rhizosphere of Tomato Plants (*Solanum lycopersicum* L.)

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A novel Gram-negative bacterial strain, designated T16R-256^T, 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 and round. The strain grew in the temperature range of 15–37°C (optimally at 28–30°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-256^T is a member of the genus *Parapedobacter*, exhibiting highest sequence similarity with *Parapedobacter pyrenivorans* P-4^T (94.2%), *Parapedobacter indicus* RK1^T (93.7%), *Parapedobacter koreensis* Jip14^T (93.7%), *Parapedobacter luteus* DSM 22899^T (93.6%), *Parapedobacter soli* DCY14^T (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-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and iso-$C_{15:0}$ 2-OH/$C_{16:1}$ $\omega 7c$. Strain T16R-256^T contained MK-7 as predominant respiratory quinone. The genomic DNA G+C content of the type strain is 55.5 mol%. On the basis of these phenotypic, chemotaxonomic and phylogenetic data, strain T16R-256^T 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-256^T (= KACC 18788^T = JCM 31602^T).

[This research was supported by RDA.]

A047

Acinetobacter sp. nov., Isolated from Swinery Waste

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A novel bacterium, designated strain JAA17^T, was isolated from swinery waste collected on Jeju Island, Republic of Korea and characterized using a polyphasic approach. Strain JAA17^T exhibited morphological, cultural and chemotaxonomic features consistent with its classification as representing a member of the genus *Acinetibacter*. Growth occurred at 15–40°C, pH 5.0–9.0 and in the presence of 0–2% (w/v) NaCl. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain JAA17^T formed a distinct clade within the genus *Acinetibacter* and was closely related to *Acinetobacter pragensis* ANC 4149 ^{T} (96.4% similarity), *Acinetobacter guillouiae* CIP 63.46^T (96.1% similarity), *Acinetobacter populi* PBJ7T^T (96.0% similarity). Based on evidence from a polyphasic taxonomical study, it was concluded that the strain should be classified as representing a new species of the class *Gammaproteobacteria*, strain JAA17^T is considered to represent a novel species of the genus *Acinetobacter*.

A048

Description of *Pelagibacterium rhizosphaerae* sp. nov., Isolated from the Rhizosphere of Tomato Plants

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A novel Gram-staining-negative bacterium, designated T20R-121^T, was isolated from the rhizosphere soil of tomato plants grown in greenhouse in Yecheon-gun, Gyeongsangbuk-do in Republic of Korea. Strain T20R-121^T were aerobic, flagellated, catalase-positive (weak) and oxidase-positive, rod-shaped bacterium. The strain grew at 15–35°C, pH 6.0–8.0 and with 0–6% NaCl (w/v). The DNA G+C content of the genomic DNA was 61.9 mol%. The major fatty acids (>10%) were $C_{18:1}$ $\omega 7c$, 11-methyl $C_{18:1}$ $\omega 7c$ cyclo and $C_{19:0}$ $\omega 8c$ cyclo. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, two unidentified glycolipids and five unidentified lipids. The major respiratory quinone is Q-10. 16S rRNA gene sequence of strain T20R-121^T showed the highest similarity with *Pelagibacterium lixinzhangensis* H642^T (98.7%), *Pelagibacterium halotolerans* B2^T (98.0%), '*Pelagibacterium nitratireducens*' JLT2005^T (97.0%) and *Pelagibacterium luteolum* 1_C16_27^T (96.2%). The phylogenetic tree showed that strain T20R-121^T formed a clade with the genus *Pelagibacterium* being separated from the other genus. DNA-DNA hybridization values with the closely related *Pelagibacterium* species were less than 70%. On the basis of phenotypic, genotypic and phylogenetic evidence, strain T20R-121^T represents a novel species of the genus *Pelagibacterium*, for which the name *Pelagibacterium rhizosphaerae* sp. nov. is proposed. The type strain is T20R-121^T (= KACC 18791^T).

[This study is supported by RDA.]

A049

Mycobacterium tuberculosis Rv21xx Promotes Bacterial Survival within Macrophages

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Pathogenicity of mycobacteria is tightly linked to their survival in host macrophages. Although mycobacteria are classical intracellular pathogens of macrophages, the mechanisms by which they invade and persist in host cells are still not well understood. Identification and characterization of the mycobacterial proteins modulating macrophage function are essential for understanding tuberculosis pathogenesis and bacterial virulence. We found a novel protein Rv21xx that induce macrophage activation from *Mycobacterium tuberculosis* (Mtb) culture filtrate proteins. To verify the immunostimulatory nature of Rv21xx, we produced recombinant Rv21xx from *Escherichia coli*. The recombinant Rv21xx increased the secretion of pro- and anti-inflammatory cytokines in dose-dependent manner, such as TNF-α and IL-10 but not IL-12p70, and the expression of surface molecules in bone marrow-derived macrophages (BMDMs). Rv21xx triggered the MAPKs and NF-κB which were dependent on TLR4 signaling pathways in macrophages. In addition, we assessed the survival of Mtb in Rv21xx-treated BMDMs. Unexpectedly, Mtb survival in Rv21xx-treated BMDMs were significantly enhanced when compared with that in non-treated BMDMs. Moreover, survival of non-pathogenic *Mycobacterium smegmatis* overexpressing Rv21xx was enhanced in comparison to the vector expressing strain when infected in host macrophages. These results suggest that *M. tuberculosis* Rv21xx influences bacterial virulence and promotes survival in host.

A050

Identification and Characterization of Yeasts Isolated from Korean Nuruk

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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 *Saccharomycopsis fibuligera* etc. In most Nuruk, *Saccharomycopsis 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).]

A051

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

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A Gram-staining-variable, aerobic, rod-shaped, motile and spore-forming bacterial strain, designated CJ11^T, was isolated from the tidal flat sediment in Ganghwa-do, South Korea. Strain CJ11^T grew optimally on R2Aat 30°C and pH 7.0. Sequencing results of the 16S rRNA gene revealed that strain CJ11^T possesses two copies of the 16S rRNA gene varying at 5 positions. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain CJ11^T belonged to the genus *Paenibacillus*, within the family *Paenibacillaceae* and was most closely related to *Paenibacillus tarimensis* KACC 14087^T (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 CJ11^T represents a novel species in the genus *Paenibacillus*, for which the name *Paenibacillus translucens* sp. nov. is proposed. The type strain is CJ11^T.

A052

Flavobacterium foetidum sp. nov., Isolated from Ginseng Soil

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A yellow-pigmented, rod-shaped, Gram-staining-negative, aerobic and devoid of flagella, but showed gliding motility bacteria strain, designated CJ42^T, was isolated from the ginseng soil in Anseong, South Korea. Strain CJ42^T 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^T belonged to the genus *Flavobacterium* within the family *Flavobacteriaceae* and was most closely related to *Flavobacterium phragmitis* DSM 23314^T (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^T represents a novel species in the genus *Flavobacterium*, for which the name *Flavobacterium foetidum* sp. nov. is proposed. The type strain is CJ42^T.

A053

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

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Strain GI5^T 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 GI5^T was affiliated to the family *Alcanivoracaceae* and is most closely related to *Alcanivorax dieselolei* B-5^T (92.0% similarity) and *Alcanivorax marinus* R8-12^T (91.7%). The major cellular fatty acids in strain GI5^T were $C_{16:1}$ *ω6c*/$C_{16:1}$ *ω7c* (28.73%), $C_{18:1}$ *ω6c*/$C_{18:1}$ *ω7c* (26.26%) and $C_{16:0}$ (11.98%) and the profile was distinct from those of the closely related species. Major respiratory quinone of strain GI5^T was Q-8. The main polar lipids were phosphatidylethanolamine and phosphatidylglycerol. The G+C content of the genomic DNA of strains GI5^T was determined to be 51.2 mol%. Based on phenotypic, chemotaxonomic, and phylogenetic studies, strain GI5^T 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 GI5^T (= KCTC 52659^T = JCM 31835^T).
[Supported by the Ministry of Oceans and Fisheries, Korean.]

B001

Microbial Community in Produced Water Samples

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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 OTUs were associated with Clostridiaceae (18.4%), Methanosaetaceae (13.1%), and Pseudomonadaceae (16.8%), which have been recognized to be thermophilic, thermotolerant. The composition of the separator sample deviated greatly from the well samples. These observations suggest the importance of temperature as the dominant driving force for microbial selection under high temperature regimes.
[Supported by KETEP and MOTIE]

B002

Methanobactin Secreted by *Methylosinus trichosporium* Strain OB3b Interferes with N_2O Reduction in *Pseudomonas stutzeri* Strain DCP-Ps1

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Methanobactin (Mb) is a Cu chelator secreted by methanotrophs for savenging 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 biogeochemical reactions that require Cu for their activities. N_2O reduction mediated by nitrous oxide reductase (N_2OR) is one of such Cu-dependent reactions, which has an environmentally significant function of reducing N_2O emissions. This study investigated the effect of methanobactin excretion by *Methylosinus trichosporium* strain OB3b on N_2O reduction activity in *Pseudomonas stutzeri* strain DCP-Ps1. N_2O evolution from strain DCP-Ps1 was monitored upon incubation in a minimal salts medium amended with 450 μmoles NO_3^-, 1000 μmoles acetate, 0.2 μM $CuCl_2$ and varying concentrations of Mb-OB3b (0, 1, 5 μM). In a separate set of experiments, strain DCP-Ps1 was incubated in an O_2-depleted culture of strain OB3b (OD_{600}~0.3) grown in the same medium. In the absence of Mb or strain OB3b cells, transient N_2O accumulation maxed at 70 ± 4 nmoles N_2O-N/bottle while cultures amended with Mb-OB3b accumulated 435.7 - 453.3 μmoles of N_2O-N. A permanent N_2O production of 288.12 ± 5.7 μmoles was observed in the co-culture experiments, supporting the results from the experiments with purified Mb. These observations suggest a key role of methanotrophs and secreted methanobactin in regulation of N_2O reduction activity.
[Supported by NRF (2016004832) and MOLIT as "U-City Program."]

B003

Comparing Bacterial Communities Associated with Soft Coral *Scleronephthya gracillimum*

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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 gracillima* 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 Hahellaceae, 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).]

B004

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

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Despite the high potential of metanotrophs 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% CH_4 (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, *Methylomonas* spp. was remarkably enriched and only one methanotroph OTU remained in the reactor, which grouped with *Methylomonas* 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."]

B005

Physiological and Genomic Features of a Novel Acidophilic Arsenite-oxidizing Bacterial Strain AS8 belonging to the Genus *Herminiimonas*

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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 (optimum 25–30°C), pH 3.0–9.0 (optimum 5.0–6.0), and 0–1% NaCl (w/v, optimum 0.2–0.4%). Under chemolithoheterotrophic 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/monosaccharide 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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B006

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

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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.

[Supported by grants from Rural Development Administration and Nongwoobio Co., Ltd.]

B007

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

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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 (PJT200620) from Ministry of Oceans and Fisheries and by the BK21 Plus.]

B008

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

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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 (ENRs) homologues, AcrB efflux pumps, and ENR substitutions. We found that most of the human and soil-borne plant pathogens contained TCS resistance determinant 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]

B009

Compartmentalizing the Role of Rhizosphere Microbiome and Endophytic Microbiome against Bacterial Wilt Incidence on Tomato Plants

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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]

B010

Genome Analysis of Flavobacteriales Bacterium Strain UJ101

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Flavobacteriales bacterium strain UJ101was 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-Douddoroff pathway were impared. 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.

B011

Cloning of Cytosine N4-methyltransferases from Marine Alphabacteria

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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 S-adenosyl methionine as a co-substrate. Using single molecule real-time sequencing method (SMRT), methylation patterns of *Celeribacter marinus* IMCC12053 and *Novosphingobium pentaromativorans* 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 exocylic 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.

B012

B013

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

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Acinetobacter oleivorans DR1 can utilize C_{12}–C_{30} alkanes as a sole carbon source, but not short-chain alkanes (C_6, C_{10}). Transcriptomic and qRT-PCR analyses under triacontane (C_{30}) condition suggested that genes participating in the synthesis of trehalose, poly 3-hydroxybutyrate (PHB), siderophore, and unsaturated fatty acid were highly upregulated. Induced *ack*A, and *pta* genes suggested unusual ATP synthesis during C_{30} degradation. Growth assay of *ace*A knock out mutant indicates that glyoxylate shunt pathway is essential metabolism under C_{30} condition. Further expression and mutant analyses of AHs showed that *alk*B1 and *alk*B2 are major AH-encoding genes under C_{12}–C_{30} alkanes, but inducible *alm*A genes on LC alkanes help to degrade C_{30}. 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.

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B014

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

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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 influenced by individual-specific qualities. We questioned how the skin microbiome differs according to the degree of urbanization, under influences of assembly process. Here, skin microbiome was heterogeneous according to socioeconomic status but irrelevant to climate or geographical distance. Dominance test, β-null deviation method, and edge-length abundance distribution analysis confirmed that the skin microbiome community in megacity (defined as a city with more than 10 million people) was assembled mostly by the niche-based process. Bacterial network in skin microbiomes of the megacity has a looser network structure and the skin disease-causing bacteria were tend to be autonomous in the fragile megacity skin microbiome. Understanding the skin microbial community assembly will provide a new insight into the skin microbiome as the first line of defense against skin diseases.

[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

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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 parahaemolyticus* (*n* = 1,757) were ubiquitous in both tidal water and mud year round, whereas *Vibrio cholerae* (*n* = 24) and *Vibrio vulnificus* (*n* = 130) were seasonally specific to summer. While all *V. cholerae* isolates were nontoxigenic (non-O1 and non-O139), more than 88% of *V. vulnificus* isolates possessed the virulence factors elastolytic protease (*vvp*). Interestingly, *V. parahaemolyticus*, 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.

B016

B017

Monitoring of Various Antibiotic Resistance in Three Rivers of South Korea

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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-usage 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 rRNA 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 (2.92×10^4). 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)]

B018

B019

Microbial Community Structures of Activated Sludge in Anaerobic/Anoxic/Oxic Reactors Operating at Full-scale Wastewater Treatment Plants Revealed by 454 Pyrosequencing

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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/518R 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 Euryarahaeota (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.

B020

Characterization of a Novel Plant Growth Promoting Dormant Bacterium from Tomato Rhizosphere

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Seung Yeup Lee, and Seon-Woo Lee*
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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 (Zuiken), 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 Zuiken 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]

B021

Metagenomic Analysis on the Influence of Ocean Acidification on Microbial Communities in a Mesocosm Experiment

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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, CO_2 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 CO_2 condition, the abundances of diatom and genes for primary production increased, whereas increase at high CO_2 was not observed for some phototrophic taxa that are present at low CO_2. Dynamics of the genes associated with nitrogen cycle and dimethylsulfide production indicated that the concentration of $NH4^+$ increase and that of dimethylsulfide 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 will make the ocean more acidic and this finally will decrease of microbial diversity in the future.

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B022

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

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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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B023

Comparative Metagenomic Analysis of Wheat-based *Nuruk*

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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 composition, 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.

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B024

Genomic Analyses of the Three Extremophiles: Hyperthermophilic and Extremely Halophilic Archaea and an Anaerobic Bacterium, Isolated Based on Culturomics

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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^T had been isolated from solfataric soil. The genome of strain CBA1501^T 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 CRISPRs and 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.

B025

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

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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: *Protoeobacteria* (*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: *Protoeobacteria* (*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.

B026

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

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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, sunblock 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.

B027

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

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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.]

B028

Evaluation of Suitability and Safety in Commercial Probiotics for Animal Using Barcoded Pyrosequencing and Multiplex-PCR

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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.]

B029

A Taxonomic Analysis Based on Functionally Equivalent Proteins

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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)].

B030

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

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To investigate the effects of *Tetragenococcus halophilus* MJ4, 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 *Salimicrobium album*, *Alkalibacillus saliacus*, *T. muriaticus*, and *Marinilactibacillus piezotolerans* appeared as major populations after 25 days. Metabolite analysis using ^{1}H 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

B031

Interplay between Tomato's Disease Resistance and Its Rhizosphere Microbiota

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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 rDNA reads, which have been extracted from the whole metagenome data using blastn against the SILVA database, revealed that the proportion of *Flavobacteriia* 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.

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B032

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

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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 *Kluyveromyces* in *Mankyua chejuens* and *Vanderwaltozyma* (40 isolates), *Cryptococcus* (40 isolates), and *Kluyveromyces* (one isolate) in *Dendropanax morbifera.* Two *Kluyveromyces* 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 *Kluyveromyces* isolates produced high bioethanol.

B033

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

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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 carbons 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 54.3% 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 NH_4Cl, 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-methy 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

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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 acI 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 $\geq$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 acI strains (previously isolated from Lake Soyang) and the results showed specific distribution patterns of the acI sub-clades.
[This study was supported by a grant of the Mid-Career Research Program through the NRF.]

B035

Genome Analysis of Marine Bacterial Strains belonging to the OM60/NOR5 Clade of *Gammaproteobacteria*

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The OM60/NOR5 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 photoheterotrophs (AAnP), represented by *Congregibacter litoralis* KT71 and HTCC2080. In this study, three isolates from coastal surface waters, IMCC3088 (NOR5-4 subclade), IMCC14385 (*Halioglobus* sp.) and IMCC14734 (unclassified subclade), in the OM60/NOR5 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 4,835,928 bases, 4418 predicated ORFs, and 54.2% G+C content. 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/NOR5 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 (PJT200620) funded by the MOF, Korea.]

B036

Investigation of Soil Microbial Community of Dokdo and Ulleungdo Islands by Pyrosequencing Analysis

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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 10^6 ~ (2.4 ± 0.3) x 10^7 CFU/g dry wt. and those of of Ulleungdo island were in the range of (5.6 ± 0.3) x 10^7 ~ (3.8 ± 0.5) x 10^8 CFU/g dry wt. during investigation, respectively. As a result of the bacterial community by pyrosequencing analysis, the numbers of OTUs obtained were 3,197 and 2,892 from Ulleungdo and Dokdo islands, respectively. And by statistical analyses, Proteobacteria was the most dominant phylum [Dokdo (41.85%) 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.

B037

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

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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 (*Ignavibacteriaceae*) and anaerobic ammonium oxidation (*Brocadiaceae*) bacteria also contributed major nitrogen removal near the separator in unit-1. Various sulfate- (e.g., *Desulfobulbaceae*) 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-2015R1A2A1A15054528).]

B038

Microbial Diversity in Secondary Calcite, Moonmilk, from Baeg-nyong Cave

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Cave is an important environment preserving past climatic information effectively. Calcite formation in cave is induced by abiotic and biotic 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. Thus, we suggest that *Lysobacter* group could influence the microbial diversity of moonmilk by their lytic activity against other microorganisms. Among 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-2017R1 D1A1A02061743]

B039

The Effect of Long-term Fertilizations on Altering the Bacterial Community Composition and Its Consequences on Microbial Methane Cycling in Rice Paddy Soils

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Long-term repeated fertilizations may alter the microbial community composition in agricultural soils. However, the role of soil microbes 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.

B040

Wolbachia Inhabited by the Insect Vector of Pine Wilt Disease

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Wolbachia, the obligate endosymbiotic bacteria influencing the reproduction of its host, is of interest in their 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-horned 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-horned 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)]

B041

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

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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 acI-A1, -A4, -A7, and -C1. The four acI strains, initially isolated by dilution-to-extinction culturing, eventually failed to be maintained as axenic cultures. However, the first complete genomes of the acI lineage were successfully obtained from these initial cultures through whole genome amplification applied to more than hundreds of cultured acI cells. The genome sequences exhibited features of genome streamlining and showed that the acI strains are aerobic chemoheterotrophs sharing central metabolic pathways, with some differences among tribes that may underlie niche diversification within the acI lineage. Actinorhodopsin 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.]

B042

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

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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 (10^5 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 *Flavobacteriaceae* (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.]

B043

Primers for Amplification and Deep Sequencing of *Alphacoronavirus* Genomes

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Rapid full genome sequencing is essential for identification of unknown viral pathogen, 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, T_m, 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)]

B044

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

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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 dibenzofuran (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-submits 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 (PJT200620) & KIOST (PE99514)]

B045

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

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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)]

B046

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

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Bacterial DNA methylation 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 methylome 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

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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 concern 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 dioxides.
[Supported by grants from KOPRI]

B048

Comparative Analysis of Skin Microbiome between Young and Old Women

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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 microbiomes showed there were a significantly separation by age groups. Interestingly, opportunistic pathogens have a potential to cause skin diseases in immune-compromised persons such as *Streptococcus* and *Corynebacterium* were higher relative abundance in the old than young group through LEfSe analysis. Predicted functional composition of skin microbiome indicated the KEGG pathways related to degradation of amino acids, and xenobiotics were dominant in the old group while DNA repair and recombination were frequently found in the young group. A dominance test based on Sloan's neutral theory showed the skin microbial community of the old group was assembled heavily by niche-based process compared with the young group. 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

Distinct Soil Bacterial Distribution in Four Different Farming Management Soils

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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]

B050

Microbial Community Structure and Composition in Rhizosphere and Endosphere of Tomato Plants Cultivated in Greenhouses

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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 tanscribed 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]

B051

Characterization of Spatial Distribution of the Bacterial Community in the South Sea of Korea

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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]

B052

Bacterial Community Structure of Surface Snow in Antarctica

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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 16S rRNA gene by pyrosequencing, overall 13,504 sequence reads were obtained, and 412 operational taxonomic units (OTUs) were generated with 97% similarity cutoff. *Gammaproteobacteria* (0.0~70.1%), *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.

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B053

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

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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 toutilize high amounts of phenol of either up to 1,000 ppm with cell vigorous growth. Isolate was able to grow in pure culture and in defined mixed culture with other aromatics degrader on phenol compound as a sole source of carbon and energy. 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.

B054

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

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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 funcitonal analysis were profiled using HUMAnN2. Shotgun metagenomic reads were mapped to 'ChocoPhlAn' pan-genome database and MetaPhlAn2 database for organism specific functional profiling. We then analyzed the gene families and pathways using the extended databases UniProt Reference Clusters and MetaCycmetabolic 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 NH_4^+ could 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 —asparagine* 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.

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B055

To Study the Effect of Change in NaCl Concentrations on Virulence of *Streptococcus parauberis*

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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 SpOF 3k 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 *Streptococcaceae* family. In the present work, the growth rates of *S. parauberis* were determined in BHI medium containing diverse array of salinity 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.

B056

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

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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 PM16030).]

B057

Control of Soil Community Structure with Oxygen and Organic Substrates

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Large amounts of herbicide mainly including TCDD (Tetra-chlorinated dibenzo-p-dioxin) had been sprayed during Vietnam War. Due by 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 microcoms 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 13 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)]

B058_9th ASME

Bacterial Community in Enrichment Culture of Cut Grass Residue Where Simultaneously Occurred both Cellulose Degradation and Nitrogen Fixation

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We obtained the stable bacterial enrichment culture of cut grass residue where occurred both cellulose degradation and nitrogen fixation after having repeated the sub-culturing. When a filter paper was added to the culture, paper degradation was observed within 3 days while simultaneously detecting the nitrogen fixation. After 15 days incubation, bacterial DNA was extracted from the enrichment culture and the extract was used for the community analysis. The next generation sequencing (NGS) targeting V3 and V4 regions of 16S rRNA genes showed that enrichment culture included 11 genera as major bacterial groups included both nitrogen fixing and cellulose degrading bacteria. Based on the NGS information, we isolated four nitrogen fixing bacteria, which sequences were corresponded with the major bacterial groups. The sequence of 16S rRNA gene were closely affiliated with *Azospira* sp., *Azoarcus* sp., *Pseudomonas* sp. and *Azospirillum* sp. They are commonly known as endophytic nitrogen fixing bacteria. Thus, these nitrogen fixing bacteria suggested to be key member in this bacterial consortia through nitrogen cycling while obtained the carbon products from cellulose degrading bacteria.

B059 _9th ASME

Isolation, Identification and Growth-promotion Effect of Phosphate Solubilizing and Potassium Decomposing Bacteria Isolated from Mangrove Soil

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The study aimed to abtain microorganisms that can convert insoluble inorganic phosphate and potassium to an accessible form to promote the growth of plant for increasing yields. Phosphate solubilizing and potassium decomposing bacteria were screened and isolated from mangrove soil in Shankou 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 phylogenetic characteristics were tested. Phosphate-solubilizing strain P225, P238, P379 and P402 were identified as *Bacillus aerophilus*, *Bacillus oceanisediminis*, *Bacillus cereus*, *Sphingomonas jaspsi*, and potassium-solubilizing strain K119, K355, K375, and K395 were identified as *Bacillus marisflavi*, *Sphingomonas sediminicola*, *Bacillus vietnamensis*, and *Halobacillus trueperi* according to the results of polyphasic taxonomy. The sequences of eight strains were deposited in the GenBank nucleotide sequence data library in NCBI with the accession numbers KX982790, KX982805, KX982816, KX982825, KX982843, KX982738, KX982746, KX982739. The inorganic phosphate-solubilizing activity was determined by the molybdenum blue method at 30°C using NBRIP medium to measure calcium phosphate [$Ca_3(PO_4)_2$]-solubilizing activity, while NBRIP replaced with aluminum phosphate ($AlPO_4$) and iron phosphate $FeSO_4$ to measure $AlPO_4$ and $FeSO_4$-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 biofertilizer applying for maize , the growth-promoting effect in soil was P402 (83.33%) > P238 (65.42%) > K119 (65.42%) > K355 (59.58%)> K225 (39.17%) > P379 (35.83%) > K375 (16.25%) > K395 (5.83%) , while for biofertilizer applying for soybean, the growth-promoting effect in soil was P379 (64.68%) > K119 (59.00%) > K225 (53.32%) > P402 (43.67%) > K355 (32.31%) > K375 (30.61%) > P238 (11.30%) > K395. In summary, we isolated several potential for plant growth-promoting bacteria for further research of maize and soybean biofertilizer.
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B060 _9th ASME

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

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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 understory 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 *Ruminococcuceae* and *Prevotellacea*, and also exhibited higher community diversity (P<0.001). Consequently, these features may contribute to the flexibility of Yaku sika's dietary shift and to maintain nutritional status under high density conditions.

B061 _9th ASME

Species-specific Interaction Using Membrane Vesicles in Enterobacteria

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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 unknown 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 *Buttiauxella agrestis* specifically interacted with *Buttiauxella* 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.
[This work support by JSPS KAKENHI Grant Numbers JP15K21043 and JP15H01315 to Y. T.]

B062 _9th ASME

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

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It is usually thought that a competitive inhibition occurred between *Dehalococcoides* and *Methanogens* under highly H_2 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 H_2 concentration. However, it is known that the activity of reductive-dehalogenase depends on the activity of hydrogenase that is positive correlation with H_2 concentrations. Furthermore, although the concentration of H_2 was sufficient for PCE/TCE-dechlorination under highly H_2 conditions, why were PCE/TCE-dechlorinating activities inhibited? Reverse transcription-PCR was used to evaluate the transcriptional efficiency (*rdhA*s mRNA per *rdhA*s). 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 H_2 concentrations. These results indicated that PCE/TCE-dechlorinating activities of *Dehalococcoides* in enrichment culture TUT2264 are regulated by different two stages using hydrogen.

B063 _9th ASME

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

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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 koningiopsis* FCD3-1 and *Hypocrea cremea* 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

B064 _9th ASME

Mechanism of Growth-repression Induced by *Pseudomonas* sp. C8

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It is important for understanding complex microbial ecosystems to analyze an interspecies interaction. In our previous study, a supernatant (C8SP) 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 C8SP and the amount of growth was 20% to 50% less than that under absence of the C8SP. KEIO library was used to unveil the growth-inhibiting mechanism of the C8SP. 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 C8SP 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 C8SP, resulting in growth inhibition based on the imbalance between catabolic and anabolic processes.

B065 _9th ASME

Microbial Diversity on the Leaves of Japanese and Taiwan Teas

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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* bacteria were found in sample A, D, E and F, 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, *Devosia* and *Chryseobacterium* in Tw2, *Aurantimonas* and *Pedobacter* in Tw3, *Brevundimonas*, *Edaphobacter*, *Propionibacterium* and *Streptomyces* in Tw4, respectively. Therefore, these results on their microbial diversities might be useful information for utilizing them as bioresource.

B066 _9th ASME

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

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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.

B067 _9th ASME

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

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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 radiorsisten* 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]

B068 _9th ASME

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

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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.

B069 _9th ASME

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

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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 Nangan, 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 5μg/L respectively, the EMMC systems have demonstrated excellent performance in pretreatment of micro-polluted source water .

B070 _9th ASME

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

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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 NH_4^+-N in the influent, the MFCs using carbon nanotube-coated sponge could effectively remove 81 ± 0.1% COD and 76 ± 0.2% NH_4^+-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% NH_4^+-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% NH_4^+-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 felt could effectively remove 82 ± 0.1% COD and 73 ± 0.2% NH_4^+-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.

B071_9th ASME

Multiple Advanced Cultivation Techniques for Isolation of Fastidious Microorganisms Associated with the Marine Sponge

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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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B072

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

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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 10 rRNA operons were predicted in the circular chromosome.
[Supported by grants from KOPRI]

B073

Reconstruction Archaeon Genome from Anoxic Intertidal Mud Flat

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The Bathyarchaeota group were composed ~46% of microbial population on the anoxic black mud flats at ~2 m depth of the garolim bay. For investigate this group, all of 113 Gb (50.7 Gb from JW200 and 62.3 Gb from GS200) microbial metagenomic data sets was generated. De novo assembly and binning of this data was produced abundant 14 archaeal draft genomes and 11 bacterial draft genomes by coverage based binning process. Phylogenetic assignments of ribosomal protein sets (rpl2, 3, 4, 5, 6, 14, 15, 18, 22, 24 and rpS3, 8, 10, 17, 19) and 16S rRNA sequences showed that most of archaea bins were belongs to various subgroups of Bathyarchaeota. Among them 5 bins were belongs to BA1,2 clade of bathyarchaeota which expected involved in methane metabolism. Most of Bathyarchaeota bins encoded acetogenic pathway using tetrahydromethanopterin as C1 carrier cofactor. But, gene for key enzyme (Mcr) for final step of methanogenesis, the methyl-coenzyme M reductase complex, was not detected in contigs as well as metagenome raw reads on present study. This genome analysis bridge the gap of metabolic potential between various Bathyarchaeota groups. This research was a part of the project titled "Long-term change of structure and function in marine ecosystems of Korea", funded by the Ministry of Oceans and Fisheries, Korean
Key words: metagenome, bathyarchaeta

B074

Characterization of Methanotrophic Community Enriched Soil Acidic Soils

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Soils represent a significant sink for methane through the activity of methane-oxidation bacteria. Difference in methane oxidation were also related to changes in the methane-oxidizing communities in acidic soils. We investigated the methanotrophic communities enriched from acidic soils using the sequencing batch reactor at pH 4 and pH 3. Illumina sequencing for 16S rRNA gene analysis indicates that the families Methylocytaceae might be key members involved in methane oxidation in acidic soils. The methanotrophic strains was iolated by using floating culture and extinction culture techniques from the enrichment culture. Isolates belonging to the genus Methylovirgula of the familiy Beijerinckiaceae and the genus Methylobacter of the family Methylococcaceae were obtained from the enrichment cultures. Our findings suggest that various methanotrophs in diverse phylogenetic clades are involved in methane oxidation in acidic soils. Metagenomic analysis of the genome and metabolic pathways of the dominant members in the acidic methanotrophic enrichments is may give insight into the methane oxidation in acidic soils.
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Key words: soil acidification, acidophilic methanotrophs, enrichment, isolation, metagenomic sequencing.

C001

Synthetic Small Regulatory RNA-based Efficient Gene Knockdown in *Clostridium acetobutylicum*

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Clostridium is a promising industrial microorganism for the production of valuable chemicals. However, current inefficient genetic manipulation techniques retard metabolic engineering of *Clostridium*. Here, we report a gene expression regulation system for *Clostridium 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 *buk*-mutant *C. acetobutylicum* strain PJC4BK (PJC4BK (pPta-HfqEco)) allows production of 16.9 g/L butanol, which is higher than that (14.9 g/L) produced by the original PJC4BK strain. The strain PJC4BK (pPta-HfqEco) produced 105.5 g solvents (70.7 g butanol, 20.5 g acetone, and 14.3 g ethanol) in fed-batch fermentation with *in situ* gas stripping, demonstrating the stable fermentation of a strain harboring the sRNA system.

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C002

Production L-arginine by Metabolically Engineereed *Corynebacterium glutamicum*

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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 *opcA*, *pgl*, *tal*, *tkt*, 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.

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C003

Production of 3-Aminopropionic Acid by Metabolically Engineered *Escherichia coli*

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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 asparatase-catalyzed reaction, the native promoter of the *aspA* gene was replaced with the strong *trc* promoter. The *aspA* and phosphoenolpyruvate carboxylase (*ppc*) genes were additionally overexpressed, and the ammonium sulfate was supplemented in the medium, which resulted in the production of 3.49 g/L 3-AP. Optimization of PPC expression level by using synthetic promoter and RBS sequences 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 32.3 g/L of 3-AP in 39h with an overall yield and productivity of 0.135 g 3-AP/g glucose and 0.828 g/L/h, respectively.

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C004

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

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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* knock-out simulation was carried out to identify additional gene knock-out targets to further improve ALA production. The *gcvTHP* genes (glycine cleavage system) were predicted as knockout targets. The JW01 strain (WL3110 ΔgcvTHP pKE112hemA) produced 1.17g/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 *iclR* and *sdhAB* genes, while the TCA cycle flux was reinforced by the deletion of *ptsG* gene. The JW03 strain (JW01 Δ*iclR* Δ*sdhAB* Δ*ptsG* pKE112hemA) was able to produce 1.72g/L of ALA. Fed-batch culture of the JW03 (pKE112hemA) strain resulted in the production of 5.77 g/L of ALA in 41 h.

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C005

Microbial Production of Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) from Unrelated Carbon Source

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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.

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C006

Metabolic Engineering of *Ralstonia eutropha* as a Platform Strain for Production of 2-Hydroxyacid Containing Polyhydroxyalkanoates

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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.

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C007

Performance Evaluation of A&A™ TB/MDR/NTM(5) qK-CAP™ Kit for Detection of Mycobacteria and Anti-tuberculosis Drug Resistance

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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 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 test, 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.]

C008

A Simple Genotyping of NTM Using New Method for Multiplexing Molecular Diagnosis

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NTM is known to about 150 species. This is why Multiplexing molecular diagnosis is recommended. We developed a simple Method for multiplexing molecular diagnosis, called Ampli & Array system, which integrates the Real-Time PCR and DNA microarray.
Probes identifying genotype of TB/NTM are immobilized on the surface of K-CAP™ platform. We can recognize clinically important targets, 20 species of *mycobacterium*. These are composed of *M. tuberculosis*, *M. intracellulare*, *M. avium*, *M. abscessus*, *M. kansasii, M. fortuitum, M. gordonae, M. chelonae, M. genavense, M. haemophilum, M. immunogenum, M. malmoense, M. marinum, M. mucogenicum, M. scrofulaceum, M. simiae, M. smegmatis, M. szulgai, M. terrae complex and M. ulcerans.*
We achieved the evaluation of the analytical performance following. 1) Range of Detection (ROD), 2) Limit of Detection (LOD), 3) Interference effects, 4) Cross reactivity.
As a result, ROD of A&A™ TB/NTM(20) Genotyping qK-CAP™ Kit was confirmed to be possibly used up to the range of 10^1 to 10^9 copies/μl. But, Genotyping for several Mycobacterium were detected in the range of 10^2 to 10^8 copies/μl. Also, there was no interference effect and cross-reactivity. we expect that the Ampli &Array system will replace the multiplexing molecular diagnosis market.
[This work was supported by the MOTIE, KIAT through the Encouragement Program for the Industries of Economic Cooperation Region (R0002888)]

C009

Metabolic Engineering of *Escherichia coli* for Production of Bio-isoprene

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Isoprene (2-methyl-1,3-butadiene) is a C5 terpenoid with the formula $CH_2=C(CH_3)CH=CH_2$. 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 methylerythritol 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, Pueraria 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.
[This work was supported by C1 Gas Refinery Program through the NRF funded by the MSIP (NRF-2016M3D3A1A0193246).]

C010

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

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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.
[Supported by NRF (No. 2016936920) and IPET, MAFRA (500-20160226)]

C011

Economic Production of D-Psicose and D-Mannitol via Combination of Whole Cell Conversion Processes

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Rare sugars, which exist only limited quantities in nature, have received considerable attention because of its various clinical effects and specific biological functions. Likewise, D-psicose (D-ribo-2-hexulose or D-allulose), a C-3 epimer of D-fructose, also has many uses which include reducing intra-abdominal fat accumulation, protecting pancreas beta-islets and improving insulin sensitivity. Especially, calorie of D-psicose is close to zero, only 0.3% calories, while it has 70% relative sweetness compared to sucrose. In 2012, D-psicose was approved as a food additive and designated as Generally Recognized As Safe (GRAS) by Food and Drug Administration (FDA). Despite such abundant advantages, there is no economical way of mass production of D-psicose. Recently, biological production of D-psicose from D-fructose using D-psicose 3-epimerase (DPE) has been developed. However, the conversion yield is below 30%, which causes an increase of purification cost because of the similar solubility of D-psicose and D-fructose. Thus, we addressed the problem by converting the residual fructose of the D-psicose production reaction to D-mannitol which has a low solubility.

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

C012

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

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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 cattles. 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 chimerical multi-epitopic recombinant protein (5BT), 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 5BT, it was conjugated with BmpB, the membrane protein B of *Brachyspira hyodysenteriae* such as fusion protein (B5BT). Our results showed that 5BT was susceptible to degradation by host protease and produced with fraction of inclusion body. The stability and solubility of 5BT was greatly increased by conjugating to BmpB. 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 study was supported by grant from Academic Research Affairs Division, Ministry of Education.]

C013

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

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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.) CJLP-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.

[Supported by the Strategic Initiative for Microbiomes in Agriculture and Food (Grand ID:914005-04), the National Research Foundation (NRF-2016 R1C1B2016246), and BK21 Plus Program from South Korea.]

C014

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

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Previous studies have shown that *P. anomala* mz-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 Phenefhyl 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.

C015

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

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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_06642 (Tor1) and CNAG_05220 (Tlk1, TOR-like kinase 1), in *Cryptococcus neoformans*. Both Tor1 and Tlk1 have rapamycin-bindidng (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 discernable 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.

C016

Metabolic Redesign of Corynebacteria for Isoprenoid Production Based on Carotenoids

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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 *dxs* gene of the endogenous MEP pathway or by introducing the whole MVA pathway into *Corynebacterium glutamicum*. We constructed lycopene production plasmid by putting heterologous *crtEBI* operon and *idi* 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 *crtY* 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.]

C017

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

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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 diphosphate (IPP) and dimethylallyldiphosphate (DMAPP), which are generated via either the MEP pathway or the MVA pathway. Farnesyl pyrophosphate (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.]

C018

C019

Antibacterial Compound Produced by Bacteria Isolated from Soil

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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 60~100°C for 30 min and in the pH range of 2-10. The antibacterial substance was not affected by carbohydrase.

C020

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

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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.

C021

C022

C023

Microbial Distribution and Potential Abundances of Anammox Bacteria in the Rice Paddy Soil

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Anaerobic 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 79% 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.

C024

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

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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 might cause environmental problems. In this study, a non-ureolytic CCP bacterium *Lysinibacillus* sp. YS11 was isolated from rhizosphere of *Miscanthus sacchariflorus* near artificial stream. CCP using phase-contrast microscopy and ion-selective electrode was observed. Strain YS11 showed CCP in both aerobic and hypoxia conditions. Energy dispersive X-ray spectrometry mapping confirmed the presence of calcium carbonate. Field emission scanning electron microscopy analysis indicated morphologically distinct mineral formation under those conditions. Resistance at high pH and in high salt concentrations, as well as the spore-forming ability of strain YS11, suggests its potential for application in self-healing concrete. Monitoring of bacterial growth, pH changes, and Ca^{2+} concentrations suggested that strain YS11 could induce alkaline conditions up to a pH of 8.9 and utilize 95% of free Ca^{2+} under aerobic conditions. Unusual Ca^{2+} binding and its release from cells were observed under hypoxia conditions. This is the first report of a non-ureolytic bacterium capable of CCP under both aerobic and hypoxia conditions.

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C025

Genetic and Physiological Characterization of Poly-β-hydroxybutyrate Production in *Sphingobium chungbukense* DJ77

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Motakatla Venkateswer Reddy, and Jong-Chan Chae*

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In this study, the conversion of wide range of monoaromatic compounds i.e., 4-methoxybenzoic acid, ferulate, veratrate, isovanillate and 4-hydroxybenzoic acid into the aliphatic biopolymer poly-β-hydroxybutyrate (PHB) was investigated with *Sphingobium chungbukense* DJ77. *S. chungbukense* showed highest PHB production (54%) with 4-methoxybenzoic acid among different aromatic compounds as carbon source at 5 mM concentration, but the production was low compared with acetate at 5 mM (68%). Two important nutrients nitrogen and phosphorous concentration was optimized at 5 mM 4-methoxybenzoic acid concentration. Optimization of nutrients concentration improved PHB production by over 3.5%. The functional groups, thermal and physical properties of the produced PHB were analyzed. These results indicate that the strain *S. chungbukense* is excellent candidate for conversion of different aromatic compounds into useable biological polyesters.

C026

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

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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 tiliing 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.

C027

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

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

C028

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

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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 molybdic 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.

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C029

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

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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.]

C030

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

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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)-β, and INF-γ. 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.

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C031

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

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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 1105 exceptively formed fuitbody 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 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

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The current study describes the biosynthesis of polyhydroxyalkanoates (PHAs) from Benzene, 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 copolyesters of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV). Contrastively, PHAs from *m*- and *p*-xylenes were polyesters consisting of 3HB, 3HV and medium chain length 3-hydroxyalkanoates. The amounts of PHAs accumulated in the cells from these substrates were in the range of 8–34 wt% 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

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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 bioresources 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% oxgall), 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

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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.

C035

Isolation of *Paenibacillus amyloticus* JS3-20 with Antimicrobial Activity

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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 methicillin-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^T (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.

C036

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

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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.

C037

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

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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 virus 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.

C038

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

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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.

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D001

Engineered Attenuated Salmonella Kills Cancer Cells through Activating Endothelium Reticulum Stress

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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_2IR△*pts*I*crr* (KST0650) using the combination of genetic manipulation and radiation mutation technology. In mice sepsis model, we found that its LD_{50} in mice sepsis model was about 1×10^6 CFU which was slightly less than *Salmonella* △ppGpp strain (KST0651) which has been most widely used in cancer therapy. But, 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.

D002

The Study on Pathogenic Bacteria in Coral, *Screlonepthya gracillimum*

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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 choleras 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 shilonii*, *Vibrio coralliiilyticus*, and *Vibrio carchariae.* The assay was applied to coral samples from the Jeju Island, Japan and Taiwan (Jeju Island -Sungsan, Munsum; Japan - Wakayama, 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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D003

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

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Since the first description of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in 1895, it is known to cause paratuberculosis or Johne'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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D004

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

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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 (*fimCDH*) and *ler*-controlled toxin genes (*espD*, *escJ*, *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.

D005

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

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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*

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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 ^{14}C-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

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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 Δ NC is 4 times higher than 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.

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D008

Induction of Apoptosis through ER Stress by Shiga Toxins in Human Retinal Pigment Epithelial Cell

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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 ($\Delta\Psi$) 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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D011

Investigation on Genotyping and Multi-drug Resistance of Diarrheageni *E. coli* Isolated in Northern Gyeonggi-do Region

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This study was isolated 125 strains from a total of 3,864 sample diarrhoea patients for 2012-2016 in northern Gyeonggi-do region. A total of 125 isolates were characterized by pathogenicity tying. Among them, EPEC to 88 isolates was the most prevalent, followed by the ETEC to 32 isolates and EHEC to 5 isolates. By antimicrobial susceptibility tests, the 36 strain (28.8%) were susceptible to all of the 17 antimicrobial agents used in this study. 23 strains (18.4%) were resistant to only one microbial agent and 66 strains (52.8%) to two or more agents, showing multi drug resistance and the isolates resistant to the 11 antibiotics were 2 strains. Ampicillin (AM) were shown to have the highest to the 72 strains, in that order of resistant agents having more than 10 strains is cephalothin (CF) and tetracycline (Te) to 39 strains, ampicillin/sulbactam (SAM) to 36 strains, nalidixic acid (NA) to 32 strains, amoxicillin/clavulanic acid (AMC) to 30 strains, trimethoprim/ sulfamethoxazole (SXT) to 26 strains, cefazolin (CZ) to 15 strains and cefotaxime (CTX) to 10 strains.

D012

Isolation and Characterization of *Acinetobacter baumannii* Associated with Tooth Decay in Children of Cambodia

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There is an increasing risk of tooth decay in children of Cambodia. Here we showed that the percentage of decayed teeth with untreated caries in primary teeth is high in children aged 2~5 years in the rural areas of the Battambang city. Glucose-utilizing bacteria were isolated from oral swabs of children in order to reveal the relations with tooth decay. Through 16S rRNA gene analysis, two *Acinetobacter* strains, CAM121 and CAM180-1, were revealed to be the most abundant species. Particularly, *A. baumannii* strain CAM180-1 which isolated from a girl with severe tooth decay was found to be codominant with *Cupriavidus* strain CAM180-2. Biochemical tests and *in vitro* tooth decay experiments showed that the mixed, but not individual, culture of strains CAM180-1 and CAM180-2 with *Streptococcus mutans* produced a large amount of acid and developed caries. In the absence of *S. mutans*, the mixed strains CAM180-1 and CAM180-2 caused the browning of dentin in a non-fermenting condition without enamel breakdown. It is important that strain CAM180-1 plays a dominant role in the early stage during fluctuations of oxygen tension and pH of the medium and contributes to biofilm formation by extracellular matrix production, co-aggregation and metabolic cooperation with other bacteria.

D013

Identification of NDM-9, a Variant of New Delhi Metallo-β-lactam-producing *Klebsiella variicola* in Gwangju River

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Since the first identification of an NDM-1-producing strain in India (2008), bla_{NDM-1}-positive pathogenic bacteria have been detected globally, raising concern because of the limited treatment options. The first NDM-9-positive *Klebsiella pneumoniae* was first isolated in 2014, followed by an *Escherichia coli* strain carrying NDM-9 and MCR-1. In this study, three carbapenem-resistant *Klebsiella variicola* isolated from Gwangju tributary were found to possess bla_{NDM-9} genes. Antimicrobial susceptibility test indicates resistance of these strains to aminoglycosides, carbapenems, cephems, folate pathway inhibitors, fosfomycin, and penicillins but susceptible to fluoroquinolones, phenicols, tetracycline and miscellaneous agents. Whole genome sequencing revealed that the 108-kb IncFII(Y)-like plasmids carry bla_{NDM-9} which were sandwiched in between IS*15* for GJ1 strain, IS*26* for GJ2 strain, IS*15D1* for GJ3 strain and IS*Vsa3*, and further bracketed by IS*26* and Tn*AS3* along with mercury resistant operon upstream and class 1 integron composed of gene cassettes of *aadA2*, *dfrA12*, and *sul1* downstream. An *aph(3')-Ia* gene conferring resistant to aminoglycosides located after the integrons. Chromosomally encoded bla_{LEN-13}, *fosA*, *aqxA*, and *oqxB* genes, as well as plasmid-mediated bla_{TEM-1B} and $bla_{CTX-M-65}$ encoding extended-spectrum β-lactamase, *ant(3')-Ia*, and *mph(A)* genes were identified too.
[Supported by MOE and MOF]

D014

Effects of Probiotics and Sodium Butyrate on Atopic Dermatitis

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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 ears 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.

D015

Regulatory Characteristics of the *cabA* Gene Encoding a Calcium-binding Protein Essential for Biofilm Development in *Vibrio vulnificus*

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Vibrio vulnificus, a food-borne pathogen, forms biofilms on different biotic surfaces to enhance survival in natural environments. *V. vulnificus* CabA is a structural protein distributed throughout extracellular matrix and essential for biofilm development. BrpT and BrpR, homologues of VpsT and VpsR in *Vibrio cholerae*, respectively, are putative transcriptional regulators involved in biofilm development. To examine roles of the two regulatory proteins, the *brpT* mutant, *brpR* mutant, and *brpRbrpT* double mutant were constructed from parental strain JN111 and their effects on the gene expressions and biofilm development were evaluated. Transcript analysis of the *V. vulnificus* strains in biofilms revealed that the *cabA* expression is activated by BrpT, and the *brpT* expression is activated by BrpR. The dual plasmid system assay showed that BrpT binds upstream of *cabA*, while BrpR binds upstream of *brpT*, suggesting that BrpR and BrpT function sequentially in a regulatory cascade. Crystal violet staining revealed that the level of biofilm formation is reduced in the isogenic mutants relative to that in JN111 under elevated c-di-GMP condition. Microscopy analyses of the colony morphology and biofilm structure produced by the *V. vulnificus* strains showed that only JN111 formed rugose colony morphology and well-structured biofilm. The combined results indicated that BrpT and BrpR positively regulate the *cabA* expression in a regulatory cascade, leading to the development of robust biofilm.

D016

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

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Cholera is an acute intestinal infectious disease caused by *Vibrio cholerae*. *V. cholerae* is endemic in many low-income countries, particularly in areas of inadequate sanitation and food hygiene practices. Up to date, two oral cholera vaccines, Dukoral and Shanchol, are qualified from WHO and licensed in several countries. Although the pathogenesis caused by *V. cholerae* takes place in the intestine, anaerobic environment, two oral vaccines are formulated with *V. cholerae* cultured in aerobic condition. Moreover, anaerobiosis-induced microbial gene regulation and virulence are not fully elucidated in *V. cholerae*. In this study, we investigated colonization of *V. cholerae* grown under anaerobic conditions in intestinal epithelial cells. Anaerobic growth of *V. cholerae* O1 significantly increased bacterial adhesion compared to aerobically grown bacteria in human epithelial cells. A similar adherent property of *V. cholerae* O1 was also observed in polarized Caco-2 cells. 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.

[Supported by grants from NRF]

D017

Activities of MMA01, a Novel Oxazolidinone, against *Mycobacterium abscessus*

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M. abscessus is the most pathogenic and chemotherapy-resistant RGM (rapidly growing mycobacterium) and pulmonary infections. But the problem is that no antibiotic class has been shown to produce long-term sputum smear conversion. Therefore, treatment of pulmonary infection is very difficult. In recent years, linezolid has become the first oxazolidinone antibacterial agent to be applied to the treatment of NTMs (nontuberculous mycobacteria). However, linezolid therapy has the potential risk of decreasing platelet, red blood cell, and white blood cell. MMA01 was found to effectively inhibit *M. abscessus* growth *in vitro,* in mouse lungs *in vivo* compared with linezolid. In addition, we were able to select spontaneous mutants that were resistant to MMA01. Sequencing analysis revealed *rplC* T460C and G419A mutation, as well as nucleotide insertion at the 503 position. Therefore, we conclude that MMA01 is a crucial candidate with better safety to be further developed for the treatment of *M. abscessus* infections.

D018

Identification of Anti-TB Agent from Newly Assembled Drug Library

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Tuberculosis (TB) is 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, 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 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.

D019

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

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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.

D020

Evaluation for Inactivation of Foot and Mouth Disease Virus

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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.

D021

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

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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 through polyethylene glycol (PEG), ammonium sulfate (AS) treatment or ultrafiltration (UF). Several methods for purification and concentration of 146S particles were evaluated by spectrophotometer to measure the amount of the purified 146S particles. The purified FMDV 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.

D022

Chromatographic Analysis of Foot and Mouth Disease Virus

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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.

D023

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

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Probiotics in livestock feed supplements are considered as a replacement for antibiotics for the mucosal immunity of gastrointestinal tract. Although several effector molecules such as bacterial cell wall components have been proposed to be associated with the function of probiotics, little evidence is suggested that the effector molecules of probiotics are responsible for strain-specificity of probiotic functions. 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. Lp.LTA, but not lipoprotein or peptidoglycan 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, dealanylated 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.

D024

Recombinant BCG to Avoid Innate Immunity in Bladder Cancer

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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 tree 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 administering small amounts of BCG and reducing side effects.

D025

Lipid Composition of Membrane and Bacteria-host Interaction

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Ornithine 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, PGE_2, 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.

D026

Screening of Synthetic Compounds for Anti-biofilm- and Anti-quorum Sensing Activities

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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.*

D027

Probiotic Effect of Attenuated *Pseudomonas aeruginosa* in Brine Shrimp

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Xi-Hui Li[1,2], and Joon-Hee Lee[1,2]*

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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 shrimps 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 we 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.

D028

Quorum Sensing-dependent Extracellular Activation of Proteases in *Pseudomonas aeruginosa*

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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.

D029

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

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

D030

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

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Whole genomes of 123 clinical isolates which consisted of 34 drug susceptible isolates, 14 non-MDR drug resistants, 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 clades, 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 PhyResSE SNP analysis. This may suggest that some of SNPs which were not included in PhyResSE 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

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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 bacillary necrosis in oysters. *V. coralliilyticus* 58, formerly reported as *V. splendidus* biovar II 58, was originally isolated from inactive 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 *cytolysin/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*

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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 Δset1 strain to identify the role of Set1 in *C. albicans* pathogenesis. In *Δset1*, 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 *Δset1* strain is more sensitive to hydrogen peroxide (H_2O_2) or menadione which induces oxidative stress. The survival assay in macrophages indicated that the survival rate of *Δset1* 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*

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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 glycerol-3-phosphatase that involved in glycerol biosynthetic pathway. Growth of the *gpp2* deletion strains was affected at 39°C in spot dilution growth assay. In addition, the *gpp2*△ strains exhibited increased sensitivity to Congo Red, Calcofluor White and DTT. *In vivo* studies demonstrated that the *gpp2* deletion mutant was attenuated compared to the wild type strains in the murine inhalation model. Genetic epistasis analysis discovered that Gpp2 and the zinc finger transcription factor Crz1 play a role in a branched calcineurin pathway that orchestrates stress survival and fungal virulence. These results propose the function of Gpp2 in cell-wall integrity, fungal pathogenicity in *C. neoformans*.

D034

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

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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 resistant 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*.

D035

The Role of Mkt1 in Sexual Reproduction in *Cryptococcus neoformans*

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The Mkt1-Pbp1 complex is required for mating-type switching in *Saccharomyces cerevisiae* by regulating posttranscriptional regulation of *HO* expression. Here, we demonstrated that the poly(A)-binding protein-binding protein Pbp1 interacts with Mkt1 by employing a *in vivo* immuno-pull-down and mass spectrometry analyses. The association of Pbp1 with Mkt1 was confirmed by co-immunoprecipitation. Result of spot dilution growth assay revealed that the *mkt1* deletion strains, unlike *pbp1* deletion strains, did not show resistant phenotype to heat stress as compared to the WT. The *mkt1* deletion mutants, similar to *pbp1* deletion mutants, exhibited a defect in dikaryotic hyphal production. In addition, the *mkt1* mutation resulted in reduced expression level of the pheromone gene (MF α1) during mating. These results propose that the Mkt1-Pbp1 complex is required for activation of pheromone gene expression sexual reproduction in *C. neoformans*.

D036

Oral Treatment with Probiotics Inhibits the Atopic Dermatitis in Mice

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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 exprssion 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*

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Dandruff is a global skin disease that has effected 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.

D038

The Antibacterial Activity against Fish Pathogen of *Paenibacillus* sp. MK-11 Isolated from Jeju Coast

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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.

D039

AMPK-activated Protein Kinase Activators Inhibit Proinflammatory Responses in Macrophages during Mycobacterial Infection

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Metformin is a widely used anti-diabetic drug targeting AMP-activated protein kinase (AMPK) pathway, and reported to activate anti-mycobacterial activities in human macrophages. AICAR is a well-known AMPK activator and autophagy pathway stimulator. While several studies have shown that metformin inhibits inflammatory and autoimmune responses to various stimuli, it is unknown what effect the AMPK activating drugs may have on cytokine production during mycobacterial infection. To address this question, *Mycobacterium tuberculosis* (Mtb)-infected bone marrow-derived macrophages (BMDMs) were cultured in the presence of AMPK activators, AICAR or metformin, and analyzed for mRNA and protein expression of proinflammatory cytokines. Both AICAR and metformin inhibited Mtb-induced tumor necrosis factor (TNF)-α and interleukin (IL)-6 production in a dose-dependent manner. However, neither treatment exerted any obvious effect on the anti-inflammatory cytokine IL-10. Next, we investigated the role of AMPK in AICAR- and metformin-mediated inhibition of proinflammatory cytokines and nitrite production. The inhibitory effects of both AICAR and metformin on TNF-α, IL-6, and nitrite production were partially reversed in cells transduced with shRNA against AMPK; similar changes were not seen in cells transduced with nonspecific shRNA. These data indicate that metformin and AICAR inhibit Mtb-induced proinflammatory responses and nitrite production through AMPK activation.

D040

Natural Killer Cell-induced Anti-hepatitis C Virus Effect in HCVcc System

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Recently, hepatitis C virus cell culture (HCVcc) system has been established, where HCV is stably replicating in highly permissive human hepatoma cell line 7.5. Natural killer (NK) cells are primary lymphoid cells that recognize and kill tumor cells and virus-infected cells. In this study, the interaction between NK cells and HCVcc system were investigated. First, coculture between NK92 cells and HCV (JFH1)-infected huh7.5 cells (or navie huh 7.5 cells) was established and the change of morphology in coculture was compared in presence or absence of HCV. In addition, the production of twelve different cytokines was measured in coculture with or without HCV using Multi-Analyte ELISArray. The expression of functional receptor for NK cells was also measured using Flow cytometry. Western blot analysis was done for apoptosis-related cellular proteins (parp, caspase 3) as well as for HCV specific proteins (core and NS3).
We observed a clear aggregation between NK 92 cells and huh7.5 cells only when HCV is present in the cells. Among twelve cytokines, only IFN-γ, IL-6, IL-8, and IL-10 are detected. The coculture between NK 92 cells and HCVcc (or naïve huh 7.5 cells) obviously induced apoptosis compared to HCVcc (or naïve huh 7.5 cells) without NK 92 cells. However, the expression of proapototic molecules (cleaved parp and cleaved caspase 3) was suppressed by the presence of HCV in coculture. HCV proteins were clearly decreased in coculture with NK 92 cells.

D041

Key Residue Analysis of MgtC

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Salmonella enterica serovar Typhimurium is a Gram-negative, and pathogenic bacteria. *Salmonella* invasion and survival within the host cells causes severe systemic disease. In a point of molecular view, an interplay between bacteria and host factor derives alteration of host cell physiology, resulting in invasion and survival of the pathogen. To understand bacterial infection mechanism, unveiling how pathogen interacts with host cell is essential. *Salmonella* penetrates host microfold cell, then it allows for transport of microbes across the epithelial cell to take place immune response. Host immune system recognizes the foreign antigen and eliminate bacteria by forming phagosome. But *salmonella* can endure host defense response, surviving within the macrophage. Given that phagosome has harsh enviroments, *Salmonella* virulence proteins are related in survival mechanism within harsh host immune system. MgtC is known as a virulence protein responding to phagosome conditions and a conserved protein in many intracellular pathogens. The *Salmonella mgtC* deletion mutant shows non-pathogenicity compared to wild type in mouse injection. Following these results, MgtC virulence protein has a key role for *Salmonella* infection mechanism. In this poster, we found that a specific amino acid residue of MgtC determines *Salmonella* survival in mouse injection. We suggest that despite phagosome has a scarcity of nutrient, *Salmonella* struggles to survive by alteration own physiological phenotype.

D042

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

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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 410 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

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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*

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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 α1,2-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 α1,2-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 *ktr3cap6*Δ 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 *ktr3cap6*Δ. 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 *ktr3cap6*Δ compared to the wild-type strain upon tunicamycin treatment, indicating that *O*-glycans with extended structure are essential for Mpk1-meidated-cell wall integrity signaling. Moreover, *ktr3cap6*Δ 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-opsonic phagocytosis of *ktr3cap6*Δ 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*

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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-1. 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-1 and contribute to dimerization. Our structural study of bcPLP would provide a foundation stone for the future researches on PadR subfamily-1-mediated transcriptional regulation.

D046

Analysis of Gastric Bacterial Communities for the Study of Gastric Carcinogenesis

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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, interstinal metaplasia, and gastric cancer. As it progresses to gastric cancer, the levels of *Epsionproteobacteria* and *Bacilli* 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*.

D047

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

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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-a 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.

D048

Effect of Different Temperatures on *Streptococcus iniae* FP5228

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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 (17°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.

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D049

Temperature Dependent Biological Characteristics on *Streptococcus parauberis* SpOF3K

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S. parauberis 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.

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D050

Effect of Salinity on the Pathogenicity of *Streptococcus iniae* FP5228

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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 organisms, in other word 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 BHI 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.]

D051

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

Sejeong Kim[1], Jimyeong Ha[1], Yohan Yoon[1], and Kyoung-Hee Choi[2]*

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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 fuidity 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.
[This study supported by grants from National Research Foundation of Korea (2014-0811).]

D052

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

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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).]

D053

Effect of Intermittent PTH Administration on Diabetes Rats with Periodontitis

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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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D054

Investigation of *Helicobacter pylori* CagL Loop Domain Roles in T4SS Functions

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Helicobacter pylori CagL is one of the components consisting Type IV secretion system, which interact with host cells able to trigger the cell signaling pathway. We sequenced 56 *H. pylori* clinical isolates in Korean population and determined CagL consensus sequence of East-Asian *H. pylori*. And then we determined the crystal structure of CagL from K74 *H. pylori* strain representative Korean populations and demonstrated. Also through characterization and determined of CagL protein structure, examined whether CagL domain function to T4SS building and virulence phenotypes via *H. pylori*-host interaction.

G27 isogenic mutants complemented with alanine substituted five loop and c-terminus mutation in *cagL* gene of K74 *H. pylori.* Loop2, 3 and 5 are required to CagA phosphorylation, iL-8 induction and gastrin promoter activation. Furthermore, CagL each loops motif mutants didn't showed hummingbirds cell phenotype in loop2, loop4 and loop5 but, loop1 and loop3 mutants. Previous study reported, cell elongation phenotypes requirement to CagA phosphorylation mediated signaling. Intriguingly, 'CGISD' residues in loop4 of CagL shows that cell elongation is required to both CagA phosphorylation and CagL-host mediated cell signaling even though, phosphorylated CagA presence in host. And, 'VIV' residues in 'KVIVK' of CT are key residues to canonical T4SS function such as CagA delivery, IL-8 induction and gastrin promoter activation. Also, actual presences of T4SS in each complemented *H. pylori* were confirmed by FESEM.

In conclusion, we demonstrated new insights to structure analysis based CagL functions as a component of T4SS formation and biological functions during T4SS-mediated interactions with host cell. Moreover, we suggesting cell elongation is not sufficient to phosphorylation of CagA but also, CagL-host signaling.

D055

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

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Helicobacter pylori is a well-known pathogen that colonizes in gastric mucosa, causing chronic gastritis, peptic ulcers, and gastric cancer. *H. pylori* carrys 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 amipregulin 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

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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 biphenyl 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 HBeAg 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.

E001

Functional Characterization of an Esterase (EaEST) from *Exiguobacterium antarcticum*

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A novel microbial esterase, EaEST, from a psychrophilic bacterium *Exiguobacterium antarcticum* B7, was identified and characterized. To our knowledge, this is the first report describing structural analysis and biochemical characterization of an esterase isolated from the genus *Exiguobacterium*. Crystal structure of EaEST, 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 EaEST is occupied by a peracetate molecule, which is the product of perhydrolysis of acetate. This result suggests that EaEST may have perhydrolase activity. The activity assay showed that EaEST has significant perhydrolase and esterase activity with respect to short-chain p-nitrophenyl esters ($\leq$C8), naphthyl derivatives, phenyl acetate, and glyceryl tributyrate. However, the S96A single mutant had low esterase and perhydrolase activity. Surprisingly, immobilized EaEST 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.

E002

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

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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 belongs to the orthorhombic space group $C222_1$, with unit cell dimensions of a = 53.1 Å, b = 121.2 Å, and c = 54.4 Å. A traceable electron density map was calculated using anomalous diffraction data obtained from a crystal soaked in zinc ions.

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

E003

Functional Implications of Hexameric Assembly of RraA Proteins

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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 RraAV 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 RraAV1 was converted to an octamer and tetramer when the Cys 9 residue was substituted with an Asp residue (RraAV1-C9D), showing decreased inhibitory activity of RraAV1 on RNase EV *in vivo*. These results indicated that the intermolecular disulfide linkage contributed critically to the hexamerization of RraAV1 for its proper function. On the contrary, the RraAV2 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 RraAV1-C9D on RNase EV activity compared to wild-type RraAV1. Based on structural and functional comparison of RraA homologos, 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*

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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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E005

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

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The *glpFKD* operon, which is induced under hypoxic conditions and in the presence of glycerol, is involved in glycerol metabolism in *Mycobacterium smegmatis* mc^2155. Immediately upstream of the *glpFKD* operon is an open reading frame that is 48% identical to GylR from *Streptomyces coelicolor* A3(2). To examine the role of GylR, we constructed the *gylR* inframe deletion mutant (Δ*gylR*) of *M. smegmatis*. The expression of the *glpFKD* operon in the Δ*gylR* mutant was abolished and the introduction of the intact *gylR* gene into the mutant complemented the Δ*gylR* phenotype, suggesting that the *glpFKD* operon is positively regulated by GylR. The Δ*gylR* 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 GylR-binding sites (IR1, IR2, and IR3) with the consensus sequence GKTCGRC-N_3-GYCGAMC. IR3 was demonstrated to be essential for *glpFKD* expression by the GylR activator and that two neighboring GylR-binding sites (either IR1 and IR2 or IR2 and IR3) are required for the binding of GylR to the control region. Herein we propose the model explaining the regulation of the *glpFKD* operon by GylR.

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E006

Structural Study on a Putative Ribonuclease Z from *Bacillus subtilis*

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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 $I4_122$. bsYhfI folds into the typical αβ/βα 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.

E007

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

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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 cluster. 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.

[Supported by BK21-Plus fellowship]

E008

Structural Study on a Butanol Dehydrogenase from *Bacillus subtilis*

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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 $NADP^+$ 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 $P2_1$ 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 $NADP^+$. This study will enhance our understanding of the enzymatic mechanism of BDH at the atomic level.

E009

Characterization of the *FruBKA* Operon Regulator FruR in *Vibrio cholerae*

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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 homo-tetramer 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 *fruR* and *fruBKA*. 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*.
[This work was supported by Korean National Research Foundation.]

E010

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

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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 $Csm1_1 2_1 3_3 4_1 5_1$, 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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E011

Gene Knockdown of Essential Genes Using Artificial Small RNAs in *Escherichia coli*

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Gene silencing or gene knockdown that leads to repression of specific genes is a very useful tool for study of genes. While the gene knockdown approach can complement the gene knockout experiment for identifying specific gene functions, it is an attractive alternative for essential genes whose knockout causes the cell death. We developed an efficient and versatile gene knockdown method in *Escherichia coli* by utilizing target recognition ability of specially designed artificial small RNAs (sRNAs), which repress translation and/or reduce endogenous stability of their target mRNAs. Artificial sRNAs were designed to bind to specific 5' UTR regions containing the Shine-Dalgarno sequence of their target mRNAs. This gene knockdown system was applied to various essential genes of *E. coli*. We found that single-strandedness of target recognition sequences of artificial sRNAs was crucial for maximizing gene knockdown. The longer 5' UTR also reduced knockdown efficiencies of target genes. The data suggest that the accessibility of target recognition sequences of artificial sRNAs to their target mRNA sequences for base-pairing would be an essential prerequisite for efficient gene knockdown. Since our gene knockdown system worked well for most of essential genes, it can provide a valuable genetic tool in the functional study of essential genes in *E. coli*.
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E012

Effect of LAMMER Kinase on DNA Damage Stress in Yeasts

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LAMMER kinase is a dual specificity kinase. It has an amino acid motif, EH**LAMMER**ILG, in kinase subdomain X and highly conserved from yeast to human. It has various functions in eukaryotes: development, cell differentiation, metabolism, and regulation of alternative splicing. According to recent study, LAMMER kinase (Lkh1) in *Ustilago maydis*, basidiomycete fungus, is required for maintaining genome stability (de Sena-Tomás *et al.*, 2015). In this study, we investigated the effect of LAMMER kinase in genome stability in fission yeast, *Schizosaccharomyces pombe*, budding yeast, *Saccharomyces cerevisiae* and opportunistic pathogen, *Candida albicans*. We performed sensitivity test using DNA damaging agents (hydroxyurea, benomyl and methyl methanesulfonate) with LAMMER kinase deletion mutants. The deletion mutants showed different spectra of sensitivity to DNA damaging agents. In addition, analysis of DNA profile of *S. pombe* revealed the involvement of Lkh1 in genome stability because *lkh1* deletion mutant showed increase of cells with <2C DNA contents. When Cds1, a major effector kinase of DNA checkpoint, was over-expressed, amount of Cds1 protein was increased in *lkh1* deletion mutant compared to wild type. It suggests that LAMMER kinase affects DNA damage response by modulating Cds1 stability. The regulation of DNA damage response by LAMMER kinase in *S. cerevisiae* and *C. albicans* (scKNS1 and caKNS1) on DNA check point will be discussed at molecular level.

E013

Rsd Regulates SpoT-dependent Stringent Response in *Escherichia coli*

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Bacteria respond to various stresses by modulating the level of alarmone (p)ppGpp (guanosine tetraphosphate and pentaphosphate) through a process called the stringent response. In *Escherichia coli*, the level of (p)ppGpp is regulated by stringent factors, RelA and SpoT proteins. RelA catalyzes the synthesis of (p)ppGpp, whereas SpoT catalyzes both the synthesis and hydrolysis of (p)ppGpp, serving as a bifunctional enzyme. SpoT is particularly important in balancing the intracellular level of (p)ppGpp since it is the only enzyme responsible for (p)ppGpp hydrolysis. However, the underlying mechanisms for the (p)ppGpp hydrolase activity regulation of SpoT still remain unknown in *E. coli*. In this study, we conducted ligand-fishing experiment and found Rsd as a novel interaction partner of SpoT. Furthermore, we confirmed that the (p)ppGpp hydrolase activity of SpoT could be directly stimulated by Rsd. We recently reported 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 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*.

E014

Dual Action of LAMMER Kinase on G1/S Cell Cycle in Fission Yeast

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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 then wild type. These results suggest a positive effect of phosphorylation on Rum1 such as stimulation of the CKI activity of Rum1. Therefore, we performed CDK inhibitor assay to confirm the effect of Lkh1-dependet 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 (*Mlu* I 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.

E015

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

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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 enconding gene (*pclip1* 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 conding sequence of *pclip1* 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.

E016

Crystal Structure of DinB2 Protein from *Deinococcus radiodurans*

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Jong-Hyun Jung, and Sangyong Lim*

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Deinococcus radiodurans is a polyextremophilic bacteria that is capable of withstanding up to 15 kGy gamma radiation, several weeks of desiccation, 500 J/m^2 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 *C*2 space group with unit cell dimensions of a = 1083624 Å, b = 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.

E017

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

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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, $EIIBC^{Nag}$, 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 $EIIBC^{Nag}$ in *V. vulnificus*. In the presence of *N*-acetylglucosamine, the cytosolic domain of $EIIBC^{Nag}$ transfers phosphate to the incoming sugar. The dephospho-form of $EIIB^{Nag}$ 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 $IIBC^{Nag}$, 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*

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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 was 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 Paf1 Complex Components on H3K4 Methylation in *Saccharomyces cerevisiae*

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Histone modifications regulate chromatin structure dynamics and consequently influences 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*, Paf1 complex consists of five proteins and functions as a platform for recruiting many types of transcription factors to elongating RNA polymerase II. Components Paf1 and Rtf1 of Paf1 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 Paf1 complex on this histone crosstalk largely remained to be identified. In this study, we constructed the deletion mutants of Paf1 complex components and observed their effects on H3K4 mono-, di- and tri-methylation as well as H2BK123 monoubiquitination. As a result, in each △paf1, △rtf1 and △ctr9 strain, we observed dramatic defects in H3K4 monomethylation, which are independent of H2B ubiquitination. This suggests that Ctr9 as well as Paf1 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

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In eukaryotes, when a ribosome complex is stalled during translation process, mono-ubiquitination of several ribosomal proteins has been recently known to be critical for ribosome quality control. Here, we report that the mono-ubiquitination of yeast Rps3 is tightly modulated by the reciprocal action between 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 K212 of 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 in the Thermotolerant Methylotrophic Yeast *Hansenula polymorpha*

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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 grant from NRF.]

E022

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

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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.
[Supported by grants from NRF and KHID]

E023

Antioxidant Effects of *Humulus japonicus* Extracts

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Humulus japonicas (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 determinated. 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.
[Supported by grants from KRF]

E024

Difference in Cellular Environments Affects the *in vivo* Sensitivity of PerR to H_2O_2

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In many Gram positive bacteria, the metal-dependent transcriptional repressor PerR senses intracellular H_2O_2 and controls the genes involved in H_2O_2 resistance. *Bacillus licheniformis*, a close relative to the well-studied model organism *Bacillus subtilis*, contains $PerR_{BL}$. As demonstrated by *B. subtilis* PerR ($PerR_{BS}$), $PerR_{BL}$ uses either Fe^{2+} or Mn^{2+} as a corepressor and only the Fe^{2+}-bound form of $PerR_{BL}$ senses low levels of H_2O_2 by iron-mediated histidine oxidation. Interestingly, despite the similar H_2O_2 sensitivity between $PerR_{BL}$ and $PerR_{BS}$, *B. licheniformis* expressing $PerR_{BL}$ or $PerR_{BS}$ could sense lower levels of H_2O_2 and was more sensitive to H_2O_2 than *B. subtilis* expressing $PerR_{BL}$ or $PerR_{BS}$. 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_2O_2 sensing ability of PerR inside the cell and the H_2O_2 resistance of cell. In contrast, *B. licheniformis* and *B. subtilis* expressing *Staphylococcus aureus* PerR ($PerR_{SA}$), which has higher intrinsic H_2O_2 sensitivity than $PerR_{BL}$ and $PerR_{BS}$, exhibited increased resistance to H_2O_2 than those expressing either $PerR_{BL}$ or $PerR_{BS}$. This result indicates that the sufficient difference in H_2O_2 susceptibility of PerR proteins can override the difference in cellular environment and affect the H_2O_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*

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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 from ecFliD to generate ecFliD^{cent1} and chemically modified ecFliD^{cent1} by reductive methylation. An ecFliD^{cent1} crystal belonged to space group P21, 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^{cent1} structure by SAD phasing using a selenomethionine-incorporated ecFliD^{cent1} crystal. The atomic resolution structure of ecFliD^{cent1} 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

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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

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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.
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E028

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

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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*$^{+}$ null mutant. While disruption of the *gas2*$^{+}$ gene was not lethal and reduced the flocculation activity of the △lkh1 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*$^{+}$ is upregulated in transcription factor *mbx2*$^{+}$ overexpression mutant. We identified the regulation of *mbx2*$^{+}$ expression by *lkh1*$^{+}$ 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*$^{+}$ through Mbx2.

E029

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

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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 Δ*yrdC*, we have isolated temperature-sensitive mutant of YrdC protein.

E030

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

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An extracellular medium-chain-length polyhydroxyalkanoate (MCL-PHA) depolymerase gene ($phaZ_{DSH}$) was cloned from the genomic DNA of *Variovorax* sp. DSH1 which was isolated from a soil sample. The $phaZ_{DSH}$ 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 $phaZ_{DSH}$ belongs to the type IV depolymerases family. The $phaZ_{DSH}$ 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 45^0C. Its hydrolyzing activity was significantly sensitive to phenylmethylsulfonyl fluoride, EDTA, *N*-bromosuccinimide, 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 $PhaZ_{DSH}$ 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.

E031

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

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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 demonsated that two genes are differentially regulated under our tested conditions: redox cycling compounds (RACs), iron, and H_2O_2. Addition of exogenous RACs increased the expression of *glcB*, however, the expression of *aceA* was upregulated under H_2O_2 or 2,2'-dipyridyl. Deletion of *aceA* made the strain more sensitive to H_2O_2 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 H_2O_2 and iron limitation, indicating the *aceA* mutant underwent more radical-mediated killing. Measurement of intracellular iron contents, amounts of pyoverdine, 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 H_2O_2 and iron limitation in *P. aeruginosa*. DNA-affinity chromatography will be used to identify transcriptional regulators that control the expression of the *aceA* under either H_2O_2 and iron-limited condition.

E032

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

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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 (GDH). *S.cerevisiae* has two isoforms of $NADP^+$-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.

E033

A Novel Mitochondrial Serine *O*-Acetyltransferase, Encoded by *SAT1*, Plays a Critical Role in Sulfur Metabolism in the Thermotolerant Methylotrophic Yeast *Hansenula polymorpha*

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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*.

E034

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

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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 μl/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 anbibiotics. 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.

E035

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

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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.

E036

Interactions of Glyphosate Herbicide with Antibiotics

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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.

E037 _9th ASME

Characterization of a Chemoreceptor, PctC, on the Growth and Chemotactic Responses to Triton X-100 in *Pseudomonas nitroreducens* TX1

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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 ethoxylted surfactants. This study is the first to investigate the bacterial chemotaxis responses toward surfactants.

E038 _9th ASME

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

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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 fermentated 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_{av} = 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.

F001

Multiple Modes of Signaling by Redox-active Compounds as Monitored by Iron-based and Thiol-based Sensor-regulator Systems

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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 time 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.
[supported by by a grant from MSIFP and a grant for ISBC of GFP]

F002

Analysis of Nitric Oxide Production and Its Role during Fungal Differentiation

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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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F003

Indole-induced Activities of β-Lactamase and Efflux Pump Confer Ampicillin Resistance in *Pseudomonas putida* KT2440

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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 *rpoS*, *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*.

F004

Genome Comparison of *Enterococcus faecium* Isolated from Chicken Feces and Plant

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Enterococcus faecium is a Gram-positive bacteria found in the gastro-intestinal tracts (GIT) and frequently found in the soybean and chicken gut. To characterize *E. faecium* genomes, we isolated and identified 12 strains 6 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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F005

Gene Regulation by Nuclear Metabolic Enzyme Aconitase in the Fission Yeast *Schizosaccharomyces pombe*

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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.
[This work was supported by a grant to JHR from National Research Foundation of Korea (2014R1A2A1A01002846). Soo-Yeon Cho received BK21 Plus fellowship from SNU Life Sciences.]

F006

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

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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. Multiplex real-time RT-PCR(rRT-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, Oesltamivir (Tamiflu), Zanamivir (Relenza) and Peramivir. But both A(H1N1)pdm09 and A(H3N2) and 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.

F007

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

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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 SNPs from 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.
[This study was supported by the Strategic Initiative for Microbiomes in Agriculture and Food (Grant ID: 914005-04), the National Research Foundation (NRF-2016RICIB2016246), and BK21 Plus Program from South Korea.]

F008

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

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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. slonacearum* 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°Cand 30°C. Sequence analysis revealed that Tn5 was inserted in the upstream regulatory sequences of *rpoE*1 gene which encodes an alternative sigma factor of RNA polymerase, involved in transcription initiation. However, *rpoE*1 gene has not been characterized in *R. solanacearum* in terms of EPS production. In order to characterize the role of *rpoE*1 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 *rpoE*1 gene with its downstream two *orf*s successfully restored EPS production. This result suggests that regulated expression of *rpoE*1 and its downsteam two *orf*s are necessary to produce EPS in a temperature-dependent manner in *R. solanacearum*.
[Supported by grant from Rural Development Administration of Republic of Korea]

F009

Replication of *Vibrio cholerae* Classical CTX Phage

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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-1, 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*.

F010

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

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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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F011

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

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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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F012

Complete Genome Sequence of *Lactobacillus helveticus* LH5, a Korean Probiotic Isolate

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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* LH5, 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 rRNAs operons. The genome sequence of *Lactobacillus helveticus* LH5 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

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Bacterial ribonucleases regulate gene expression through RNA processing and decay. Among them, RNase G (Rng) is an endoribonuclease, which is involved in rRNA processing and degradation of a subset of mRNAs in *Escherichia coli*. However, its physiological role remains largely uncharacterized. Here, we report that RNase G controls expression levels of histone-like nucleoid structuring protein (H-NS) encoded by *hns*, which were strongly associated with the pathogenicity of *S*. Typhimurium cells in both epithelial cells and mice. In fact, RNase G expression in *S*. Typhimurium cells was 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 *rng* 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 *S*. Typhimurium in host cell.
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F014

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

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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. vulnifcus* 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.]

F015

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

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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 VFDB (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 the 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 subsequent 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.]

F016

Genome and Transcriptome Analyses of *Vibrio vulnificus* FORC_037, a Food-borne Pathogen Isolated from Soft-shell Clam (*Mya arenaria oonogai)*

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To study *Vibrio 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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F017

The C_2H_2 Zinc Finger Protein AslA is a Novel Transcriptional Regulator of Development and Secondary Metabolism in *Aspergillus nidulans*

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In *Aspergillus nidulans*, multiple regulatory factors are involved in coordinated regulation of development and secondary metabolism. Here, we report functional features of the putative C_2H_2 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, Δ*aslA* 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 terraquinone biosynthesis, *tdiA* and *tdiB*. 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, *AfuaslA* and *AflaslA* from *Aspergillus fumigatus* and *Aspergillus flavus*, respectively, complemented the phenotypes of Δ*aslA* 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*.

F018

Inactivation Efficiency of Plasmid-encoded Antibiotic-resistant Genes during Oxidative Water Treatment

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This study assessed the deactivation efficiency of pUC4K plasmid encoding ampicillin and kanamycin resistant genes in phosphate buffered and wastewater effluent matrices during water treatment with chlorine, UV (254 nm), and UV/H_2O_2. The extracted plasmid (i.e., extracellular ARG) as well as the plasmid present in a host *E. coli* (i.e., intracellular ARG) were examined and compared for the deactivation efficiency. A quantitative real-time PCR (qPCR) assay was used to determine ARG damage to amp^R (850 bp) and kan^R (806 bp). The plate count and flow cytometry methods were also used to determine the bacterial inactivation parameters, such as cultivability and membrane damage, respectively. Overall, chlorine, UV and UV/H_2O_2 were able to achieve significant deactivation of the model ARGs (e.g., more than 4-logs deactivation levels) albeit the required oxidant or UV dose was much larger than needed for *E. coli* (ARB) inactivation.

F019

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

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Cryptococcus neoformans is an opportunistic fungal pathogen that causes cryptococcosis in both immunocompromised and immunocompetent individuals. Due to its clinical importance, revealing the factors that can affect its life cycle is critical. Among the various factors, pseudouridylation of RNA is the most abundant type of post-transcriptional modification. Pseudouridylases isomerize uridine into pseudouridine, therefore can affect the stability of RNA structure. In *S. cerevisiae*, 8 proteins exist as stand-alone pseudouridylases, and each protein has specific pseudouridylation sites and roles. To discover the characteristics of pseudouridine 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 pseudouridine 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*.

[Supported by grants from MSIP]

F020

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

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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 *bud32*Δ, *kae1*Δ, and *pcc1*Δ mutants were constructed by homologous recombination and biolistic transformation. Similar to the *bud32*Δ mutant, the *kae1*Δ and *pcc1*Δ mutants showed severe growth defects, indicating that each component of the EKC/KEOPS complex is critical for its function in *C.neoformans*. Previously, it was shown that the *bud32*Δ 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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F021

Systematic Functional Analysis of Phosphatases in the Fungal Pathogen *Cryptococcus neoformans*

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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.
[supported by grants from MAFRA]

F022

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

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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* GH1-13 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.

F023

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

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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 Rad53 was required for DNA damage response and gamma radiation and was phosphorylated by both PI3K-like kinases Tel1 and Mec1 in response to DNA damage stress. Furthermore, *Cryptococcus* Chk1, which is another effector kinase like Rad53 in the budding yeast, was involved in the DNA damage stress. In addition, 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*.

F024

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

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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 spcies 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 *dur1/2Δ*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.

F025

Overexpression of *PMA1* Enhances Tolerance to Variable Environmental Conditions and Constitutively Activates the MAPK Pathways in *Saccharomyces cerevisiae*

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PMA1 encodes a transmembrane polypeptide that functions to pump protons out of the cell. Ectopic *PMA1* 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 osmolarity glycerol (HOG) pathway, but not the MAPK Slt2 of the cell wall integrity (CWI) pathway; however, a *PMA1* overexpression constitutively activated both Hog1 and Slt2. The constitutive Hog1 activation required theMAPK kinase kinase (MAP3K) Ssk2 of the HOG pathway, but not Ste11 and Ssk22, two other MAP3Ks of the same pathway. The constitutive Slt2 activation did not require Rom2 and the membrane sensors of the CWI pathway, whereas Bck1 was indispensable. The *PMA1* overexpression activated the stress response element but not the cyclic AMP response element and the Rlm1 transcription factor. *PMA1* overexpression may facilitate the construction of industrial strains with simultaneous tolerance to weak acids, ROS, and ethanol.

[Supported by grants from BK21]

F026

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

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Cryptococcus neoformans is an opportunistic fungal pathogen. Among its virulence factors, melanin is important to protection 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, BZP4, GSK3, KIC1, CBK1, MEC1, MET3, 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]

F027

Deletion of Dfg5 Glycosylphosphatidylinositol-anchored Membrane Protein Confers Improved Heat Resistance in *Saccharomyces cerevisiae*

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The protein product of *Saccharomyces cerevisiae DFG5* gene is a glycosylphosphatidylinositol (GPI)-anchored plasma membrane protein and a putative glycosidase/glycosyltransferase that links other GPI-anchored proteins to β-glucans in the cell wall. Upon exposure to heat (41°C), *DFG5* deletion mutant *dfg5Δ* displayed significantly enhanced heat tolerance as well as lowered level of reactive oxygen species and decreased membrane permeability compared with those in the control (BY4741). Comparative transcriptome profiles of BY4741 and *dfg5Δ* revealed that 38 and 23 genes were up- and down-regulated in *dfg5Δ* respectively. Of the 23 down-regulated genes, 11 of 13 viable deletion mutants were identified to be tolerant to heat, suggesting that the down-regulation of those genes might have contributed to the enhanced heat tolerance in *dfg5Δ*. Deletion of *DFG5* caused slight activation of mitogen-activated protein kinases Hog1 in the high-osmolarity glycerol pathway and Slt2 in the cell wall integrity pathway. Therefore, a model is proposed on the signal transduction pathways associated with deletion of *DFG5* upon heat stress.

[Supported by grants from NRF]

F028

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

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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.

F029

Unveiling of Complex Signaling Networks Involved in the Developmental Process of *Cryptococcus neoformans*

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The fungal pathogen *Cryptococcus neoformans* causes cryptococcosis by the inhalation of infectious spores generated by unisexual or bisexual reproduction. To understand complex signaling networks modulating the developmental process, 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—MAT**a** 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*.

F030

Inhibition of Adipocyte Differentiation and Adipogenesis by Fermented Sea Tangle in Mouse 3T3-L1 Preadipocytes

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Adipocyte differentiation and fat accumulation are closely related to various diseases such as coronary artery disease and obesity. Sea tangle, *Laminaria japonica*, has long been used as a Korean folk remedy to promote health. This seaweed is commonly consumed in Korea as a dietary supplement and is well known for having several positive biological effects including antioxidant and antibacterial activity. However, the mechanisms of their inhibitory effects on adipocyte differentiation and adipogenesis are poorly understood. In this study, the effects and mechanisms of GABA (gamma-amino butyric acid)-enriched sea tangle juice by *Lactobacillus brevis* BJ-20 fermentation (FST) on adipocyte differentiation and adipogenesis in 3T3-L1 preadipocytes were investigated. FST significantly suppressed differentiation of 3T3-L1 cells in a dose-dependent manner as confirmed by a decrease in lipid droplet number and lipid content through Oil Red O staining. In addition, FST tended to decrease accumulation of cellular triglyceride, which was confirmed by significant inhibition of key pro-adipogenic transcription factors including PPAR γ and C/EBP α. These results provide important new insight that FST might inhibit adipogenesis by suppressing the pro-adipogenic transcription factors in 3T3-L1 cells.

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F031

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

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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 *tor1*Δ mutant up to that in *sir2*Δ 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 *tor1*Δ mutant, suggesting that the decrease in the RLS of *tor1*Δ 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.

F032

Large Scale Metagenomic-based Study of Enzyme Profiling with Next-generation Sequencing; the FINDER Strategy

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Excavation of the molecular details required to convert metagenomes deposit into useful gene signatures remains a daunting task in the high throughput enzyme screening field. Herein we presented a versatile platform method that greatly accelerates enzyme profiling for highly selective gene capture in metagenomes using Next-Generation Sequencing (NGS). The combination of PCR with NGS is based on the sets of short identifying degenerate sequences which are specific for the superfamilies of industrial enzymes, followed by multiplexed DNA-barcode sequencing. This approach enabled us to generate target enzyme profiling datasets in metagenomes, thereby allowing minimal hands-on time and high-throughput screening. This provided us with a target inventory of the predicted proteins on a metagenomics scale. We anticipate that the high throughput and sensitivity of this approach will help accelerate the decryption of the diverse protein profiles in metagenomes.

F033

The Zinc Finger Transcription Factor Cas5 Represses Genes for Hyphal Growth under Yeast Growth Condition in *Candida albicans*

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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 Cas5 is known to activate expression of many cell wall integrity genes, but it remains unknown whether Cas5 affects hyphal growth in *C. albicans*. Interestingly, we found that *cas5Δ/Δ* mutant cells could not maintain yeast form under non-hyphal inducing condition. Analysis of Cas5 expression revealed that *CAS5* transcription is significantly downregulated upon hyphal initiation in wild type, which suggests that Cas5 is a kind of transcription factor repressing genes required for hyphal growth. Consistently with the traits, the *cas5*Δ/Δ mutant highly expressed hypha-specific *ALS3*, *ECE1* and *HWP1* genes under non-hyphal inducing condition. In addition, the *cas5*Δ/Δ mutant showed decreased transcription of several genes involved in ergosterol biosynthesis pathway. Collectively, this study suggests that Cas5 represses transcription of genes responsible for hyphae formation during yeast-form growth in *C. albicans*.

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F034

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

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In this study, we identified two genes in the thermotolerante 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 in KmPdc5p. The single deletion of *KmPDC1* diminished most of Pdc activity, preventing the growth of *Kmpdc1Δ* 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 *Kmpdc1Δ* in the presence of antimycin A. The *Kmpdc1Δ* single and *Kmpdc1Δpdc5Δ* 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.

F035

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

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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*-demethly-FK506 that have lower immunosuppression 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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F036

Involvement of a Yeast NPL4 Ortholog NplD in *Aspergillus nidulans* Hypoxic Adaptation

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Sterol regulatory element-binding proteins (SREBPs) are essential ER-tethered transcriptional regulators for hypoxia adaptation in fungi. We have screened hypoxia-sensitive mutants (HSMs) in A. nidulans. Sequence analysis of HSM52 revealed that a mutation in nplD a yeast NPL4 ortholog (a component of the Cdc48-Ufd1-Npl4 complex) was responsible for the defective growth of the strain under hypoxic conditions. To determine if NplD plays a role in SrbA SREBP cleavage, Western analysis was conducted. While both precursor and activated nuclear forms of SrbA were detected in wild type, only SrbA precursor was observed in the nplD1 strain. In addition, expression of the N-terminus of SrbA restored growth of the nplD1 strain in hypoxia, indicating that NplD is essential for the SrbA cleavage-activation process. We investigated if impaired NplD1-UfdA complex formation was the reason for the hypoxia-sensitivity of the nplD1 strain using yeast two hybrid system. The yeast results showed that NplD interacts with UfdA at both 25°C, 30°C and 37°C, whereas the mutant NplD1 interacts with UfdA at only 25°C. This suggests that growth of the nplD1 strain was blocked at 37°C in hypoxia because NplD1 was not able to form a complex with UfdA under the conditions and thus SrbA cleavage did not occur. Our results suggest that NplD is important for the cleavage of SrbA in order for hypoxia adaptation.

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F037

Unravelling of the Polysaccharide Capsule Regulatory Signaling Pathways in the Human Fungal Pathogen *Cryptococcus neoformans*

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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(FBS), 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.

[Supported by grants from MSIP]

F038

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

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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 *hob1*Δ and *sre*1Δ mutants to confirm whether the functions of both genes are conserved in most serotypes. As a result, *hob1* Δ 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.

F039

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

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Hahella 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*, *yopN*, *yscO*, and *yscV* of *Yesinia* 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*.

F040

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

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The order Bacillale, one of the most thriving and diverse microorganisms, has actively evolved under various extreme environments. Hence many taxanomic and physiological studies on individual strains in the order Bacillale have been performed, but their genetic traits for thermal adaptation still remain unclear. Herein, 75 thermophiles and mesophiles in the order Bacillale, comprised of ten genera based on 16S rRNA gene, were selected for comparative genomics to understand molecular evolution for thermal adaptation. Firstly, comparative phylogenic 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 rDNA, 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 Bacilliae.

F041

A Putative Transcription Factor, MoAfo1, is Involved in Sensing during Appressorium Formation in the Rice Blast Fungus

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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 MoAfo1 is involved in sensing multiple host signals such as hydrophobicity from artificial surfaces, cutin monomers, and long chain primary alcohols. The Δ*Moafo1* 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, MoAfo1 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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F042

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

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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 $homC^{L}$ 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

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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

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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_2H_2 Transcription Factor DsdA Is a Novel Regulator of Differentiation in *Aspergillus nidulans*

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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_2H_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 conidiation 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*.

G001

Isolation and Fermentation of Novel *Bacillus* spp. for Stereospecific Production of (R,R)-2,3-BD

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2,3-Butanediol (2,3-BD) has great potentials in the diverse industries including cheimcal, 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* nomally generate (R,R)-2,3-BD. In this study, isolation of novel *Bacillus* spp. from soil samples was carred out to find efficient produer 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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G002

Application of 2,3-Butanediol for Cosmetics, Personal Cares, and Home Cares and Characterization of *Klebsiella oxytoca* Strain

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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)-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.

[This work was supported by the Biochemical Industry Promoting Technology Development Project (No. 10050407) funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea).]

G003

Metabolic Engineering of *Escherichia coli* for Efficient Production of 1,3-Diaminopropane Using *in silico* Flux Analysis

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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 C_4 and C_5 pathways for 1,3-DAP production by *in silico* flux analysis revealed that the C_4 pathway employing *Acinetobacter baumannii dat* and *ddc* genes, encoding 2-ketoglutarate 4-aminotransferase and L-2,4-diaminobutanolate 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 *pfkA* was found to increase 1,3-DAP production by applying 128 synthetic small RNAs. Overexpression of the *ppc* and *aspC* genes in the *pfkA* deleted strain resulted in even higher production of 1,3-DAP. Fed-batch fermentation of the final engineered *E. coli* strain allowed production of 13 g/L of 1,3-DAP in a glucose minimal medium.

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G004

Production of 1,5-Diaminopentane by Metabolically Engineereed *Corynebacterium glutamicum*

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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).

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G005

Production of L-Ornithine by Metabolically Engineereed *Corynebacterium glutamicum*

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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, *proB* and *argF* genes were deleted to optimize the metabolic pathway. Next, *argR* 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 *argCJBD* 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 *sod* 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.

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G006

Metabolic Engineering of *Mannheimia succiniciproducens* for the Production of L-Malic Acid

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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 anaplerotic pathway under anaerobic condition rich in CO_2. 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 anaplerotic pathway under CO_2 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 anaplerotic pathway to produce malic acid in higher titer by rational metabolic engineering.

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G007

Enhanced Production of Fumaric Acid in *Escherichia coli* by Combining Metabolic Engineering and Flux Optimization Strategies

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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, CWF4N 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.

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G008

Unraveling Structure and Function of the N-terminal Domain of *Ralstonia eutropha* Polyhydroxyalkanoate Synthase, and Proposing Structure and Mechanisms of the Whole Enzyme

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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 a-helices (N-a3 to N-a7) 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.

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G009

Methods for Improving Human Gut Microbiome Analysis by Quantitative Real-Time PCR

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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 microbiomes 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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G010

Construction of Wild Type EBV Reverse Genetic System from B95-8 Strain of EBV

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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 lympoproliferative 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.

G011

Enhanced Production of Recombinant Protein with *Leuconostoc citreum* by Engineering of Shine-Dalgarno Sequence

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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]

G012

Characterization and Differential Expression of Terpene Synthase and Farnesyl Pyrophosphate Synthase from Basidiomycetous Fungus *Polyporus brumalis*

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National Institute of Forest Science

Both farnesyl diphosphate synthase (FPS) and terpene synthase (TPS) are key enzymes in the synthesis of various isoprenoid-containing compounds. Here, TPS and FPS genes were isolated from a sesquiterpene producing basidiomycetous fungus, *Polyporus brumalis* by transcriptome sequencing. The deduced amino acid sequence of the FPS and TPS cDNA exhibited a high homology with other wood rot fungal genes. The TPS isolated from *p. brumalis* contains a putative ORF of 1,185 base pairs that encodes 394 amino acids with a predicted molecular mass of 45.74 kDa and pI of 5.07. FPS contains a putative ORF of 1,021 base pairs that encodes 350 amino acids with a predicted molecular mass of 40.50 kDa and pI of 5.37. The expression profiles of TPS and FPS, and functional complementation of the genes have being performed in fission yeast (*Shizosaccharomyces pombe*) mutant system. The nucleotide sequence corresponding to the ORF of TPS was inserted into the pSLF 273 expression vector and transformed into *S. pombe* cells. Recombinant pSLF273-TPS, which had an approximate size of 40.50 kDa, was isolated to near purity as determined by SDS-PAGE. The clones (pSLF273-TPS) were transformed into FPS deletion mutant in order to identify the function of FPP and TPS. Recombinant proteins will be analyzed as further study.

G013

Indigo Production Using Recombinant *Escherichia coli* Cloning a Flavin-containing Monooxygenase Gene (*fmo*) from *Celeribacter* sp. TSPH2

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Gyeongbuk Institute for Marine Bio-Industry (GIMB)

Previously, we reported *Celeribacter* sp. TSPH2 as a novel strain which produce blue pigments, indigo. TSPH2 produced indigo with indole as a substrate in 1% NaCl and 0.5% yeast extract medium. TSPH2 belongs to α -proteobacteria, gram stain negative and is known to degrade polycyclic-aromatic-hydrocarbons. In order to find the enzyme involved in the production of indigo, the genome sequence of TSPH2 was analyzed and the *fmo* (*cfmo*) gene was found. cFMO is 448aa and very similar to FMO(mFMO) from *Methylophaga aminisulfidivorans* MP (65% identity) and is matched all characteristic consensus sequences with mFMO. Recombinant *E. coli* cloning a *cfmo* was constructed on pBluescript II KS(+) and also produced indigo in both 0.2% tryptophan medium and 2mM indole medium.

[This work was supported by Gyeongsangbuk-do R&D Program.]

G014

Development of High MK-7 (Menaquinone-7) *Cheonggukjang* by *Bacillus subtilis* SRCM100757 Isolated from Meju

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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 25.38ppm 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*

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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_{BAD}) of the *E. coli* arabinose 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 Detction

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^{MCR}), the affinity for HuNoV (GII.4) diminishes, demonstrating that this Ca^{2+} and Mn^{2+} coordinated region is responsible for the observed virus-protein interaction. The mutated carbohydrate binding region of Con A (mCon A^{CBR}) 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 polyacrylate beads (Con A-PAB), for rapid detection of genotypes from genogroups I and II (GI and GII).

G017

Purification and Characterization of Regulatory and Reductase Component from Type II Methanotrophs

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Methane hydroxylation through methane monooxygenases (MMOs) is a key aspect due to their control of the carbon cycle in the ecology system and recent applications of methane gas in the field of bioenergy and bioremediation. Methanotropic bacteria perform a specific microbial conversion from methane, one of the most stable carbon compounds, to methanol through elaborate mechanisms. MMOs express particulate methane monooxygenase (pMMO) in most strains and soluble methane monooxygenase (sMMO) under copper-limited conditions. The mechanisms of MMO have been widely studied from sMMO included in the bacterial multicomponent monooxygenase (BMM) superfamily. Mechanism studies of sMMO have been intensively studied 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 *Methylocystis* sp. *Strain M*.

G018

Cloning and Expression of a Putative Chitinase-encoding Gene from a Thermophilic Marine Bacterium *Rhodothermaceae* sp. MEBiC09517

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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 (ROT0835) among glycoside hydrolases affiliated with variety of GH families was selected. The ROT0835 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%). ROT0835 was cloned and expressed through *E.coli* system using T-easy vector and pET-24a vector. An expressed protein was purified by affinity chromatography & size exclusion, and optimal condition for enzyme activity was estimated.
[Supported by MOF (PJT200620) & KIOST (PE99514)]

G019

Artificial ncRNAs Leading to Resistance to Cinnamaldehyde in *Escherichia coli*

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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.*

G020

Maximal Production of Antifungal Fusaricidins from *Paenibacillus kribbensis* CU01 through Fed-batch Fermentation

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In our previous studies, a bacterial strain showing strong antifungal activity was isolated from yellow loess 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, Fe^{2+} and Mn^{2+} to M9 medium. Maximal production concentrations of fusaricidin 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.

G021

Characterization of Transcripts Encoding Isolectins Based on RNAseq from an Edible Mushroom *Hericium erinaceus*

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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 glycoconjuages 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 erinaceum*. 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 (2013R1A1A1061657).]

G022

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

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Broad substrate specificity of sugar isomerases provides novel biological catalysts for the production of rare sugars. L-Arabinose isomerase (AI), that catalyzes the isomerization of L-arabinose to L-ribulose, can also convert D-galactose to D-tagatose. Hence AI with higher specificity toward D-galactose is more preferable for the production of D-tagatose. However, an intricate sugar isomerization assay hampers a rapid screening of improved enzymes with high substrate specificity for D-galactose. To develop a screening system of AI, we constructed a *de novo* D-tagatose catabolic pathway in *Escherichia coli* BL21 (DE3) lacking D-galactose utilization by the implementation of a D-tagatose 1,6-bisphosphate aldolase. Subsequently, expression of the *araA* gene enabled the D-tagatose auxotroph to grow on D-galactose. To further investigate the efficacy of enzyme activity-based cell growth, we performed an error-prone PCR to generate an AI mutant library, which was expressed in the D-tagatose auxotroph. After several rounds of sub-cultures on D-galactose as the sole carbon source in minimal media, cells with higher growth rates were dominant over cells containing proto-type enzyme activity. Detailed biochemical and biophysical analyses with purified AI variants clearly indicated that higher affinity of AI toward D-galactose accelerated the growth of its expression host, suggesting that such an *in vivo* screening system can be a powerful tool for directed evolution of sugar isomerases.

H001

Introduction of a Stem-loop at the 5′ Untranslated Region Stabilizes and Enhances Gene Expression in *Clostridium acetobutylicum*

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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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H002

Enhanced Production of 5-Aminovaleric Acid by Metabolically Engineereed *Corynebacterium glutamicum*

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5-Aminovaleric acid is a promising and important five-carbon platform chemical especially synthesis of polymers and other chemicals of industrial interest. Employment of lysine 2-monooxygenase encoded by the *davB* gene and 5-aminovaleramidase encoded by the *davA* gene has succeeded in enzymatic conversion of l-lysine to 5AVA. For the bioconversion of l-lysine to 5AVA, a recombinant *Escherichia coli* strain expressing the *davB* and *davA* genes was developed. Previously, direct fermentative production of 5AVA from glucose by metabolically engineered *E. coli* strains was examined to use glucose and xylose derived from lignocellulosic biomass rather than l-lysine as substrates. However, the developed recombinant *E. coli* strains' yield and productivity of 5AVA remain very low. C*orynebacterium glutamicum* is a highly efficient l-lysine producing microorganism and thus highly promising in the development of direct fermentative production of 5AVA using l-lysine as a precursor for 5AVA. In this work, *Corynebacterium glutamicum* was metabolically engineered to enhance the fermentative production of 5-Aminovaleric acid from glucose.

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H003

Biosynthesis of Polylactic Acid and Other 2-Hydroxyacid Containing Copolymers by Metabolically Engineered *Escherichia coli*

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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 polyhydroxyalkanoate (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 *adhE, frdB, pflB* and *poxB* to prevent byproduct formation. Moreover, an evolved propionyl-CoA transferase converts d-lactate and glycolate to d-lactyl-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 *dld*, or increasing the proportion of glycolate by deleting *aceB* and *glcDEFGB*. Production of 2-hydroxybutyrate integrated polymers can be controlled by either deleting *ilvA* or feeding strains with l-isoleucine.

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H004

Introduction of the Process of Human Gut Microbiome Analysis System

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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

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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, flavonoid, 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. Autooxidation 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

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Viroids 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) in the family *Avsunviroidae* and *Chrysanthemum stunt viroid* (CSVd) in the family
the family *Pospiviroidae*. 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

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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 July. PCoA results showed the different separation according to the seasons. April groups were seperated two clusters, however, July groups were formed one cluster. Further research is required to confirm that the affection of the seasonal condition on perilla leaf's microbiota.
[This research was supported by a grant (14162MFDS972) from Ministry of Food and Drug Safety in 2017.]

H008

Biological Activities and Chemical Characteristics of Extracts from *Kaempferia parviflora*

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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,α'-diphenyl-β-picrylhydrazyl) free radical scavenging activity, Cu/Fe reducing power, peroxidation of rat hepatocyte microsome] with water, ethanol and methanol extracts from *Kaempferia parviflora*(KP) 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, ClR

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Yonsei University

Recently, light-driven sodium pump rhodopsin (NaR/KR2/NDQ rhodopsin) and chloride pump rhodopsin (ClR/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 (ClR) from Nonlabens marinus S1-08, at resolutions of 1.57 Å. Like structured Halorhodopsin (HR), ClR can transfer chloride ion from extracellular to cytosol. Although both ClR and HR are same light-driven chloride pump rhodopsin, we found some evidences that ClR 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 ClR has structural differences such as ECL1 and Helix 8. These results suggest that together with NaR, ClR is a unique and new molecules for optogenetic study.

H010

Marine Fungal Resource Bank

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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.]

H011

Synergistic Effects of Co-treatment with Quercetin and *Ganoderma lucidum* in EBV-associated Gastric Carcinoma

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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 *helicobactor 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 QST 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.

H012

Korea Mushroom Resource Bank

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

H013

Isolation and Characterization of Quorum Quenching Bacteria for Biofouling Control in MBR

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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.

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H014

Biofilm Inhibition Effects of the Ginger Compounds Oleic and Linoleic Acid

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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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H015

Phylogenetic Analysis of *Sinonovacula constricta* Based on Mitochondrial Cytochrome Oxidase I Gene

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Sinonovacula constricta is a benthic clam and one of the important economic shellfish. The mitochondrial cytochrome oxidase subunit 1(CO I) 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 Gangjin, Korea and Wenzhou, China.

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H017

Anti-Inflammatory Activity of *Streptomyces* sp. MJ12405E in LPS-stimulated RAW 264.7 Macrophages

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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 MTT 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.

H018

Genome Information of *Dokdonella koreensis* DS-123 and Comparative Analysis of *Rhodanobacteraceae* Genomes

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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 phylogenomic 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-limiting 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.

H019

Subtype Distribution of Influenza Laboratory Surveillance during 2016/2017 Season in Korea

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The active nationwide surveillance network termed the KINRESS has been implemented since 2009 in Korea. The nation-wide influenza laboratory surveillance was expanded into KINRESS to analyze pattern 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 (0.84%) cases were Influenza B viruses. Influenza A (H3N2) viruses (826, 99.2%) has 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 of A (H3N2), additional cases were continuously detected and positive rate reached up to 15.9% in week 4. January, 2017. The highest number of influenza A (H3N2) viruses have come from the 7-18 (30.4%) years age group. Antigenic analysis showed that influenza A viruses were similar 2016/2017 season vaccine strains and resistant analysis for some viruses, all viruses were sensitive to NA inhibitors. However A (H3N2) and A (H1N1) pdm09 isolates were all resistant to M2 inhibitor. Our results show that the A (H3N2) subtype is uniquely prevalent in the 2016/2017 season, unlike the last two seasons.

[supported by grants of the KCDC]

H020

Detection of Antiviral Drugs Resistant Influenza Viruses in Korea during 2016-2017 Season (2017, 10 weeks)

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Antiviral drugs are an important supplement to vaccination for reducing the public health impact of influenza virus infections. Antiviral monitoring for emergence of drug resistant variant has been important to control and prevent of influenza. Currently, neuraminidase inhibitors have been widely used as main drugs against influenza infection. Therefore, we report the survey results of antiviral drugs resistance based on KINRESS during 2016/2017 season. We investigated well-known mutations in matrix ion channel (M2e) and NA genes related with antiviral drug resistance of randomly selected 113 influenza isolates by RT-PCR and sequencing analysis. Of them, a total of 80 isolates were tested for phenotypic assay using NA inhibition assay (NAI). The IC_{50} values were measured by NAI assay using the NA inhibitors such as oseltamivir and zanamivir. In genotypic analysis, we did not find any known mutations related with resistance in NA gene of all isolates. However, all influenza A isolates harbored S31N mutation in M2e gene, which was known to be related with the resistance to M2e inhibitors. Phenotypic assay showed that A/H1N1pdm09, A/H3N2 and B isolates did not showed reduced susceptibility to oseltamivir and zanamivir. As it is possible to emerge antiviral drug resistant strain like previous seasonal A(H1N1)and A(H1N1) pdm09 viruses, it is required to consistently strengthen the monitoring in the community surveillance system.

[supported by grants of the KCDC.]

H021

Changes in Quality Characteristics and Biogenic Amine Content in Raw Milk Gouda Cheese during Ripening

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This study was to evaluate the changes in quality characteristics and biogenic amine content of gouda cheese made from pasteurized and raw milks during ripening for 90 days. As a result, the value of pH, moisture, fat, protein and salt did not change in pasteurized and raw milk cheese. The number of total bacteria and lactic acid bacteria were slightly decreased in pasteurized milk cheese but increased in raw milk cheese as the ripeing period increased. On day 90, the counts of total bacteria and lactic acid bacteria were 2 log units higher in raw milk cheese than in pasteurized milk cheese. We also analyzed the content of eleven biogenic amines in cheeses made from raw and pasteurized milk. The most abundant amines were cadaverine, putresine, phenylethylamine, tyramine, tryptamine, and histamine in raw milk cheese and tryptamine, histamine, spermine, spermidine, putresine, and cadaverine in pasteurized milk. The total biogenic amine concentration in cheeses made from pasteurized and raw milk were increased during ripening. In particular, raw milk cheese showed 13 times higher biogenic amine compared with pasteurized milk cheeses. Therefore, it is considered to prepare a method that can reduced the content of biogenic amine in raw milk cheese.

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H022

Screening of Antigenic Epitopes in Zika Virus Nonstructural Protein 1 (ZIKV-NS1)

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The current outbreak of Zika virus has resulted in a massive effort to accelerate the development of ZIKV-specific diagnostics and vaccines. A diagnostic test that can detect past ZIKV infection is required for monitoring the current ZIKV outbreak. Development of the ZIKV-specific diagnostics requires identification of specific B-cell epitopes from the target strains. In addition to the E protein, NS1 is an important antigenic protein that elicits protective antibody responses in animals and can be used for the serological diagnosis of ZIKV infection. Although more than 90% of epitopes are estimated to be conformational, identification of the linear epitope from sero positive sample is important to the development of diagnostics. We analyzed sequences conservation among different ZIKV lineage and synthesized linear peptides from of ZIKV NS1. From antibody binding test using 23 of human sera with various ZIKV NS1 peptides, we found linear B-cell epitope targets that are most antigenicity within ZIKV NS1 peptides. These epitopes showed high sensitivity and specificity when used for serological diagnostics.

H023

Inhibition of Influenza Virus Infection by *Poncirus Trifoliata* Rafin Seeds

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The emergence of drug resistant variants of the influenza virus has led to a need to develope 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, trifoliata 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 trifoliata 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 inhibite the viral attachment and penetration into the host cells. In conclusion, an ethanol extract from seeds of the trifoliata orange might be a promising source for the development of new antiviral drugs to fight influenza viruses pndemics.

H024

Development of the Chimeric ZIKA Virus Vaccine Using SA14-14-2, Attenuated Japanese Encephalitis Virus Vaccine Strain

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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

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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.
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H026 _9th ASME

Structural Characterization of Mannosylerythritol Lipids from *Pseudozyma aphidis* B1 and *Pseudozyma hubeiensis* TS18 Strains

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Mannosylerythritol lipids (MEL) are glycolipids that are surface active compounds with variable biochemical functions that are produced from certain species of yeasts. MELs shows 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 eryhritol, 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 by micro-Raman spectroscopy and near infrared (NIR) absorption produced from *Pseudozyma aphidis* B1 and *Pseudozyma hubeiensis* TS18 strains. 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 vibrational bands in the ranges of 800-1800 cm^{-1} and 2700-3100 cm^{-1}. The Raman bands in the ranges of 840-940 cm^{-1} and 1250-1350 cm^{-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 can be indicators to discriminate MEL-A, -B, and –C compounds.

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H027 _9th ASME

Overlooked Gene Transfer Mediator between Organisms

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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^T), 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.

H028 _9th ASME

The Isolation of the Symbiotic Bacteria from the Accessory Nidamental Glands and Eggs of the Pharaoh Cuttlefish, *Sepia pharaonis* (Cephalopoda: Sepiidae)

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The nidamental glands (NGs) and accessory nidamental glands (ANGs) are two accessory reproductive organs in the female pharaoh cuttlefish, *Sepia pharaonis* and both play 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 *Phaeobacter* and *Leisingera* species and the other one was *Vibrio* group. *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.

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H029

RtxA1 Toxin Binds Filamin A to Regulate Pak 1- and MAPK-dependent Cytoskeleton Reorganization and Cell Death

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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 ($RtxA1_{ERM}$) 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 $RtxA1_{ERM}$ 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.

H030

An Ethyl Acetate Fraction from *Dendropanax morbifera* Leaves Increases EL-4 T Cell Growth by Upregulating NF-AT-mediated IL-2 Secretion

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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 effect of 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.