

A001

***Kaistella soli* sp. nov., Isolated from Oil-contaminated Soil**Dhiraj Kumar Chaudhary¹, Ram Hari Dahal², Dong-Uk Kim³, and Yongseok Hong^{1*}

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A light yellow-colored, rod-shaped bacterial strain DKR-2^T was isolated from oil-contaminated experimental soil. The strain was Gram-stain-negative, catalase and oxidase positive, and grew at temperature 10–35°C, at pH 6.0–9.0, and at 0–1.5% (w/v) NaCl concentration. The phylogenetic analysis and 16S rRNA gene sequence analysis suggested that the strain DKR-2^T was affiliated to the genus *Kaistella*, with the closest species being *Kaistella haifensis* H38^T (97.6% sequence similarity). The chemotaxonomic profiles revealed the presence of phosphatidylethanolamine as the principal polar lipids; iso-C_{15:0}, antiso-C_{15:0}, and summed feature 9 (iso-C_{17:1} ω9c and/or C_{16:0} 10-methyl) as the main fatty acids; and menaquinone-6 as a major menaquinone. The DNA G + C content was 39.5%. In addition, the average nucleotide identity (ANIu) and *in silico* DNA–DNA hybridization (dDDH) relatedness values between strain DKR-2^T and phylogenically closest members were below the threshold values for species delineation. The polyphasic taxonomic features illustrated in this study clearly implied that strain DKR-2^T represents a novel species in the genus *Kaistella*, for which the name *Kaistella soli* sp. nov. is proposed with the type strain DKR-2^T (= KACC 22070^T = NBRC 114725^T).

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A002

Chitinibacter bivalviorum* sp. nov., Isolated from the Gut of Freshwater Mussel *Anodonta arcuiformis

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A novel Gram-negative, aerobic, rod-shaped bacterium with a single polar flagellum, designated strain 2T18^T, was isolated from the gut of the freshwater mussel *Anodonta arcuiformis*. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain belonged to the genus *Chitinibacter*. Strain 2T18^T formed a monophyletic clade with *C. fontanus* KCTC 42982^T, *C. tainanensis* KACC 11706^T, and *C. alvei* KCTC 23839^T. Strain 2T18^T exhibited optimal growth at 30°C, pH 8, with 0.5% (w/v) NaCl. The major isoprenoid quinone was ubiquinone-8. The predominant fatty acids were summed feature 3 (C_{16:1} ω6c and/or C_{16:1} ω7c) and C_{16:0}. The polar lipids comprised phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, one unidentified lipid, three unidentified phospholipids, and two unidentified aminophospholipids. The G+C content of the genomic DNA was 50.6 mol%. The average nucleotide identity and digital DNA–DNA hybridization values between strains 2T18^T and *C. fontanus* KCTC 42982^T were below the thresholds used for the delineation of a novel species. Based on the phylogenetic, phenotypic, chemotaxonomic, and genotypic characteristics, strain 2T18^T represents a novel species of the genus *Chitinibacter*, for which the name *Chitinibacter bivalviorum* sp. nov. is proposed. The type strain is 2T18^T (= KCTC 72821^T = CCUG 74764^T).

[This work was supported by a grant by Ministry of Food and Drug Safety (20172MFDS195), Republic of Korea.]

A003

Agave Virus T, a Novel Tepovirus, Identified in the Blue Agave Transcriptome Data

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The genome sequence of a novel RNA virus was identified by analyzing transcriptome data obtained from the stem sample of a blue agave (*Agave tequilana*) plant. Sequence comparison and phylogenetic analysis showed that the RNA virus, Agave virus T (AgVT), was a new member of the genus *Tepovirus* in the family *Betaflexiviridae*. AgVT genome had three open reading frames: a 1605-amino acid (aa) replicase (REP), 355-aa movement protein (MP), and 220-aa coat protein (CP). Phylogenetic analyses based on the REP, MP, and CP sequences of AgVT, previously reported tepoviruses, and other *Betaflexiviridae* viruses revealed that tepoviruses could be classified into two subclades: “potato virus T (PVT)-clade” and “Prunus virus T (PrVT)-clade.” PVT, the type species and founding member of the genus *Tepovirus*, belonged to “PVT-clade” along with AgVT, while the other five tepoviruses belonged to “PrVT-clade.” The genome sequence of AgVT may be useful for studying the phylogenetic relationships between tepoviruses and other closely related viruses.

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A004

Zostera Associated Varicosavirus 1, a Novel Negative-sense RNA Virus, Identified in the Common Eelgrass Transcriptome Data

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Varicosaviruses (genus *Varicosavirus*) are bipartite, negative-sense, single-stranded RNA viruses that infect plants. We analyzed a transcriptome dataset isolated from the common eelgrass (*Zostera marina*) and identified a novel varicosavirus named Zostera associated varicosavirus 1 (ZaVV1). The ZaVV1 genome consists of two genomic segments: RNA1 (6,632-nt) has an open reading frame (ORF) encoding a large multi-functional polymerase protein (L), while RNA2 (4,304-nt) has four ORFs: one for a nucleocapsid protein and three for proteins with unknown functions (P2, P3, and P4). Sequence comparison and phylogenetic analysis using L proteins showed that ZaVV1 is a novel member of the genus *Varicosavirus* of the family *Rhabdoviridae*. The conserved regulatory elements involved in transcription termination/polyadenylation and transcription initiation were identified in the ZaVV1 gene-junction regions with the consensus sequence 3'-UAAUUAUUCUUUUUGCUCU-5' (in the negative-sense genome). The ZaVV1 genome sequence may be useful for studying the phylogenetic relationships of varicosaviruses and genome evolution of rhabdoviruses.

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A005

A Novel Acetate-producing *Bacteroides* Species, Isolated from Human Faeces

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Two obligately anaerobic, Gram-stain-negative, non-motile, non-spore-forming, and short rod, and catalase-, oxidase-negative bacteria, designated KGMB07931^T and KGMB10229, were isolated from the faeces of a healthy Korean. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains KGMB07931^T and KGMB10229 were very similar to each other (99.9 %) and the two strains were grouped within the genus *Bacteroides*, displaying the highest similarity with *B. uniformis* ATCC 8492^T (97.5%), *B. rodentium* JCM 16496^T (96.6%), and *B. fluxus* YIT 12057^T (94.5%). Both strains grew optimally at 37°C and pH 7.5 in the presence of 0.5% (w/v) NaCl. The draft genome of KGMB07931^T comprises 3,435 putative genes with a total of 4,196,373 bp and an average G + C content of 46.3 mol%. The major fatty acids were C_{18:1 cis}9 (26.4%) and anteiso-C_{15:0} (22.5%); the predominant respiratory quinone were MK-9 and MK-10; the major fermentation end products acetate, isobutyrate. On the basis of polyphasic taxonomic data, strain KGMB07931^T and KGMB10229 represent a novel species of the genus *Bacteroides*. The type strain is KGMB07931^T (= KCTC 25160^T).

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A006

Microbiome Analysis of Sponge in the Genus *Discodermia* Using Illumina Miseq

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The symbiotic microbial community structures of *D. calyx*, *D. japonica*, *D. emarginata*, and *Microscleoderma* sp. sponges collected from Munseom Island (October 2019) and Gapa Island (May 2020) in Jeju Island were compared using Illumina Miseq.

The gDNA of each of five specimens from four species of sponges were extracted, and the V3-V4 region was amplified using a pair of Bacterial 16S rRNA gene primers and used for analysis.

In alpha-diversity analysis, *D. calyx* sponge was the most abundant in symbiotic microbial species than the other sponges. The result of beta-diversity analysis, revealed differences in the symbiotic microbial communities of different species of sponges and comparative analysis of the sponges showed the specificity according to the species of the sponge and the specificity according to the habitat environment.

Microbiome analysis results of *D. emarginata*, *D. calyx*, *D. japonica* collected from Gapado. There were significant differences in microbiome such as *Chloroflexi*, *Proteobacteria*, and *Actinobacteria*.

The difference between species of sponges in *Discodermia* was not significant in the rich taxa, but was shown in the sparse taxa.

This analysis showed that the microbiome associated with marine sponges not only had host specificity but also the distribution of dominant species by environment varied.

A007

Description of Novel Strain *Actibacterium* sp. 188UL27-1 from Nostoc Commune

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A Gram-stain-negative, coccus-shaped, aerobic, pink-pigmented bacterium, designated strain 188UL27-1, was isolated from the nostoc commune collected from the Ulleungdo coast of the Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain 188UL27-1 belonged to the family *Rhodobacteraceae* and showed the highest 16S rRNA gene sequence similarity with *Actibacterium pelagium* JN33^T (96.32%). Strain 188UL27-1 grew at 18–30°C, with an optimum of 25°C. The pH range for growth was between 5.5–9.0, with optimum of pH 7.0–7.5. The range of NaCl concentration for growth was between 1.0–7.0% (w/v), with an optimum of 3.0–4.0% (w/v). The DNA G+C content of strain 188UL27-1 was 59.60 mol%. The major fatty acids were C_{18:1} ω_{7c} / C_{18:1} ω_{6c} (74.99%), C_{16:0} (17.06%). The major respiratory quinone was ubiquinone-10. The polar lipids were phosphatidylglycerol, aminolipids, four unidentified polar lipids, and five unidentified phospholipids. On the basis of the polyphasic analyses, strain 188UL27-1 is considered to represent a novel species of the genus *Actibacterium*.

A008

***Calidifontibacter carri* nov., Isolated from Car Air Conditioner System**

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A bacterial strain, designated DB2511S^T, was isolated from car air conditioner system in South Korea. Cells of this strain were strictly aerobic, Gram-stain-negative, non-spore-forming, oxidase-negative and catalase-positive, coccoid-shaped, convex, round and formed creamish-white colored colonies. Growth occurs at 18–37°C (optimum, 28°C), at pH 6.0–8.0 (optimum, pH 6.5), and in the presence of 0–7% NaCl (w/v) (optimum, 3.0%). Strain DB2511S^T contained C_{17:1} ω8c (38.1%), iso-C_{16:0} (12.8%) and C_{17:0} (10.9%) as major fatty acids. The polar lipid profile consisted diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, aminophosphoglycolipid and several uncharacterized lipids. The G + C content of the genomic DNA of DB2511S^T was 71.2 mol%. The 16S rRNA gene sequence analysis showed that strain DB2511S^T belonged to the genus *Calidifontibacter* and phylogenetically related to *Calidifontibacter indicus* PC IW02^T and *C. terrae* R161^T (96.3% and 95.6% sequence similarity, respectively). The results of genotypic and phenotypic data showed that strain DB2511S^T could be distinguished from its phylogenetically related species, and that this strain represented a novel species of the genus *Calidifontibacter*, for which the name *Calidifontibacter carri* nov. (type strain DB2511S^T = KCTC 49354^T = NBRC 114621^T) is proposed.

A009

Genome Based Reclassification of *Desulfovibrionaceae* Family

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Desulfovibrionaceae is one of the most actively studied taxa of sulfate-reducing bacteria. However, due to the absence of definite classification criteria, the family currently contains a number of misclassified species and numerous synonyms. Although species belonging to *Deltaproteobacteria* have been reclassified recently, *Desulfovibrionaceae* strains remain misclassified, requiring further reclassification. The development of NGS facilitated rapid expansion of genome sequence databases, which proved to be a potential classification standard by overcoming the limitations of 16S rRNA sequencing. In this study, a reclassification was performed based on genome. The AAI cut-off value, from 63.13 to 63.45, was calculated to be an appropriate criterion for genus delineation of this family. By applying the AAI cut-off value, the 84 genomes were divided into 27 genera, which is consistent with the core gene phylogeny result. In this process, four novel genera (*Alkalidesulfovibrio*, *Petrodesulfovibrio*, *Litoridesulfovibrio*, and *Lutosidesulfovibrio*) were proposed. In addition, by applying the 96% ANI and the 70% dDDH standard values for species, six strains were reorganized as independent species. After verifying that the classification was properly performed through RSCU analysis, all taxa belonging to *Desulfovibrionaceae* that have not yet been genome sequenced were reclassified and their common characteristics were listed. [Supported by grants from KIOST (PE99922) and MOF (20170411)]

A010

***Streptomyces daejeonensis* sp. nov., Isolated from Garbage Incineration Plant Soil**

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This study aims to classify a new actinobacterial species candidate designated NR30^T belonging to the genus *Streptomyces*, which was isolated from garbage incineration plant soil, using a polyphasic taxonomic approach. Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that the strain showed the highest sequence similarities to *Streptomyces cyaneus* NRRL B-2296^T (98.62%), *Streptomyces acidiscabies* ATCC 49003^T (98.55%), *Streptomyces aquilus* GGCR-6^T (98.49%), *Streptomyces griseoruber* NRRL B-1818^T (98.48%), and *Streptomyces caeruleatus* NRRL B-24802^T (98.48%). Growth occurred at 10–40°C (optimum, 30°C), at pH 6.0–9.0 (optimum, pH 8.0), and in the presence of 0–3% (w/v) NaCl (optimum, 0%). Good growth occurred on ISP agar media 2 and 3, and also on modified Bennett's agar. The predominant menaquinones were MK-9(H₆) and MK-9(H₈). The diagnostic polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositolmannoside, or phosphatidylinositol. The major cellular fatty acids were anteiso-C_{15:0}, C_{16:0}, iso-C_{16:0} and anteiso-C_{17:0}. NR30^T exhibited antimicrobial activity against several Gram-negative bacteria and yeasts. Based on both phenotypic and phylogenetic evidences, strain NR30^T should be classified as a novel species of *Streptomyces*, for which the name *Streptomyces daejeonensis* sp. nov. (type strain = KCTC 49653^T) is proposed.

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A011

Description of *Fervidibacillus* gen. nov. in the Family *Fervidibacillaceae* with Two Species, *Fervidibacillus albus* sp. nov., and *Fervidibacillus halotolerans* sp. nov., Isolated from Tidal Flat Sediments

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Two Gram-stain-positive, motile, endospore-forming, facultatively anaerobic strains, designated MEBiC13591^T and MEBiC13594^T, were isolated from tidal flat sediment of the Incheon City, West seaside of Korea. 16S rRNA gene sequence analysis revealed that the novel strains were affiliated to the genus *Bacillus*, a member of the *Fervidibacillaceae*, showing sequence similarities of 96.1–96.3% with respect to the type strain *Bacillus kwashiorkori*. Growth was observed at pH 5–9 (optimum, pH 7.5) for strain MEBiC13591^T, and pH 5–9 (pH 7) for strain MEBiC13594^T. Both strains displayed growth in 0–8% NaCl with an optimum at 2% for strain MEBiC13591^T; 3% for strain MEBiC13594^T. Strains MEBiC13591^T and MEBiC13594^T grew optimally at 49.8°C, (37.5–56.1°C) and 43.8°C (20.7–50.7°C), respectively. The main cellular fatty acids of strain MEBiC13591^T were iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0} and anteiso-C_{17:0}, while those of for strain MEBiC13594^T were C_{14:0}, iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0} and C_{16:0}. DNA G+C contents were 35.4 and 36.5 mol% for MEBiC13591^T and MEBiC13594^T respectively. Based on the phenotypic, genomic and biochemical data, strains MEBiC13591^T and MEBiC13594^T represent two novel species in the genus *Bacillus* for which the names *Fervidibacillus albus* sp. nov. [MEBiC13591^T (=KCCM 43317^T = JCM 33662^T)], and *Fervidibacillus halotolerans* sp. nov. [MEBiC13594^T (= KCTC 43182^T = MCCC 1K06222^T)] are proposed.

A012

Two New Species of Root Endophytic *Mucilaginibacter*, *Mucilaginibacter artemisicola* sp. nov. and *Mucilaginibacter chungnamensis* sp. nov. Isolated from the Root of Mugwort

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Two Gram-negative bacterial strains designated UR6-1^T and UR6-11^T were isolated from the roots of mugwort (*Artemisia princeps*). Based on the 16S rRNA gene sequencing analysis, UR6-1^T was mostly related with *Mucilaginibacter defluvii* A5^T (98.26%), and UR6-11^T with *Mucilaginibacter panaciglaebae* BXN5-31^T (98.13%). The optimal growth medium for both strains was R2A. The growth temperature range of UR6-1^T was 15–30°C (optimum, 30°C) and that of UR6-11^T was 10–30°C (optimum, 25°C). The NaCl concentration for growth was 0–1% (optimum, 0%) for UR6-1^T, but UR6-11^T could grow only in the absence of NaCl. The major fatty acids of both strains were a summed feature consisting of C_{16:1} w6c and/or C_{16:1} w7c, and iso-C_{15:0}. The major polar lipids of both strains were phosphatidylethanolamine, diphosphatidylglycerol, and an unknown aminophospholipid. Both strains were positive for catalase activity, whereas only UR6-1^T was positive for amylase and DNase, and only UR6-11^T was positive for hydrolysis of tyrosine. The strains could be differentiated from related species in enzyme activities. Based on these results, each of the two strains UR6-1^T and UR6-11^T merits recognition as a new species, for which the names *Mucilaginibacter artemisicola* sp. nov. (type strain = UR6-1^T = KCTC 82787^T = LMG 32370^T) and *Mucilaginibacter chungnamensis* sp. nov. (type strain = UR6-11^T = KCTC 82786^T = LMG 32371^T) are proposed. [Supported by a research grant from the National Institute of Biological Resources (NIBR).]

A013

Polyphasic Taxonomic Analysis of *Nocardioides okcheonensis* sp. nov. Isolated from Riverside Soil

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An actinobacterial strain designated MMS20-HV4-12^T was isolated from a riverside soil sample, and characterized by polyphasic taxonomic analysis. Based on the 16S rRNA gene sequencing analysis, MMS20-HV4-12^T was mostly related with the type strains of *Nocardioides alpinus* (98.33% sequence similarity), *Nocardioides furvisabuli* (98.14%), and *Nocardioides zeicaulis* (97.95%). MMS20-HV4-12^T showed optimal growth on R2A. The temperature growth range was 10–37°C (optimum, 30°C), the NaCl concentration 0–4% (optimum, 0%), and the pH range pH 7–9 (optimum, 8). MMS20-HV4-12^T was catalase-positive and formed creamy white-pigmented colonies. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, unidentified phospholipids, and an unidentified glycolipid. The major fatty acids were iso-C_{16:0}, C_{17:1} w8c and 10-methyl-C_{17:0}. The major isoprenoid quinone was MK-8(H₄). The diagnostic cell wall sugars were galactose, ribose and xylose. MMS20-HV4-12^T had better hydrolytic activities than related species, as the strain was able to hydrolyze starch, casein, Tween 40, Tween 80, DNA and tyrosine. Based on the genotypic and phenotypic information, strain MMS20-HV4-12^T evidently represents a novel species of genus *Nocardioides*, for which the name *Nocardioides okcheonensis* sp. nov. (type strain = MMS20-HV4-12^T = KCTC 49651^T = LMG 32360^T) is proposed.

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A014

The Characteristics of Saemangeum Soil Microbial Community with Land Use Type

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Paddy-upland rotation fields are unique from other soils, because they are associated with frequent cycling between wetting and drying under anaerobic and aerobic conditions; such rotations biological effectiveness of soil nutrient elements varied with seasons, increase the diversity of soil organisms. The objective of this study was to evaluate characteristics of soil microbial community in Saemangeum reclaimed land. The analysis was carried out using the illumine Mseq system for DNA sequencing of the bacterial 16s rRNA genes in soil. Lang use type of Saemangeum reclaimed land was found to have significant influences on the bacterial community diversity and composition. *Proteobacteria*, *Actinobacteria*, *Acidbacteria* were found in higher abundance in all treatments. In the PUU treatment, Proteobacteria increased by 4.2% compared to before the experiment. It showed a positive correlation with calcium and magnesium. Our results indicated that differences observed in bacterial diversity and composition were related to land use types.

A015

Effect of Short-term Intake of Fermented Red Ginseng in Elderly Korean Women

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Red ginseng has a potential effect on the improvement of various metabolic diseases, and the pharmacological superiority of red ginseng has been proven by the identification of chemical components (saponins) newly created by heating during the manufacturing process of red ginseng. Although the exact mechanism is not known, these red ginseng saponins are metabolized by human intestinal bacteria after ingestion and express functional effects in the body. The red ginseng concentrate used in this study was fermented and used probiotics added. We performed control termination as a transient series that included a control period 3 weeks prior to probiotic intake, FRG intake for 3 weeks, and a washout period of 3 weeks. The fecal microbiota composition was analyzed by sequencing the V3-V4 hypervariable region of 16S rRNA. After all subjects consumed fermented red ginseng (FRG) for 3 weeks, 22 species changed significantly. In addition, the levels of glucose, TG, cholesterol, and LDL, which are lipid-based indicators, were improved, and the liver enzymes, ALP and LDH, were improved. All subjects were classified as enterotyping due to their unique gut microbiota composition, and showed different responses to FRG depending on the enterotype. Therefore, it is necessary to consider conducting an individual enterotype test before ingestion of FRG and determining the intake dose and method according to the enterotype.

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A016

Comparing Sampling Reagents and Storage Temperatures with RLU and CFU Values

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Sampling with a swab is a method widely used in clinical and forensic testing. When taking a sample with a swab, it is important to preserve the composition of bacteria and the proportion of microbial species present in the initial sample. So, we observed the reagent and storage temperature that will preserve the samples well. In this study, we selected *Escherichia coli* and *Staphylococcus aureus* as the representative strains of Gram-negative and Gram-positive bacteria. We applied *E. coli* and *S. aureus* to the desk by using sterilized cotton. The bacteria were collected with three sampling reagents (DW, Phosphate buffer saline [PBS], Tris-EDTA) and stored at three storage temperatures (22°C, 4°C, -70°C). After that, RLU and CFU results were observed at every three-time points (0, 4, 8 weeks). Except when kept at room temperature for 4 weeks, at most reagents and time points, it was best maintained when we stored samples at -70°C. For long-term storage, it was effective when sampling using Tris-EDTA. We can identify the correlation between log RLU/ml and log CFU/ml for each reagent. Seven cases, excluding two (When collected using PBS and Tris-EDTA and stored at 22°C), showed correlation above 0.9. To maximize recovery of samples during long-term storage, samples should be taken by Tris-EDTA and stored at -70°C.

A017

Nocardioides macrobrachii* sp. nov., Isolated from the Gut of the Lake Prawn, *Palaemon paucidens

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

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A018

Ramlibacter algicola* sp. nov., Isolated from a Freshwater Alga *Cryptomonas obovoidea

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A Gram-stain-negative, strictly aerobic, catalase-negative, oxidase-positive and non-motile rod bacterium, designated strain CrO1^T, was isolated from a freshwater alga *Cryptomonas obovoidea* in Nakdong river of South Korea. Colonies of strain CrO1^T were white, convex and circular and growth was observed at 25–40 °C (optimum, 37 °C) and pH 6.0–9.0 (optimum, pH 7) and in the presence of 0–0.5 % (w/v) NaCl (optimum, 0 %). Strain CrO1^T contained C_{16:0}, summed feature 5 (comprising C_{18:0} ante and/or C_{18:2} ω6,9c), C_{18:0}, summed feature 3 (comprising C_{16:1} ω7c and/or C_{16:1} ω6c), summed feature 8 (comprising C_{18:1} ω7c and/or C_{18:1} ω6c) as the major cellular fatty acids (>5 %) and ubiquinone-8 as the sole respiratory quinone. Phosphatidylethanolamine was detected as the major polar lipid. The G+C content of strain CrO1^T calculated from the whole genome sequence was 69.6 mol%. Strain CrO1^T was most closely related to *Ramlibacter humi* 18x22-1^T with a 97.6 % 16S rRNA sequence similarity and shared less than 97.4 % 16S rRNA sequence similarities with other type strains. Phylogenetic analyses based on the 16S rRNA gene and whole genome sequences revealed that strain CrO1^T formed a distinct phyletic lineage within the genus *Ramlibacter*. On the basis of phenotypic, chemotaxonomic and molecular analysis, strain CrO1^T clearly represents a novel species of the genus *Ramlibacter*, for which the name *Ramlibacter algicola* sp. nov. is proposed. The type strain is CrO1^T (=KACC 19926^T=JCM 33302^T).

A019

Parasphingorhabdus toreuma* sp. nov., Isolated from the Intestine of a Korean Limpet, *Cellana toreuma

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

[This work was supported by a grant from the Mid-Career Researcher Program (NRF-2020R1A2C3012797) through the National Research Foundation of Korea (NRF).]

A020

Diversity of Endolichenic *Penicillium* Isolated from *Cladonia* and *Xanthoparmelia* with the Report of New *Penicillium* Species

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Endolichenic fungi are the fungal symbionts living inside of lichen thallus like the endophytes in plants. *Penicillium* is one of the most common fungal genus found in various environments as saprotrophic or endophytic fungus. The diversity of endolichenic *Penicillium*, however, is not studied well. We isolated the endolichenic fungi and investigated the diversity of *Penicillium* from the lichen *Cladonia* and *Xanthoparmelia* collected from Changwon, South Korea. A total of eight *Penicillium* species was identified using a molecular phylogeny based on the beta-tubulin region. Additionally, one species is potentially new species, thus we conducted a polyphasic analysis for morphology and multi-gene phylogeny. Based on the combination of beta-tubulin, calmodulin, and RNA polymerase II second largest subunit regions, we identified that this species belongs to the series *Restricta* in the section *Exilicaulis*, but it formed a distinct clade from other species. Our results suggest that the lichens have a high potentiality as the source of new species.

A021

***Knoellia carri* sp. nov., Isolated from Air Conditioner**

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A bacterial strain, designated DB2414S^T, was isolated from air conditioner. Cells of this strain were Gram-stain-positive, nonmotile and non-spore-forming cocci. Growth occurs at 18–42°C and pH 6.0–8.5, with 0–1% (w/v) NaCl concentration. The major fatty acids of strain DB2414S^T were iso-C_{16:0}, C_{17:1} ω8c and iso-C_{15:0}. Mk-8(H₄) is a predominant quinone of genus *Knoellia* and polar lipid profile contained diphosphatidylglycerol, phospholipids. The G + C content of the genomic DNA of DB2414S^T was 71.9 mol%. The 16S rRNA gene sequence analysis showed that strain DB2414S^T was phylogenetically related to *Knoellia remsis* JCM 15662^T and *K. locipacati* KACC15114^T (97.72% and 97.44% sequence similarity, respectively). Also, the average nucleotide identity (ANI) with the closely related species, *Knoellia remsis* JCM 15662^T, was 76.13%, which was a low value. The results of genotypic and phenotypic data showed that strain DB2414S^T could be distinguished from its phylogenetically related species, and that this strain represented a novel species within the genus *Knoellia*, for which the name *Knoellia carri* sp. nov. (type strain DB2414S^T = KCTC 49355^T = NBRC 114620^T) was proposed as a first reported *Knoellia* species.

A022

Taxonomic Analysis of *Pseudomonas palusis* sp. nov., Isolated from Wet Soil

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Pseudomonas is one of the most diverse and prevalent genera present in natural environments. In this study, a novel Gram-negative, aerobic bacterium designated *Pseudomonas* sp. MMS21 TM103^T was isolated from a wet soil sample of a saltwater wetland. Strain MMS21 TM103^T could grow at 4–37°C (optimum, 30°C) and pH 5–9 (optimum, pH 7), and in the presence of 0–6% NaCl (optimum, 0%). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain MMS21 TM103^T was mostly related to *Pseudomonas taeanensis* MS-3^T (98.56% sequence similarity), *P. taetrolens* DSM 21104^T (97.88%) and *P. borbori* R-20821^T (97.88%). The strain was oxidase and catalase positive, and hydrolyzed DNA and Tween 20. Ubiquinone Q-9 was present as the predominant quinone, and C_{16:0}, iso-C_{17:0} cyclo and summed features consisting of C_{16:1} ω7c and/or C_{16:1} ω6c and C_{18:1} ω7c and/or C_{18:1} ω6c were the main fatty acids. The diagnostic polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol. The combination of physiological and biochemical properties clearly distinguished the isolate from related species of *Pseudomonas*. On the basis of these results, strain TM103^T evidently represents a novel species of *Pseudomonas*, for which the name *Pseudomonas palusis* sp. nov. is proposed (type strain = MMS21 TM103^T = KCTC 82785^T).

[Supported by a research grant from the National Institute of Biological Resources (NIBR).]

A023

***Roseomonas oryzae* sp. nov., a Purple Phototrophic Bacterium Isolated from Oxidized Rice Paddy Soil**

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During a study to evaluate the enumeration and isolation of purple phototrophic bacteria (PPB) from rice paddy soil treated with the chemical fertilizer. We examined the purple phototrophic bacteria, which were detected by PCR amplification of the *pufLM* gene. Based on phylogenetic characterization, some of these PPB were considered to represent novel species. The aim of the present work was to determine the phylogenetic position of strains NPKOSM-4^T and NPKOSM-8 using a polyphasic approach. Two Gram-stain-negative, non-motile coccobacilli-shaped and aerobic bacteria. The strain NPKOSM-4^T grew at 20-37°C (optimum, 30°C), at pH ranges of 3.0–10.0 (optimum pH 5.0-7.0) and in the presence of 0–1.0% (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that two isolates belonged to the genus *Roseomonas*, showing the highest sequence similarities to *R. terricola* EM0302^T (97.8%), *R. wooponensis* WW53^T (96.3%) and less than 95 % with other closely related species. DNA–DNA relatedness between two isolates and closely related type strains in the genus was below 70%. The major fatty acids of the two strains were C_{18:1} ω7c and C_{16:0}. On the basis of these genotypic and phenotypic characteristics, the two strains are assigned to represent novel species of the genus *Roseomonas*, for which the name for which the name *Roseomonas oryzae* sp. nov. is proposed with the type strain NPKOSM-4^T (=KACC 22135^T).

[Supported by grants from iMAF (Project No. 918016-4).]

A024

***Pleomorphomonas oryzae* sp. nov., a Nitrogen-fixing Bacterium Isolated from Rice Paddy Soil**

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We cultured a collection of 81 isolates from rice paddy soil treated with the chemical fertilizer by 10 different culture conditions, some isolates belonged phylogenetically novel bacteria. The aim of present study was to determine the exact taxonomic position of a bacterial strain, COJ-58^T, by using polyphasic approach. A novel Gram-stain-negative, aerobic, pleomorphic-rod, non-motile and nitrogen-fixing bacterial strain, designated COJ-58^T, was isolated from rice paddy soil treated with the chemical fertilizer. The strain grew optimally at 20–30°C, at pH 5.0–8.0 and with 0–1.0% (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences indicated that, strain COJ-58^T belonged to the genus *Pleomorphomonas*, and showed the highest sequence similarities to *Pleomorphomonas oryzae* DSM 16300^T (96.0%), *Pleomorphomonas koreensis* Y9^T (95.7%), *Pleomorphomonas carboxyditropha* SVCO-16^T (95.6), *Pleomorphomonas diazotrophica* R5-392^T (95.3%). DNA–DNA relatedness values between strain COJ-58^T and its closest phylogenetic neighbours were much lower than 70%. The major fatty acids were C_{18:1} ω7c, C_{16:0} and C_{19:0} cyclo ω8c. Differential phenotypic and genotypic characteristics of the strain COJ-58^T should represent a novel species of the genus *Pleomorphomonas*, for which the name *Pleomorphomonas oryzae* sp. nov. is proposed, with strain COJ-58^T (= KACC 22108^T) as the type strain.

[Supported by grants from iMAF (Project No. 918016-4).]

A025

Biocontrol Activity of Rhizospheric *Bacillus velezensis* and Endophytic *Bacillus cereus* against Brown Patch Disease of Turfgrass Caused by *Rhizoctonia solani*

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A total of 300 bacteria were isolated from the rhizosphere and roots of turfgrass in Hwasun County, Jeonnam province in Korea during 2020. All bacterial isolates were screened against *Rhizoctonia solani* AG2-2(IIIB) causing brown patch disease on turfgrass. One isolate (CMML20-20) from rhizosphere and one isolate (CMML20-21) from the roots of turfgrass showing strong antifungal activity to *R. solani* were selected for further study. The two isolates and one negative control isolate were tested against 19 plant pathogens and the isolates CMML20-20 and CMML20-21 showed over 70% inhibition rates against *R. solani*. The phylogenetic analysis of 16S rRNA and gyrB gene sequences indicated that the isolates CMML20-20 and CMML20-21 were identified as *Bacillus velezensis* and *Bacillus cereus*, respectively. The two isolates strongly inhibited the mycelial growth of *R. solani* AG2-2 (IIIB) and altered the morphology of fungal hyphae. Detection of dead cells of *R. solani* AG2-2 (IIIB) using Evans blue and Neutral red staining suggested that the two bacterial isolates had antifungal activity. The isolates and commercial fungicide, azoxystrobin, were applied to control brown patch on creeping bentgrass. The application of CMML20-20 alone (1×10^7 CFU/ml), CMML20-21 alone, or the mixed two isolates showed significant disease reduction in planta, suggesting that the bacterial isolates could serve as potential biocontrol agents to control brown patch in the field.

[Supported by IPET]

A026

LasB, a Protease at the Top of the Hierarchy of Activation of LasA and PIV, is not Auto-activated in *Pseudomonas aeruginosa*

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LasB (elastase B) is a quorum sensing (QS)-regulated protease in *Pseudomonas aeruginosa*. Previous studies suggested that LasB is auto-activated post-secretionally and successively activates other proteases, protease IV (PIV) and LasA (elastase A), by degrading their propeptides. However, our findings showed that although LasB overexpressed in a QS mutant has a reduced activity, the SDS-PAGE analysis revealed that LasB in the QS mutant was in its mature form, suggesting that the reduction of LasB activity in the QS mutant was not caused by the incomplete processing of LasB. This means that LasB is not auto-activated, but requires some QS-dependent factors for activation. The purified LasA and PIV could not restore the reduced LasB activity in the QS mutant, but the addition of whole culture supernatant from a QS⁺ *lasB*⁻ strain could restore the LasB activity, indicating that there is an unknown QS-dependent factor that activates LasB. Interestingly, the purification of LasB from the QS mutant also restored the LasB activity gradually. This means that an unknown inhibitor inhibits LasB in the QS mutant. Taken together, we propose that a QS-independent inhibitor represses LasB activity, and another QS-dependent activator eliminates the function of this inhibitor, thereby activating LasB.

[This work was supported by a grant from the National Research Foundation of Korea funded by the Korean government (NRF-2019R1A2C1010087) + BK.]

A027

***Fibrella aquatilis* sp. nov., *Fibrella rubiginis* sp. nov. and *Fibrella forsythiae* sp. nov., Isolated from Samples Collected in Yongin-si, Korea**

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Three novel strains, designated as HMF5036^T, HMF5335^T, and HMF5405^T, were isolated from freshwater, rusty iron and forsythia flower, in Yongin-si, Republic of Korea, respectively. They were Gram-staining-negative, strictly aerobic, non-motile, reddish-pigmented and rod-shaped bacteria. The predominant fatty acids of three strains were summed feature 3 (comprising C_{16:1} ω7c and/or C_{16:1} ω6c) and C_{16:1} ω5c. They contained MK-7 as the predominant menaquinone. The major polar lipids were phosphatidylethanolamine, two unidentified aminophospholipids and several unidentified lipids. Strains HMF5036^T, HMF5335^T, and HMF5405^T exhibited the highest 16S rRNA gene sequence similarities of 91.8, 92.6, and 93.6% to *Fibrella aestuarina* BUZ 2^T and less than 88.7 % with other members of the family *Spirosomaceae*. Among themselves, the values were 94.9–96.6%. Phylogenetic analysis based on the 16S rRNA gene sequences of three isolates revealed that they formed a distinct clade within the family *Spirosomaceae*. The genome sizes of strains HMF5036^T, HMF5335^T, and HMF5405^T were 6.78, 6.35, and 7.76 Mbp, and their DNA G+C contents were 54.9, 54.0, and 52.0 mol%, respectively. Based on the results of polyphasic analysis, three novel species, *Fibrella aquatilis* sp. nov., *Fibrella rubiginis* sp. nov., and *Fibrella forsythiae* sp. nov. are proposed. The type strains are HMF5036^T (= KCTC 82476^T = NBRC 115092^T), HMF5335^T (= KCTC 82477^T = NBRC 115093^T), and HMF5405^T (= KCTC 82478^T = NBRC 115094^T), respectively.

A028

***Aquibacillus saliphila* sp. nov., a Moderately Halophilic Bacterium Isolated from a Gray Saltern**

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A novel moderately halophilic bacterium, designated strain KHM2^T, was isolated from the sediment of a gray saltern from located in Sinui island at Shinan, Republic Korea. Cells are found to be rod-shaped, endospore forming, positive for Gram's stain, catalase, oxidase and negative for urease. Moderately halophilic, growing over a wide range of NaCl concentrations (5–20.0% NaCl, w/v), motile and facultative anaerobe. It grew at wide range of pH (6.0–10.0) and temperatures (10–40°C). Based on 16S rRNA gene sequence similarity and chemotaxonomic properties, the isolate was assigned to the genus *Aquibacillus*, with the high 16S rRNA gene sequence similarities to *Aquibacillus halophilus* B6B^T (98.21%), *Aquibacillus salifodinae* WSY08-1^T (96.84%), *Aquibacillus albus* YIM 93624^T (96.77%), *Aquibacillus koreensis* BH30097^T (96.44%) and *Aquibacillus sedimins* BH258^T (95.78%). The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, glycolipid, and an unidentified phospholipid. Major fatty acids were anteiso-C_{15:0} and anteiso-C_{17:0}. The DNA–DNA hybridization experiments revealed low levels of relatedness between strain KHM2^T and *Aquibacillus halophilus* B6B^T (%). Based on the polyphasic analysis (phylogenetic, chemotaxonomic, and biochemical), strains KHM2^T can be suggested as two new bacterial species within the genus *Aquibacillus* and the proposed name *Aquibacillus saliphila*. The type strain is KHM2^T (= KACC 19068^T = NBRC 112577^T).

A029

***Mucilaginibacter rivuli* sp. nov. Isolated from a Water Rivulet**

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Strain HMF5004^T was isolated from a rivulet located on Yongin, Republic of Korea. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain HMF5004^T belonged to the genus *Mucilaginibacter* and highest similarity with *Mucilaginibacter paludis* (97.7%) and *Mucilaginibacter gracilis* (97.2%). The average nucleotide identity (ANI) between strain HMF5004^T and *M. paludis* was 72.8%, which is far below the suggested threshold for species demarcation. Cells of strain HMF5004^T were Gram-stain-negative, rod-shaped, non-motile, catalase and oxidase positive. The DNA G + C contents of strain HMF5004^T was 42.4%. Strain HMF5004^T had menaquinone-7 (MK-7) as a major quinone. The major cellular fatty acids included iso-C_{15:0}, summed feature 3 (C_{16:1} ω7c and/or C_{16:1} ω6c) and anteiso-C_{15:0}. The polar lipids of strain HMF5004^T contained phosphatidyletanolamine, five unidentified aminolipids, two unidentified aminophospholipids, three unidentified lipids on the basis of the evidence presented in this polyphasic taxonomic study, strain HMF5004^T is considered to be a novel species *Mucilaginibacter rivuli* sp. nov. (= KCTC 82633^T = NBRC 115091^T).

A030

Isolation and Genomic Analysis of *Limnobacter profundus* sp. nov., a Phenol-degrading Betaproteobacterium Isolated from Deep-seawater in the Northwest Pacific Ocean

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Although culture-independent studies have shown the abundance of the order *Burkholderiales* in deep-sea environment, deep-sea members of the order have been rarely cultured and characterized. In the present study, a Gram-stain-negative, rod-shaped, aerobic and motile by a single flagellum, designated SAORIC-580^T, was isolated from deep seawater (4,000 m) in the Northwest Pacific Ocean. The 16S rRNA gene sequence analysis showed that the strain is affiliated to the genus *Limnobacter* and most closely related to *L. thiooxidans* CS-K2^T sharing 99.9% sequence similarity. Whole genome sequence of strain SAORIC-580^T was 3.3 Mbp of genome size with 52.5 mol% of the DNA G + C content. The SAORIC-580^T genome shared less than 79.4–85.7% of the average nucleotide identity and 19.9–29.5% of digital DNA-DNA hybridization values to the *Limnobacter* genomes, indicating that the novel strain represents a novel species in the genus. The isoprenoid quinone of strain SAORIC-580^T was ubiquinone-10. The major cellular fatty acids were summed feature 8 (C_{18:1} ω7c), C_{18:1} ω7c 11-methyl and C_{16:0}. The major polar lipids constituted phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol. On the basis of taxonomic data obtained in this study, it was concluded that strain SAORIC-580^T represented a novel species of the genus *Limnobacter*, for which the name *Limnobacter profundus* sp. nov. is proposed. The type strain is SAORIC-580^T (= KACC 21440^T = NBRC 114111^T).

[Supported by grants from HNIBR.]

A031

Taxonomic and Genomic Characterization of *Chryseobacterium paludis* sp. nov., Isolated from Ungok Wetland, South Korea

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A Gram-staining-negative, aerobic, yellow-pigmented and non-motile bacterial strain, designated NUG5-1^T, was isolated from wetlands from Ungok, South Korea. Strain NUG5-1^T grew optimally at 30°C on tryptic soy agar. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain NUG5-1^T belonged to the genus *Chryseobacterium* within the family *Flavobacteriaceae* and was most closely related to *Chryseobacterium piperi* CTM^T with 98.47% similarity. The average nucleotide identity values between strain NUG5-1^T and two closely related type strains *C. piperi* CTM^T and *C. soldanellicola* DSM 17072^T were 82.4% and 80.4%, respectively. The digital DNA–DNA hybridization values between strain NUG5-1^T and the related type strains were 25.3% and 22.9% respectively. The genome of strain NUG5-1^T was assembled to a single contig that consists of 5,050,391 bp with a G + C content of 34.42%. The genes related with the biosynthetic pathway for menaquinone (MK-6), pathways for the assimilation of arabinose, gluconate, glycogen, and phenylacetic acid, the degradation pathway for acrylonitrile, and a metallo-β-lactamase were predicted. Based on polyphasic taxonomy study, strain NUG5-1^T represents a novel species in the genus *Chryseobacterium*, for which name *Chryseobacterium paludis* sp. nov. is proposed. The type strain is NUG5-1^T.

[This work was supported by a project of the National Institute of Biological Resources (NIBR) to survey Korean indigenous species.]

A032

Strain LPB0316^T sp. nov., belonging to the Genus *Thalassotalea*Hyoseok Jang^{1,2} and Hana Yi^{1,2*}¹*Interdisciplinary Program in Precision Public Health, Korea University,* ²*Integrated Biomedical and Life Sciences, Korea University*

Strain LPB0316^T, a Gram-staining-negative, aerobic, motile, cream-colored, catalase-negative and oxidase-positive bacterium, was isolated from beach sand of Kkotji Beach of Korea. Best growth condition was observed at 25°C on marine agar. In the 16S rRNA gene sequence trees, strain LPB0316^T was belonging to the genus *Thalassotalea*. The highest sequence similarity was observed with *Thalassotalea loyana* (97.73%). The low sequence similarity and tree topology demonstrated the taxonomic independence of the strain at species-level. The isolate possessed summed feature 3 (C_{16:1} ω7c/C_{16:1} ω6c), C_{17:1} ω8c, C_{14:0}, and summed feature 8 (C_{18:1} ω7c/C_{18:1} ω6c) as the major cellular fatty acids. Strain LPB0316^T has a circular chromosome of 3.5Mb with DNA G + C content of 42.0 mol%. The genome includes 3,057 protein-coding genes and 100 RNA genes (19 rRNA genes, 77 tRNA genes, and 4 ncRNC genes), and encoded 6 copies of rRNA operon. On the basis of the phylogenetic, genomic and phenotypic data presented in this study, the strain was classified as representing a novel species of the genus *Thalassotalea*.

A033

***Mytilibacter wandonensis* gen. nov., sp. nov., Isolated from Mussel in Wando**

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A novel bacterium, designated MH4015^T, was isolated from mussel in Wando, Republic of Korea. The isolate was Gram staining-negative, aerobic, rod-shaped, oxidase- and catalase-positive. Strain MH4015^T grew at 15–30°C (optimum, 30°C) and pH 6–8 (optimum, pH 7) in the presence of 0.5–5.0% (w/v) NaCl (optimum, 1%). Based on phylogenetic analyses, the 16S rRNA gene sequence showed that strain MH4015^T belonged to the family *Rhodobacteraceae* in the class *Alphaproteobacteria* and was closely related to the type strains of *Hasllibacter halocynthiae* KCCM 90082^T, *Actibacterium mucosum* KCTC 23349^T, and *Wenxinia marina* KACC 12837^T with 95.7%, 94.7%, and 93.0% of 16s rRNA gene sequence similarities, respectively. The predominant respiratory quinone was Q-10. The major fatty acids were summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c) and C_{16:0} 2-OH. Phosphatidylglycerol was the only identified polar lipid, although other lipids were also detected. The DNA G + C content was 64.9 mol%. Strain MH4015^T represents a novel genus of the family *Rhodobacteraceae*, for which the name *Mytilibacter wandonensis* gen. nov., sp. nov., is proposed for this isolate, and the type strain is MH4015^T (= KCTC 82702^T). [This research was supported by a grant (20SCIP-C158976-01) from Construction Technology Research Program funded by Ministry of Land, Infrastructure and Transport of Korean government.]

A034

***Robertkochia sediminis* sp. nov., Isolated from the Seashore Sediment**

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The two strains 3YJGBD-33^T and 3YJGBD-35 were isolated from mud samples in seashore sediment of the West Sea, the Republic of Korea (37°13'06.3"N 126°37'36.3"E) about taxonomic position of *Robertkochia* sp. The pigment colors of strain 3YJGBD-33^T and 3YJGBD-35 were orange and yellow. Among two strains, strain 3YJGBD-33^T was a Gram-negative, catalase- and oxidase-positive and non-flagellated bacterium. Colonies were circular, smooth, yellow-orange, approximately 1 mm in diameter and cells were long rod shaped (0.5 µm wide, 2.5–2.6 µm long). Growth was observed in the presence of 1–6% (w/v) NaCl (optimum, 4%) and at 15–45°C (optimal growth, 42°C). Strain 3YJGBD-33^T showed highest 16S rRNA gene sequence similarities to *Robertkochia marina* CC-AMO-30D^T (96.86%) and *Robertkochia solimangrovi* CL23^T (95.93%). Strain 3YJGBD-33^T contained iso-C_{15:0}, iso-C_{15:1} G, and iso-C_{17:0} 3-OH (> 10.0%). The major polar lipids consisted of phosphatidylethanolamine and two unidentified aminolipids. Genome sequencing revealed that strain 3YJGBD-33^T has a genome size of 3.4 Mbp and a G + C content of 44.62 mol%. Based on phenotypic, chemotaxonomic, phylogenetic, and genomic evidence, strain 3YJGBD-33^T is proposed to represent a new species with the name *Robertkochia sediminis* sp. nov. (= KCTC 82715^T).

A035

The Genomic Characteristics of Strain SS1-8-M1-7 within Family *Rhodovibrionaceae* Isolated from Bioreactor of Closed-recirculating Aquaculture System

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The strain SS1-8-M1-7 was isolated from the lab-scale bioreactor which was designed to investigate the nitrogen and phosphorus removal efficiency of the wastewater from the closed recirculating wastewater treatment system. Among the isolated strains, there was a strain which is in the Family *Rhodovibrionaceae*. In the Arb tree, the strain was in the clade, close to genus *Tistlia* and *Thalassocola*. The strain SS1-8-M1-7 based on 16S rRNA sequence was closed to *Tistlia consotensis* (89.6% similarity) and *Thalassocola ureilytica* (88.9%). The genome size of strain SS1-8-M1-7 was 3.8 Mbp and the G + C content was 62.7 mol%. As a result of genome analysis using KEGG, the isolate was revealed to have the sulfur pathway, nitrogen metabolism such as dissimilatory nitrate reduction and denitrification. The isolate was found to have gamma-aminobutyric acid (GABA) pathway. GABA was the primary inhibitory neurotransmitter in the central nervous system (CNS), can be used for the safe animal feed as a feed additive and function as anti-hypertension, anti-diabetes, anti-cancer, antioxidant, anti-inflammation, anti-microbial, and anti-allergy. Thus, this strain can be applied to not only remove the nitrogen and sulfur in the bioreactor but also be supplied to enhance the growth of fish. Further study will be conducted to have a better understanding about strain SS1-8-M1-7 (= KEMB 1603-010).

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A036

Exploring Novel Bacterial in the Family *Fusibacter* Terpene Synthases

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The strain YDP3^T was grown at room temperatures, 72 h in anaerobic condition. Genome sequencing revealed that strain YDP3^T has a genome size of 3.5 Mbp (contigs 11 and N₅₀ 2,464,899), and the G + C content of 47.1 mol%. According to genomic analysis of strain YDP3^T, there are many genes about transporters as molybdate, spermidine, phosphate, osmoprotectant, arginine, branched-chain amino acid, nucleoside, and ribose. This is evidence to support the lack of a metabolic pathway that synthesizes various energy sources on its own, as it uses energy from nutrients such as C, N, and P from the environment. It can also be used as an energy source by taking DNA from the outside through gene transporters. Especially, strain YDP3^T was produced terpenoids by terpenoid backbone biosynthesis pathways. Terpene synthase activity was detected for 15 of these enzymes in strain YDP3^T genome. Overall, we have identified terpene synthases in Firmicutes and demonstrated that terpene synthases with substrate promiscuity are widely distributed in nature, forming a rich resource for engineering terpene biosynthetic pathways for industrial biotechnology.

A037

***Mangrovicoccus caeni* sp. nov., Isolated from a Closed-recirculating Aquaculture System**Wonkyoung Kim¹, Hong Sik Im^{2,3}, Hyekyoung Yang^{2,3}, Ha Jung Moon^{2,3}, Byung kwon Kim⁴, Jungjo Han⁴, Sukchan Lee², and Sang-Seob Lee^{2,3*}

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A Gram-staining-negative, aerobic, and cocci shaped bacterium, designated DBReWK2^T, was isolated from a Closed-Recirculating Aquaculture System which was collected from aquaculture ground, Republic of Korea. Phylogeny-based on the 16S rRNA gene sequence showed that strain DBReWK2^T formed a distinct level lineage within the family *Rhodobacteraceae* of the class Alphaproteobacteria and was most closely related to *Mangrovicoccus ximenensis* T1lg56^T (97.4%, 16S rRNA similarity) and *Poseidonocella pacifica* DSM 29316^T (96.2%). Growth occurred at 4–37°C (optimum, 30°C) and in the presence of 0–12% (w/v) NaCl (optimum, 1%). The major cellular fatty acids were C_{18:1} ω7c and C_{16:0}. The predominant isoprenoid quinone was Ubiquinone-10. Major Polar lipids were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The genome size was 7.8 Mbp with a DNA G+C content of 71.9 mol%. Based on the distinctive phenotypic characteristics and phylogenetic analysis, low average nucleotide identity (ANI) and digital DDH (dDDH) results (ANI 85.6% and dDDH 14%, respectively), it is concluded that strain DBReWK2^T represented a novel species of the genus *Mangrovicoccus*, for which the name *Mangrovicoccus caeni* sp. nov. is proposed. The type strain of DBReWK2^T is (= KCTC82714^T).

[The work is supported by the grant NRF-2017M3A9B8065734.]

A038

***Aliikangiella sediminis* sp.nov., Isolated from the Seashore Sediment**

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A Gram-staining-negative, aerobic, and long rod-shaped marine bacterium, designated G2MR2-5^T, was isolated from mud sample which was collected from the seashore sediment of the West Sea, the Republic of Korea. Phylogeny-based on 16S rRNA gene sequences showed that strain G2MR2-5^T formed a distinct species-level lineage within the genus *Aliikangiella* of the class *Gammaproteobacteria* and was most closely related to *Aliikangiella marina* GYP-15^T (96.3% similarity) and *Aliikangiella coralliicola* M105^T (94.6%). Growth occurred at 25–37°C (optimum 37°C) and in the presence of 2–5% (w/v) NaCl (optimum 2-3%). The predominant isoprenoid quinone was ubiquinone 8. Major Polar lipids were phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. The DNA G + C content based on the draft genome sequence was 41.6 mol%. Based on the distinctive phenotypic characteristics and phylogenetic analysis, it is concluded that strain G2MR2-5^T represented a novel species of the genus *Aliikangiella*, for which the name *Aliikangiella sediminis* sp. nov. is proposed. The type strain is G2MR2-5^T (= KCTC 82293^T).

A039

The Genome-scale Propionate Network Analysis of a Novel Propionogenic Gut Bacterium

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A novel Gram-stain-positive, non-spore-forming, rod-shaped and strictly anaerobic bacterium, designated AP1^T, was isolated from healthy Korean feces. Comparative analysis of 16S rRNA gene sequences showed that AP1^T was most closely related to *Merdimonas faecis* BR31^T (94.3%) and ANI value between their genomes was 75.6%. AP1^T produced the high level of propionate compared with reference strains. To investigate the metabolic pathways utilized by AP1^T to produce propionate, the genome-scale propionate network of AP1^T was reconstructed based on its annotated genes, literature, and physiological data. As the result, AP1^T did not produce propionate by pathways that most gut bacteria use. AP1^T was determined to produce a large amount of propionate through the amino acid catabolic pathway, that was also supported by increased propionate after adding amino acids. Based on the phylogenetic, phenotypic and chemotaxonomic characteristics, AP1^T represents a novel species in a novel genus within the family *Lachnospiraceae*. It is also expected that a novel propionogenic bacterium AP1^T can be used as new generation probiotics to target various metabolic syndrome as propionate-producing consortium does. [Supported by a Bio & Medical Technology Development program of the National Research Foundation of Korea funded by the Ministry of Science and ICT of the Republic of Korea (NRF-2016M3A9F3946674) and a grant from the KRIBB Research initiative program]

A040

Strain LPB0304^T, a Novel Species of the Genus *Massilia*

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Strain LPB0304^T, a Gram-staining-negative, aerobic, catalase- and oxidase-positive bacterium, was isolated from beach sand of Kkotji Beach, Korea. In phylogenetic trees, strain LPB0304^T belongs to the genus *Massilia*, but forms an independent branch apart from previously described species. *Massilia suwonensis* 5414S-25^T showed the highest sequence similarity to the isolate (98.44%), and followed by *M. niabensis* 5420S-26^T (98.35%), *M. brevitalea* byr23-80^T (98.09%), *M. jejuensis* 5317J-18^T (98.07%), *M. aurea* AP13^T (97.48%) and *M. timonae* CCUG 45783^T (96.87%). Strain LPB0304^T possesses a circular chromosome of 5.18 Mb and a single plasmid of 158.6 kb. The genomic G + C content is 64.74 mol%. Of 4,786 genes, 4,634 genes are protein-coding genes and 104 RNA (22 rRNA, 78 tRNAs, 4 non-coding RNAs) were detected. Best growth was observed at 25°C and pH 7 on R2A Agar. The isolate hydrolyzed casein, hypo-xanthine, and Tweens 20, 40, 60, and 80, but not alginic acid, chitin, cellulose, adenine, starch, L-tyrosine, or xanthine. The isolate possessed summed feature 3 (C_{16:1} ω7c/C_{16:1} ω6c) and C_{16:1} as the major cellular fatty acids. Based on the phylogenetic and phenotypic data obtained in this study, strain LPB0304^T should be classified as a novel species in the genus *Massilia*.

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A041

A Novel *Jinshanibacter* Species, Isolated from a *Allomyrina dichotoma* Larva Feces

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A novel facultatively anaerobic bacterium, strain M2-1^T, was isolated from a *Allomyrina dichotoma* larva feces. Cells of the strain M2-1^T were Gram-stain negative, non-motile and rod-shaped and around 0.5–0.7 × 1.2–1.8 μm in size. Optimum growth occurred at 25°C. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain M2-1^T belonged to the genus *Jinshanibacter* and was closely related to *Jinshanibacter zhutogyuii* CF-458^T (99.52%). The genome sequencing of strain M2-1^T revealed a genome size of 4,579,802 bp, a G+C content of 45.25 mol%. It contains a total of 4051 CDSs, 10 rRNA genes and 62 tRNA genes. According to the data obtained strain M2-1^T shared ANI value 91.14%, dDDH value 42.8% with the closest type species. Strain M2-1^T possessed Summed Feature 3 (C_{16:1} ω7c/C_{16:1} ω6c), C_{16:0} and Summed Feature 8 (C_{18:1} ω7c/ C_{18:1} ω6c) as the major fatty acids and had Q-8. Based on phylogenetic, physiological and chemotaxonomic characteristics, strain M2-1^T represent a novel species of the genus *Jinshanibacter*. The type strain is M2-1^T (=KCTC 25030^T).

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A042

***Brachybacterium* sp. EF45031 sp. nov., Isolated from Hotspring from Chungju, Republic of Korea**

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A novel Gram-positive, non-motile and moderately halophilic rod-shaped bacterium EF45031^T was isolated from hotspring in Chungju, Republic of Korea. The strain was able to grow at concentrations of 0–5% (w/v) NaCl (optimum, 3% NaCl), at pH 6.0–9.0 (optimum, pH 7.0) and in a temperature range of 20–50°C (optimum, 45°C). On the basis of 16S rRNA gene sequence analysis, strain EF45031 was closely related to *Brachybacterium nesterenkovii* CIP 104813^T (97.71%), *Brachybacterium huguangmaarensis* M1^T (97.43%), *Brachybacterium phenoliresistens* phenol-A^T (97.12%), *Brachybacterium zhongshanense* JB^T (97.10%), *Brachybacterium sacelli* LMG 20345^T (97.09%), *Brachybacterium squillarum* M-6-3^T (97.01%), and *Brachybacterium rhamnosum* LMG 19848^T (96.94%). The DNA G + C content of the strain was 70.95 mol%. On the bases of chemotaxonomic, phenotypic and genotypic data, strain EF45031^T represents a novel species of the genus *Brachybacterium*, for which the name *Brachybacterium* sp. nov. is proposed.

A043

***Microbacterium* sp. EF45047 sp. nov. and *Microbacterium* sp. EF45044 sp. nov., Isolated from Hotspring**

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Two Gram-positive, nonmotile, rod-shaped, EF45047^T and EF45044^T, were isolated from hotspring in Chungju, Republic of Korea. Strains EF45047^T and EF45044^T contained menaquinone-12 and menaquinone-13 (MK-12, MK-13) as the predominant respiratory lipoquinone and C_{17:0} anteiso as the major fatty acid. A neighbor-joining tree based on 16S rRNA gene sequences showed that the two isolates fell within the evolutionary radiation enclosed by the genus *Microbacterium*. Strains EF45047^T and EF45044^T showed highest 16S rRNA gene sequence similarities with *Microbacterium arthrosphaerae* CC-VM-Y^T (97.91%) and *Microbacterium ketosireducens* DSM 1251^T (98.2%), respectively, and the 16S rRNA gene sequence similarity between them was 99.7%. The DNA G + C contents of strains EF45047^T and EF45044^T were 71.4 mol% and 71.4 mol%, respectively. On the basis of phenotypic, phylogenetic, chemotaxonomic, and genetic data, strains EF45047^T and EF45044^T were classified in the genus *Microbacterium* as two distinct novel species, for which the names *Microbacterium* sp. EF45047 sp. nov. and *Microbacterium* sp. EF45044 sp. nov. are proposed.

A044

***Salinimicrobium* sp. HN-2-9-2 sp. nov., Isolated from Seawater from Tongyeong, Republic of Korea**

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A novel Gram-negative, non-motile and moderately halophilic rod-shaped bacterium HN-2-9-2^T was isolated from seawater in Tongyeong, Republic of Korea. The strain was able to grow at concentrations of 0.5–7% (w/v) NaCl (optimum, 3% NaCl), at pH 5.5–8.5 (optimum, pH 7.0–7.5) and in a temperature range of 18–45°C (optimum, 37°C). On the basis of 16S rRNA gene sequence analysis, strain HN-2-9-2 was closely related to *Salinimicrobium xinjiangense* BH206^T (98.22%), *Salinimicrobium terrae* YIM C338^T (97.70%), *Salinimicrobium soli* CAU 1287^T (97.16%), *Salinimicrobium sediminis* CGMCC 1.12641^T (96.95%), *Salinimicrobium gaetbulicola* BB-My20^T (96.54%), *Salinimicrobium marinum* KMM 6270^T (96.12%), *Salinimicrobium flavum* X7^T (95.29%) and *Salinimicrobium catena* HY1^T (94.81%). The DNA G + C content of the strain was 43.01 mol%. On the bases of chemotaxonomic, phenotypic, and genotypic data, strain HN-2-9-2^T represents a novel species of the genus *Salinimicrobium*, for which the name *Salinimicrobium* sp. nov. is proposed.

A045

Polyphasic Characterization of Two Novel *Ornithinimicrobium* Species Isolated from Korean Indigenous Birds

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Phenotypic and genomic analyses were performed to characterize two novel strains, H23M54^T and AMA3305^T, isolated from the faeces of the Oriental stork (*Ciconia boyciana*) and the cinereous vulture (*Aegypius monachus*), respectively. Strains H23M54^T and AMA3305^T showed the highest 16S rRNA gene sequence similarity with *Ornithinimicrobium cavernae* KCTC 49018^T (98.54%) and *O. pekingense* JCM 14001^T (98.49%), respectively. Both the strains were Gram-stain-positive, obligate aerobes, non-motile, non-spore forming, and coccoid to rod shaped. Colonies of the both strains were smooth, opaque, and convex and had an intact margin. Both the strains had iso-C_{15:0}, iso-C_{16:0}, and summed feature 9 (iso-C_{17:1} ω9c and/or C_{16:0} 10-methyl) as their major cellular fatty acids. Strains H23M54^T and AMA3305^T possessed diphosphatidylglycerol and phosphatidylglycerol as their major polar lipids. Moreover, strains H23M54^T and AMA3305^T commonly contained ribose and glucose as their whole-cell sugar components and L-ornithine, L-alanine, glycine, and aspartic acid as their whole-cell amino acid components. We propose the name *Ornithinimicrobium ciconiae* sp. nov. for strain H23M54^T (= KCTC 49151^T = JCM 33221^T) and the name *Ornithinimicrobium avium* sp. nov. for strain AMA3305^T (= KCTC 49180^T = JCM 32873^T).

[This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science and ICT (NRF-2017M3A9F3046549).]

A046

A Novel Bacterium, *Flaviflexus ciconiae* sp. nov., Isolated from the Faeces of the Oriental Stork

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A novel Gram-stain-positive, non-spore-forming, non-motile, strictly aerobic bacterium, designated strain H23T48^T, was isolated from the faecal sample of an oriental stork, *Ciconia boyciana*, collected from the Seoul Grand Park Zoo in Seoul, Republic of Korea. 16S rRNA gene sequence-based phylogenetic tree revealed that strain H23T48^T was related to the genus *Flaviflexus*, with 97.0 and 96.7% sequence similarities to *Flaviflexus salsibiostraticola* EBR4-1-2^T and *Flaviflexus huanghaiensis* H5^T, respectively. Optimal growth of strain H23T48^T occurred at 30–37°C, pH 8 and with 3% (w/v) NaCl. Strain H23T48^T possessed C_{16:0} (42.4%), C_{18:1} ω_{9c} (31.3%), and C_{14:0} (17.7%) as the major cellular fatty acids and MK-9(H₄) as the major menaquinone. The amino acid composition of the whole-cell peptidoglycan was L-alanine, L-lysine, D-glutamic acid, L-aspartic acid, and glycine. The genomic G + C content of strain H23T48^T is 59.5 mol% and the average nucleotide identity value between H23T48^T and *F. salsibiostraticola* KCT C33148^T (= EBR4-1-2^T) is 75.5%. Based on the obtained data, strain H23T48^T represents a novel species of the genus *Flaviflexus*, for which the name *Flaviflexus ciconiae* sp. nov. is proposed. The type strain is H23T48^T (= KCTC 49253^T = JCM 33282^T).

[This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science & ICT (NRF-2017M3A9F3046549).]

A047

Comparative Analysis of Three Novel *Deefgea* Strains Isolated from Korean Indigenous Fishes

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Gut microbiota has an important role in host metabolism, immune system, and health. Although the gut microbial community of mammals were well known, that of wild fishes remain largely unexplored. To investigate the gut microbiota of Korean indigenous fishes, we performed bacterial isolations from gut samples of Korean oily bitterling (*Tanakia koreensis*), Korean dark sleeper (*Odontobutis platycephala*), and kumgang fat minnow (*Rhynchocypris kumgangensis*).

We obtained three putative novel strains and they were identified as new members of the genus *Deefgea* through a phylogenetic analysis based on nearly full-length 16S rRNA gene sequences. We evaluated their optimal growth conditions and performed additional biochemical analyses. To evaluate the genomic relatedness, the digital DNA–DNA hybridization (dDDH), average amino acid identity (AAI), and orthologous ANI (OrthoANI) values were also calculated. Taken together, strain D17^T, D13, and D25^T represent three novel strains within the genus *Deefgea*. [This work was supported by a grant from the Mid-Career Researcher Program (NRF-2020R1A2C3012797) through the National Research Foundation of Korea (NRF).]

A048

***Thermomonas paludicola* sp. nov., Isolated from a Lotus Wetland**Mirae Kim¹, Jaeho Song², Miri S. Park¹, Yeonjung Lim¹, and Jang-Cheon Cho^{1*}¹Department of Biological Sciences and Bioengineering, Inha University, ²Microbial Research Division, Honam National Institute of Biological Resources

A Gram-stain-negative, aerobic, rod-shaped, motile bacterium, designated *Thermomonas* sp. IMCC34681^T, was isolated from a lotus wetland in Gapyeong, South Korea. Cellular growth occurred at 30°C, pH 7.0, and with 0–2% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain IMCC34681^T belongs to the genus *Thermomonas* and shares 95.7–96.9% sequence similarities with other *Thermomonas* species. Whole genome sequencing of strain IMCC34681^T revealed genome size of 2.72 Mbp and DNA G + C content of 66.2 mol%. The IMCC34681^T genome shared the average nucleotide identity (ANI) of 82.0% with *Thermomonas fusca* DSM 15424^T and 80.7% with *Thermomonas haemolytica* DSM 13605^T. The major respiratory quinone was ubiquinone-8 (Q-8) and predominant cellular fatty acids were iso-C_{15:0} (25.7%) and iso-C_{14:0} (20.8%). Major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine. Based on phylogenetic distinction and differential phenotypic characteristics, strain IMCC34681^T is considered to be assigned to the genus *Thermomonas* as the type strain of a new species, for which the name *Thermomonas paludicola* sp. nov. is proposed. The type strain is IMCC34681^T (= KACC 21793^T = NBRC 114635^T).

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A049

Pseudorhodobacter turbinis* sp. nov., Isolated from the Gut of the Korean Turban Shell, *Turbo cornutus

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A Gram-stain-negative, coccus-shaped, aerobic and motile bacterial strain designated S12M18^T was isolated from the gut of the Korean turban shell, *Turbo cornutus*. The phylogenetic analysis based on the 16S rRNA gene sequence showed that strain S12M18^T belonged to the genus *Pseudorhodobacter*. The 16S rRNA gene sequence similarity with the most closely related *Pseudorhodobacter* species, *P. aquimaris* HDW-19^T was 98.63%. The phylogenomic tree consistently confirmed that strain S12M18^T is a genuine member of the genus *Pseudorhodobacter*. The OrthoANIu value between strain S12M18^T and *P. aquimaris* HDW-19^T was 87.22%. The major cellular fatty acid was summed feature 8 (C_{18:1} ω7c or C_{18:1} ω6c). The major components of the polar lipids were phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine. The predominant isoprenoid quinone was Q-10. The DNA G + C content was 57.8%. The polyphasic analyses indicated that strain S12M18^T represents a novel species of the genus *Pseudorhodobacter*, for which the name *Pseudorhodobacter turbinis* sp. nov. is proposed. The type strain is S12M18^T (= KCTC 62742^T = JCM 33168^T).

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A050

***Abyssanaerobacter marinus* gen. nov., sp. nov., γ -Aminobutyrate-fermenting, Anaerobic Bacterium Isolated from the Onnuri Vent Field of the Indian Ocean and Suggestion of Clostridiales Family XIII Insertae Sedis Emended *Mogibacteriaceae* fam. nov., Proposal for Phylogenetically Reclassification of Ambiguous This Family's Genus Member**

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An anaerobic, mesophilic, γ -aminobutyrate fermenting, Gram-staining positive, rod-shaped bacteria, strain IOR16^T was isolated from a newly discovered new deep-sea hydrothermal vent (OVF, Onnuri Vent Field) area in Indian Ocean ridge. The 16S rRNA gene sequences analysis showed that strain IOR16^T was closely related to *Bacilliculturomica massiliensis* Marseille-P3303^T (94.5%), *Clostridium aminobutyricum* DSM 2634 (94.4%) *Anaerovorax odorimutans* NorPut1^T (94.2%), and *Aminipila butyrica* FH042^T (93.0%). It indicated a low similarity with less than 94.5% with other members of three genus member and others. Strain IOR16^T could grow at 23–42°C (optimum, 34°C), pH 6.0–9.0 (optimum, pH 7.0), and 0–6.0% (w/v; optimum, 3.0%) NaCl. The major fatty acids of strain IOR16^T were C_{14:0} (16.5%), C_{16:0} (22%), and C_{18:1} ω 9c (13.9%) and high exist unsaturated fatty acid (Total 49.3%). The DNA G+C contents of IOR16^T was 35.9 mol%. Strains IOR16^T, unlike the other strains (DSM 2634, NorPut1^T, and FH042^T), could utilize wide range of carbohydrate (glucose, sucrose, maltose, and trehalose) as substrates. Based on the result of phylogenetic, phenotype, and chemotaxonomic properties, *Abyssanaerobacter marinus* gen. nov., sp. nov., with type strain IOR16^T (= KCTC 25034^T = MCCC^T = JCM^T) is proposed in a new family within the Clostridiales XIII groups and the family name is suggested as *Mogibacteriaceae*.

A051

***Staphylococcus* sp. nov. SB1-57, Isolated from Human Skin**

Yu jin Choi, Hae Lim Son, Munkhtsatsral Ganzorig, and Kyoung Lee*

Changwon National University

Staphylococcus sp. SB1-57, isolated from human skin, is Gram-stain-positive, aerobic, non-motile and round-shaped. This strain grew on TSB, NB, LB, BHI medium, at the temperature of 13–45°C (optimum, 30–37°C). This strain grew a wide range of 0–23% (optimum, 0–5%) NaCl concentration, indicating very high salinity tolerance. Isolated colonies were observed as round, viscous, and white (slight ivory color) color when incubated for one day at 37°C on TSB agar plate. SB1-57 was catalase-positive, hemolysis-weakly positive, oxidase-negative, coagulase-negative. Antibiotic tests showed that SB1-57 was resistance to polymyxin B, nalidixic acid, streptomycin. Menaquinone-7 (MK-7) (73.92%) was detected as a major quinone, and MK-8 (21.73%) and MK-9 (4.34%) were also detected. The major fatty acids were C_{15:0} anteiso (54.73%), C_{17:0} anteiso (24.79%). The genome of the SB1-57 was sequenced using Illumina and Oxford nanopore sequencing technology. The genome size was 2.44 Mb with the G + C content of 32.76 mol%, and it was composed of one chromosome (2.38 Mb) and three plasmids. Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values were calculated between the most closely-related strain *Staphylococcus warneri* NCTC 11044^T. The result showed that the ANI and dDDH values were 93.88% and 55.1%, respectively. Thus, phenotypic, genotypic, chemotaxonomic and physiologic data suggested that strain SB1-57(= KCTC 43302) is a novel species belonging to genus *Staphylococcus*.

A052

***Brevundimonas* sp. nov. CS-1, Isolated from Cold Spring Water in Cheongsong**

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Changwon National University

Brevundimonas sp. CS1, isolated from cold spring water in Cheongsong-gun, Korea, is Gram-stain-negative, aerobic, motile and rod-shaped. This strain grew on NB, LB, BHI medium, at the temperature of 10–37°C (optimum, 28–32°C) and at 0–4% (optimum, 0–2%) NaCl concentration. Isolated colonies were observed as round, viscous, and clear orange color when incubated for two days at 28°C on nutrient agar plate. Major fatty acids were a summed feature of C_{18:1} ω7c/C_{18:1} ω6c (47.31%) and C_{16:0} (17.61%). Only coenzyme Q-10 was detected as the quinone of this strain. CS1 was oxidase- and catalase-positive and coagulase- and hemolysin-negative. The genome of the CS1 was sequenced using Illumina and Oxford nanopore sequencing technology. The genome size was 3.3 Mb with the G + C content of 66.1 mol%. Average nucleotide identity (ANI) values between CS1 and closely-related strains *Brevundimonas vesicularis* NBRC 12165^T, *Brevundimonas naejangsanensis* DSM 23858^T, *Brevundimonas diminuta* ATCC 11568^T, and *Brevundimonas bullata* HAMBI 262^T were 93.54%, 77.04%, 77.42%, and 77.68%, respectively. Digital DNA-DNA hybridization (dDDH) value between CS1 and the most closely-related strain *Brevundimonas vesicularis* was 56.3%. These comparative ANI and dDDH values are well below grouping of the same species. According to phenotypic, genotypic, chemotaxonomic and physiologic data, strain CS1 (= KCTC 82559) is identified to be a novel species belonging to genus *Brevundimonas*.

A053

A Report on 15 Unrecorded Bacterial Species of Korea in 2021

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In 2021, as a subset study to discover indigenous prokaryotic species in Korea, a total of 14 bacterial strains were isolated and assigned to the phylum Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria. From the high 16S rRNA gene sequence similarity (> 98.5%) and formation of a robust phylogenetic clade with the closest species, it was determined that each strain belonged to each independent and predefined bacterial species. There is no official report that these 15 species have been described in Korea; therefore, 5 strains of the Actinobacteria, 1 strain of the Bacteroidetes, 3 strains of the Firmicutes, and 6 strains of the Proteobacteria are described for unreported bacterial species in Korea. Colony and cell morphology, basic biochemical characteristics, and isolation sources are also described in the description section. Here we report 15 unrecorded bacterial species belonging to 9 orders of 6 class in the Actinomycetia, Alphaproteobacteria, Bacilli, Betaproteobacteria, Cytophagia, Gammaproteobacteria, Thermoleophilia, which were isolated in Korea; 3 strain of the Bacillales, 1 strain of the Bacteroidales, 2 strain of the Burkholderiales, 1 strain of the Cytophagales, 2 strain of the Dermatophilales, 1 strain of the Lysobacterales, 1 strain of the Micrococcales, 1 strain of the Rhizobiales, 1 strain of the Solirubrobacterales, 3 strain of the Sphingomonadales.

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A054

A Novel Bacterium Isolated from Feces of a Corb Shell

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A bacterium was isolated from the feces of Korean corb shell, *Cyclina sinensis*, collected from mud flat of Gochang, Republic of Korea. The isolate aerobically grew at 15–30°C (optimum, 25°C), pH 7.0–9.0 (optimum, pH 8.0), with 0–6% (w/v) NaCl (optimum, 0.5% NaCl). Phylogenetic consensus tree based on 16S rRNA gene sequence revealed that isolated strain was establishing a monophyletic clade with species of genus *Flavobacterium* including *Flavobacterium cucumis* DSM18830^T, *Flavobacterium aquaticum* JC164^T, and *Flavobacterium cheniae* NJ-26^T with sequence similarities of 97.77%, 97.01%, and 96.88%, respectively. The strain was grown in optimal condition of R2A medium for further analysis. The isolate showed oxidase negative, catalase positive responses and does not possess flexirubin-type pigment.

[This work was supported by a grant from the Mid-Career Researcher Program (NRF-2020R1A2C3012797) through the National Research Foundation of Korea (NRF).]

A055

***Polaribacter batillarius* sp. nov., and *Polaribacter pectinii* sp. nov., Isolated from the Gut of Shellfish in the South Korea**

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Three aerobic, motile, Gram-negative, and rod-shaped bacterial strains, designated G4M1^T, SM13, and L12M09^T, were isolated from the gut of various kinds of shellfish collected in the Korean oceans. All the strains grew in optimal conditions; 25°C, pH 7.0–8.0, and 2.0% (w/v) NaCl. According to a phylogenetic analysis based on 16S rRNA gene sequence, these three strains, G4M1^T, SM13, and L12M09^T, belonging to the genus *Polaribacter* in the family Flavobacteriaceae showed the sequence similarity with the *Polaribacter haliotis* RA4-7 (98.68–98.13%) and *Polaribacter litorisediminis* OITF-11 (98.13–97.36%). All the strains contained MK-6 as the predominant menaquinone and iso-C_{15:0} as the major fatty acids. The polar lipids detected in all strains were phosphatidylethanolamine and three unidentified aminolipids. The DNA G+C contents of three strains, G4M1^T, SM13, and L12M9^T, were 31.0%, 30.4%, and 29.7%, respectively. Based on its phenotypic, phylogenetic, chemotaxonomic, and genotypic analyses, strain G4M1^T (= KCTC 82388 = DSM 112372) and L12M9^T (= KCTC 62751 = DSM 112374) were classified in the genus *Polaribacter* as the type strain of a novel species, for which the name *Polaribacter batillarius* sp. nov. and *Polaribacter pectinii* sp. nov. are proposed.

[This work was supported by a grant from the Mid-Career Researcher Program (NRF-2020R1A2C3012797) through the National Research Foundation of Korea, Republic of Korea]

A056

Soil Microbial Co-occurrence Networks Became Less Connected with the Soil Development, in a High Arctic Glacier Foreland

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Glacier forefields provide an example of community assembly of early soil microbiota in primary succession. In this study, we investigated the bacterial and fungal communities in the retreating high-Arctic glacier Midtre Lovénbreen, Svalbard, aiming to understand how the ecological interactions within and between bacteria and fungi lineages change along the chronosequence. We found even though the network structure of bacteria became less connected with soil age, there was no significant trend in overall network co-occurrence of soil microbiota that was specifically related to soil age since the retreat of the glacier. Instead, soil properties that are highly correlated to soil development were accountable for the alternation in-network co-occurrence, while accumulation of total organic carbon (TOC) and the decrease in soil pH weakened the associations within the network of soil microbiota. Our results also indicated the ecological clusters specific to high pH and low TOC conditions played more important roles in the co-occurrence network, while soil pH and TOC were the most influencing environmental factor to the network co-occurrence patterns of bacteria and fungi community respectively. Our results overall addressed the vital role influencing the microbial network co-occurrence patterns of edaphic heterogeneities along with soil development in the glacier primary succession, but not the temporal succession itself.

A057

The Addition of Biochar Improved the Efficiency of Aerobic Composting of Pig Manure without Substantially Changing the Bacterial Community Structure

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A better understanding of the potential mechanisms by which biochar increases composting efficiency is essential for managing composting process and improving compost quality. In this study, metacommunity sequencing technology was used to investigate the effects of biochar on bacterial communities during aerobic composting of pig manure. Our results showed the biochar adding into the composting system significantly increased the absolute abundance of all bacteria phyla without having influences on their community compositions. Although Firmicutes, Bacteroides, Proteobacteria, and Actinomycetes were consistently the most dominant phyla in all composting systems, the results showed the adding of biochar prevented the composting systems from losing the bacterial diversity over time. Adding biochar also significantly increased the number of network connectors of the composting systems compared to control, suggesting more core co-occurrence of bacteria were generated by adding biochar into the composting systems, and therefore a more stable and interacting systems were resulted. Our study overall provides an in-depth understanding of the effects of biochar on the dynamics of bacterial communities during composting processes, providing theoretical support understanding the potential mechanism how biochar increases the composting efficiency.

A058

***Dyadobacter* sp. nov. Isolated from Plastic Dumped Soils**

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A Gram-stain negative, yellow-pigmented, and aerobic bacterium, designated strain U1^T, was isolated from plastic dumped soil, South Korea. Cells of strain U1^T were non-motile rods showing catalase-negative and oxidase-positive activities. Strain U1^T was shown to grow at 10–37°C and pH 6.0–9.0 and in the presence of 0–0.5% (w/v) NaCl. Strain U1^T contained C_{14:0}, C_{16:0}, iso-C_{15:0} and summed feature 3 (comprising C_{16:1} ω6c and/or C_{16:1} ω7c) as major cellular fatty acids (>5%) and MK-7 as the major respiratory quinone. Phosphatidylethanolamine, unidentified aminolipids, and an unidentified lipid were detected as major polar lipids. Strain U1^T was most closely related to *Dyadobacter bucti* QTA69^T with a 97.9% 16S rRNA sequence similarity. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain U1^T formed a distinct phylogenetic lineage within the genus *Dyadobacter*. Based on phenotypic, chemotaxonomic, and molecular features, strain U1^T represents a novel species of the genus *Dyadobacter*. The type strain of *D. pollutisoli* is U1^T (= KACC 22210^T = JCM 34491^T).

A059

Polyphasic Taxonomic Analysis of Two New Species of *Paraburkholderia* Isolated from Park Soil

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Two Gram-negative strains designated MMS20-SJTN17^T and MMS20-SJTR3^T were isolated from a soil sample taken from a public park, and investigated using a polyphasic approach. The 16S rRNA gene sequence analysis indicated that they belong to the genus *Paraburkholderia*, strain MMS20-SJTN17^T being the mostly related to *P. sprentiae* WSM5005^T (96.45% sequence similarity) and strain MMS20-SJTR3^T to *P. tuberum* STM678^T (98.59% sequence similarity). MMS20-SJTN17^T grew at 15–40°C (optimum, 25–30°C) and at pH 6.0–8.0 (optimum, pH 6.0–7.0), whereas MMS20-SJTR3^T grew at 10–40°C (optimum, 25–37°C) and at pH 6.0–8.0 (optimum, pH 6.0). Both strains grew in the presence of 0–1% (w/v) NaCl (optimum, 0%). The predominant fatty acids of MMS20-SJTN17^T were C_{16:0} and C_{19:0} cyclo ω 8c, and those of MMS20-SJTR3^T were C_{17:0} cyclo and a summed feature comprising 18:1 ω 7c and/or 18:1 ω 6c. The isoprenoid quinone of both strains was Q-8. The disagnostic polar lipids of both strains consisted of diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine. On the basis of 16S rRNA genotypic, phenotypic and chemotaxonomic data, each strain should be classified as the type strain of a novel species of the genus *Paraburkholderia*, for which the names *Paraburkholderia alba* sp. nov. (type strain = MMS20-SJTN17^T = LMG 32366) and *Paraburkholderia sejongensis* sp. nov. (type strain = MMS20-SJTR3^T = LMG 32367) are proposed. [Supported by a research grant from the National Institute of Biological Resources (NIBR).]

A060

***Nnibrimonas intestinalis* gen. nov., sp. nov., Isolated from Fish Intestine**Ji-Hye Han¹, Seoni Hwang¹, Kang Seon Lee², and Eui Jin Kim^{1*}¹Microbial Research Department, Nakdonggang National Institute of Biological Resources (NNIBR), ²Department of Bio and Brain Engineering, KAIST

Gram-stain negative, non-motile, ivory colored and designated strain FH7-10^T was isolated from fish intestine. Growth of strain FH7-10^T occurred at 4–37°C (optimum, 20–30°C), at pH 6.0–8.0 (optimum, pH 7.0), with 0–1.0% (w/v) NaCl (optimum, 0%). Phylogenetic analysis based on 16S rRNA gene sequences and phylogenomic analysis revealed that the strain FH7-10^T was assigned to the family *Neisseriaceae*, and its closest relatives are *Vitreoscilla stercoraria* DSM 513^T (94.54%) and *Kingella negevensis* Sch538^T (93.10%). The major cellular fatty acids are summed feature 3 (C_{16:1} ω7c and/or C_{16:1} ω6c), C_{16:0} and summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c). The major respiratory quinone was Q-8, and the polar lipids were phosphatidylglycerol (PG), diphosphatidyl-glycerol (DPG), phosphatidylethanolamine (PE), as well as several distinct aminolipids (AL) and lipids (L). The assembled genome of strain FH7-10^T had one contig with 421.77x genome coverage, total length 3,223,482bp and G + C content 51.34%. On the basis of the differences in the phenotypic, physiological and biochemical characteristics from its known relatives and the results of phylogenetic analyses, *Nnibrimonas intestinalis* gen. nov., sp. nov., belonging to the family *Neisseriaceae* is proposed with type strain FH7-10^T.

A061

Complete Genome Sequence of *Bacillus subtilis* E5 Isolated from Chungkukjang, Korean Fermented Soybean Food

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Bacillus subtilis is a Gram-positive and endospore-forming bacterium in the phylum *Firmicutes*. *Bacillus subtilis* E5 was isolated from Chungkukjang, a traditional Korean fermented food made from soybeans. We selected the strain E5 and sequenced the its complete genome in finding host bacteria for genetic engineering. The genome size of strain E5 is 4,083,441 bp with a G + C content of 43.83%. The genome includes 4,009 protein-coding genes, 86 tRNA genes and 30 rRNA genes. This complete genome sequence data can be useful for comparative genome research, metabolic engineering using *Bacillus* and exploring the metabolism of *Bacillus* species.

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A062

Ent-Penicilerqueinone Suppresses Acetaldehyde-induced Cytotoxicity and Oxidative Stress by Inducing ALDH and Suppressing MAPK Signaling

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Studies on ethanol-induced stress and acetaldehyde toxicity are actively being conducted, owing to an increase in alcohol consumption in modern society. In this study, ent-penicilerqueinone (EPQ) isolated from a Hawaiian volcanic soil-associated fungus *Penicillium herquei* FT729 was found to reduce the acetaldehyde-induced cytotoxicity and oxidative stress in PC12 cells. EPQ increased cell viability in the presence of acetaldehyde-induced cytotoxicity in PC12 cells. In addition, EPQ reduced cellular reactive oxygen species (ROS) levels and restored acetaldehyde-mediated disruption of mitochondrial membrane potential. Western blot analyses revealed that EPQ treatment increased protein levels of ROS-scavenging heme oxygenase-1 and superoxide dismutase, as well as the levels of aldehyde dehydrogenase (ALDH) 1, ALDH2, and ALDH3, under acetaldehyde-induced cellular stress. Finally, EPQ reduced acetaldehyde-induced phosphorylation of p38 and c-Jun N-terminal kinase, which are associated with ROS-induced oxidative stress. Therefore, our results demonstrated that EPQ prevents cellular oxidative stress caused by acetaldehyde and functions as a potent agent to suppress hangover symptoms and alcohol-related stress.

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A063

Isolation and Identification of a Novel *Alteromonadaceae* sp. BrNp21-10

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A Gram-stain-negative, catalase- and oxidase-positive, aerobic, rod-shaped marine bacterium, designated strain BrNp21-10, was isolated from coastal seawater, Boryeong of Korea. Colonies of strain BrNp21-10 on marine agar medium were cream-coloured, circular with entire margin, and had glistening surface. On API 20NE kit test, hydrolysis of esculin, β -galactosidase and assimilation of D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose and malate were positive. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain BrNp21-10 belonged to the family *Alteromonadaceae* and showed the highest 16S rRNA gene sequence similarity with *Neptunicella marina* S27-2^T (94.5%, sequence similarity). The low 16S rRNA gene similarity and physiological difference support the assignment of the strain BrNp21-10 as a novel species into the family *Alteromonadaceae*. [Supported by NRF-2017R1D1A3B04033871.]

A064

***Chryseobacterium panacisoli* sp. nov., Isolated from Ginseng Cultivating Soil with Ginsenoside Converting Activity**

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A Gram-staining-negative, non-motile, non-spore-forming, aerobic, rod-shaped, and yellow-pigmented bacterium, designated strain Gsoil 183^T was isolated from ginseng cultivating soil, in Pocheon province, Republic of Korea. Strain Gsoil 183^T grew at 10–37°C and at pH 5.0–9.0 on TSA. Strain Gsoil 183^T had β-glucosidase activity, which was responsible for its ability to convert ginsenoside Rb₁ (one of the dominant activity components of ginseng) to F₂. MK-6 was the predominant respiratory quinone and the major fatty acids were iso-C_{15:0}, iso-C_{17:0}-3OH, and C_{16:1} ω6c and/or C_{16:1} ω7c (summed feature 3). The polar lipids were phosphatidylethanolamine, six unidentified glycolipids, five unidentified aminolipids and three unidentified lipids. The G + C content of the genomic DNA was 36.6 mol%. Digital DNA-DNA hybridization between strain Gsoil 183^T and reference strains resulted in values below 70%. Strain Gsoil 183^T could be differentiated genotypically and phenotypically from the recognized species of the genus *Chryseobacterium*. The isolate therefore represents a novel species, for which the name *Chryseobacterium panacisoli* sp. nov. is proposed, with the type strain Gsoil 183^T (= KACC 15033^T = LMG 23397^T). [This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) and by a grant from the Korea Research Institute of Bioscience & Biotechnology (KRIBB) Research Initiative Program.]

A065

***Paludibacterium denitrificans* sp. nov., a Novel Denitrifying Bacterium Isolated from Sludge**

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A novel Gram-reaction-negative, facultatively aerobic, motile, non-spore-forming, rod-shaped and denitrifying bacterium, designated dN18-1^T, was isolated from sludge, Republic of Korea. This bacterium was investigated via a polyphasic approach to reveal its taxonomic position. Phylogenetic analysis based on 16S rRNA gene sequence indicated that strain dN18-1^T is belonging to the family *Chromobacteriaceae* and related to the closest species *Paludibacterium purpuratum* KCTC 42852^T (96.2% sequence similarity), *Paludibacterium yongneupense* KACC 11601^T (96.1%) and *Paludibacterium paludis* BCRC 80514^T (95.2%). The average nucleotide identity (ANI) between strain dN-18-1^T and the closely related strains were 72.5–73.1%, indicating that dN18-1^T is a novel species of the genus *Paludibacterium*. It grew at 18–37°C on R2A medium in the presence of 0–2% NaCl (w/v) and at pH 6.0–8.0. Strain dN18-1^T was characterized chemotaxonomically having Ubiquinone 8 (Q-8). The results of physiological and biochemical tests allowed phenotypic differentiation of strain dN18-1^T from other genus *Paludibacterium* species with validly published names. Therefore, an isolate represented a novel species, for which the name *Paludibacterium denitrificans* sp. nov. (type strain dN18-1^T = KACC 19537^T = CGMCC 1.16961^T) is proposed [Supported by grants from NIBR and KIRBB].

A066

***Hanamia caeni* gen. nov., sp. nov., a Member of the Family *Chitinophagaceae* Isolated from Activated Sludge in Korea**

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A novel Gram-stain-negative, aerobic, yellowish-pigmented, non-motile, rod-shaped bacterial strain, designated strain BO-59^T, was isolated from activated sludge of a wastewater treatment plant in Hanam city, South Korea. Phylogenetic study based on the 16S rRNA gene sequence positioned BO-59^T in a distinct lineage in the family *Chitinophagaceae*, sharing less than 92.8% sequence similarity with members of the closely related genera *Ferruginibacter*, *Flavitalea*, *Pseudoflavitalea*, *Flavisolibacter*, *Niastella*, and *Terrimonas*. Strain BO-59^T contained MK-7 as predominant quinone, and iso-C_{15:0}, iso-C_{17:0} 3OH, and iso-C_{15:1} G as major fatty acids (> 10%). The DNA G + C content was 39.1 mol% based on genome sequence analysis. The detected polar lipids of strain BO-59^T were phosphatidylethanolamine, one unidentified aminophospholipid and three unidentified polar lipids. 16S rRNA gene sequence similarity, physiological and biochemical characteristics indicated that strain BO-59^T represents a novel species of a new genus, for which the name *Hanamia caeni* gen. nov., sp. nov. is proposed. The type strain is BO-59^T (= KACC 19646^T = LMG 30865^T).

[This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR)]

B001

Isolation and Screening of Bacteria for Tyrosinase Inhibition from a Tidal Flat of the Yellow Sea

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Overproduction and accumulation of melanin causes skin diseases such as melasma, freckles, and lentigo. Melanin biosynthesis can be reduced by the inhibition of the tyrosinase enzyme, these inhibitors can be employed as skin whitener of cosmetics. It catalyzes the hydroxylation of tyrosine to DOPA first, then tyrosinase catalyzes the oxidation of DOPA to DOPA quinone. Therefore, this study presents newly isolated bacteria inhibiting tyrosinase activity. 149 bacterial strains isolated from a tidal flat of the yellow sea of Korea. Screening of the bacteria was carried out by measurement of tyrosinase inhibitory activity. The reaction mixture comprised each bacterial culture, tyrosinase, tyrosine in sodium phosphate buffer (pH 6.8). The absorption at 490 nm of reaction mixture was measured after incubation at 37°C for 15 min. As a result, six bacterial strains were detected to inhibit tyrosinase activity. Of these, the isolate with the highest tyrosinase inhibition effect was identified as *Bacillus vietnamensis* strain H15a, displaying 99.28% similarity of 16S rRNA sequence. Strain H15a has 54% of tyrosinase inhibition rate for tyrosine and 65% for L-DOPA. Further assays indicated that *Bacillus vietnamensis* strain H15a showed the highest inhibition ability after culture at 37°C for 7 days in Marine broth medium. Additionally identification of specific molecule showing tyrosinase inhibition effect and potential for whitening agent will be more analyzed and discussed.

B002

Bacterial Community and Metabolic Diversity of Mercury-contaminated Sediments from Hyeongsan River, Pohang, South Korea

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This study aimed to explore bacterial community and metabolic diversity and its association with mercury and other environmental parameters in sediments collected at various sites (HSR-1~HSR-6) from Hyeongsan River, South Korea. The data revealed that the highest total mercury (THg) and methylmercury (MeHg) were in HSR-2 with 4,585.3 $\mu\text{g}/\text{kg}$ and 13.4 $\mu\text{g}/\text{kg}$, respectively. The HSR-1 sample showed the lowest THg (31.9 $\mu\text{g}/\text{kg}$) and MeHg (0.1 $\mu\text{g}/\text{kg}$). The sulphate and organic matter (OM) showed a positive relationship with THg and MeHg but affect negatively to bacterial and metabolic diversities. Both the bacterial and metabolic diversities were also negatively affected by THg and MeHg concentrations. The phylum Proteobacteria was upscaled and revealed a significant positive association with THg, MeHg, and OM in HSR-2 to HSR-6 samples. The bacterial genera *Sulfurovum* and *Sulfurimonas* were predominantly reported in HSR-2~HSR-6, whereas *Ilumatobacter*, *Methylotenera*, *Nevskia*, and *Sediminibacter* were only detected in HSR-1. The physiological analysis showed the highest average well colour development in HSR-1 and the lowest in HSR-2. In conclusion, these data revealed the inhibitory effects of THg, MeHg, and other environmental variables on microbial communities and metabolic diversities.

[Supported by Korea Environmental Industry & Technology Institute (1485017197) and National Institute of Environmental Research (NIER-2020-04-02073), funded by the Ministry of Environment, Republic of Korea.]

B003

Identification of Anaerobic Styrofoam (Polystyrene) Biodegradation Pathway in Gut of Mealworm

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Until present, several studies have been reported that the waste styrofoam (polystyrene) could be biologically degraded by aerobic metabolism between several microbial communities in the environment. However, only limited information on their biodegradation pathway under anaerobic conditions has been known. To identify the possible anaerobic biological degradation pathway of styrofoam in the gut of mealworms, metagenomics-based functional gene identification has been performed under styrofoam-fed conditions. The mealworms were grown under continuously styrofoam supplemented conditions and the styrofoam degradation efficiency by mealworm were monitored every five days. Based on the styrofoam degradation efficiency, the degradation phases were defined and the debris samples in each phase were collapsed. Meta-community DNAs were directly extracted and used for the shotgun metagenome sequencing. The microbial community compositions showed the enrichment patterns of *Enterobacter* species in response to the styrofoam feeding time. Moreover, the functional gene involved in the polystyrene degradation metabolic pathway also showed different distribution patterns in each styrofoam degradation phase. These results suggested that the specific microbial communities and their functional genes were enriched by the styrofoam supplementation to the mealworm, and might be significantly involved in the anaerobic metabolic pathway of styrofoam.

B004

Seasonal Variation and Diversity of Diatoms Community in Han River, South Korea Revealed by 18S rRNA Molecular Profiling

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Diatoms have been used to investigate environmental changes and water quality in freshwater systems. In the present study, seasonal molecular profiling of the Han River in Korea was analyzed based on hypervariable regions of 18S V1-V3 rRNA and pyrosequencing. Physicochemical data including temperature, DO, pH, and nutrients showed typical seasonal patterns. In addition, high levels of cell count and chlorophyll-a have been recorded in spring compared to other seasons, which is attributed by the diatom bloom. Metagenomics data revealed a seasonal variation in the phytoplankton community composition. Diatoms were predominantly detected in spring (83.8%) and winter (69.7%). Overall, *Stephanodiscus*, *Navicula*, *Cyclotella*, and *Discostella* were the most frequent in the Han river. However, an unknown *Thalassiosirales* diatoms were also found in spring (35.5%) and winter (36.3%). The molecular profiling of Han river showed diverse diatom taxa compared to morphological observations. This is the first study of diatoms in the Han River through a molecular approach.

B005

Communities of Free-living and Particle-associated Bacteria Change Depending on the Growth Phases of *Tetraselmis suecica*

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Bacteria are remarkably associated with marine green algae *Tetraselmis* which are well known for a feed source in aquaculture, however characterize of *Tetraselmis* associated bacterial community is insufficient. We investigated the composition of free-living (FLB) and particle-associated (PAB) bacteria community coexisting with *Tetraselmis suecica* (P-039) in each growth phase (lag, exponential, stationary, and death) using pyrosequencing. The shared operational taxonomic units (OTUs) percentage between FLB and PAB communities was high ($\geq 92.4\%$), but their compositions were significantly different ($p = 0.05$). The PAB community was more diverse than the FLB community in accordance with the growth phase, and the proportions of *Marinobacter* and *Flavobacteriaceae* were considerably varied depending on *T. suecica* cell density. There were no significant differences in the FLB community composition. These imply that the PAB community may have more relation to the growth of *Tetraselmis* than the FLB community. Genus *Muricauda* and *Roseobacter* clade were dominant in both FLB and PAB regardless of the growth phase. The result also suggest that *T. suecica* co-existing bacterial communities may affect the growth of plankton and potentially enhance the *T. suecica* growth.

B006

Evaluation of Bacterial Diversity in the Feces of Hanwoo Heifers Fed Different Diets

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Division of Animal Science, College of Agriculture and Life Sciences, Chonnam National University

The objective of this study is to analyze differences in fecal microbiomes of Korean native Hanwoo heifers fed different diets at the same farm: 1) 11 breeding Hanwoo heifers (BHH) fed 37.5% oat hay plus 62.5% TMR, and 2) 11 fattening Hanwoo heifers (FHH) fed 100% TMR. From the 22 heifers, fresh fecal samples were collected and then used to extract total community DNA. The composition of fecal microbiome was analyzed using 16S rRNA gene amplicon sequencing. *Firmicutes* and *Bacteroidetes* were the dominant phyla in all 22 fecal samples in both diet groups. *Firmicutes* was more abundant ($p < 0.05$) in the FHH diet group than in the BHH diet group. At the genus level, *Butyrivibrio*, *Oscillibacter*, and *Bacteroides* represented more than 1% of the total sequence. *Oscillibacter* and *Bacteroides* were more abundant ($p < 0.05$) in the BHH diet group than in the FHH diet group whereas *Butyrivibrio* was more abundant ($p < 0.05$) in the FHH diet group than in the BHH diet group. Alpha diversity indices did not differ ($p > 0.05$) between the two diet groups. The principal coordinate analysis plot revealed that the overall composition of fecal microbiome was different between the two diet groups. This study demonstrates that the composition of fecal microbiome in Hanwoo heifers is greatly influenced by different diets.

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B007

The Impact of the Rumen Microbiome on Feed Efficiency in Growing Hanwoo Steers

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The objective of this study is to determine if the rumen microbiome has an association with feed efficiency in growing Hanwoo steers. A total of 64 growing Hanwoo steers were fed the same TMR diet at the same farm. Among the 64 steers, 32 steers including the top 25% feed efficiency group (n = 16) and the bottom 25% feed efficiency group (n = 16) were selected. Fresh rumen fluid samples were collected using the stomach tubing method, and then total community DNAs were extracted. The composition of rumen microbiome was analyzed using 16S rRNA gene amplicon sequencing on the MiSeq platform followed by bioinformatics analysis with the QIIME software package. Firmicutes and Bacteroidetes were the dominant phyla in all samples in both feed efficiency groups. These two phyla did not differ between the two feed efficiency groups ($p > 0.05$). At the family level, *Succinivibrionaceae* was more abundant ($p < 0.05$) in the top 25% group than in the bottom 25% group. At the genus level, *Aminipila*, *Hungateiclostridium*, *Lutispora*, and *Anaeroplasma* were more abundant ($p < 0.05$) in the bottom 25% group than in the top 25% group. This study demonstrates that the rumen microbiome is associated with feed efficiency in Hanwoo steers and manipulation of rumen microbiome may improve feed efficiency in Hanwoo steers.

[This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2020R1A2C101118812)]

B008

Evaluation of *In Vivo* Rumen Microbiota and *In Vitro* Rumen Fermentation Characteristics between Hanwoo and Jeju Black Steers Under the Same Dietary Condition

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The objective of this study is to assess differences in *in vivo* rumen microbiota and *in vitro* rumen fermentation characteristics of Hanwoo and Jeju Black steers under the same dietary condition at the same farm. The rumen fluids were obtained from 3 Hanwoo and 3 Jeju Black steers and used to analyze rumen microbiota using 16S rRNA amplicon sequencing. *Ruminococcus* was more abundant ($p < 0.05$) in Hanwoo steers than in the Jeju Black steers. The same rumen fluids were used to determine *in vitro* rumen fermentation characteristics and microbiota. After 24 h of incubation, the pH was lower ($p < 0.05$) in Hanwoo steers than in Jeju Black steers while DM digestibility, total gas production, total VFAs and NH₃-N production were greater ($p < 0.05$) in Hanwoo steers than Jeju Black steers. The Jeju Black steers had greater A:P ratio than Hanwoo steers. After 24 h of incubation, the PCoA plot revealed that the overall composition of rumen microbiota differed ($p < 0.05$) between Hanwoo and Jeju Black steers. This study demonstrates that rumen inocula of different breeds include different predominant microbes that affect *in vitro* fermentation characteristics and rumen microbiota.

[Supported by a grant (715003-07) from the Research Center for Production Management and Technical Development for High Quality Livestock Products through Agriculture, Food and Rural Affairs Convergence Technologies Program for Educating Creative Global Leader, Ministry of Agriculture, Food and Rural Affairs.]

B009

Isolation and Characterization of the Fungi Strains that have High Activity of Enzyme for Taste Enhancement of Soy Sauce

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Amino acids and low molecular weight peptides are key ingredients that impart a rich taste in soy sauce. In order to increase the ingredient, fungi with high activity of enzyme should be used as Meju starter. Generally, *Aspergillus oryzae* is used as a starter in the industrial field. But soy sauce made at the factory is limited to making abundant taste compared to traditional soy sauce that various microorganisms were inoculated from the atmosphere.

In the present study, 132 strains of fungi; 59 strains of *Scopulariopsis brevicaulis*, 10 strains of *S. candida*, 63 strains of *Penicillium solitum*; were isolated from Traditional Meju. The Isolated strains were assayed for 8 kinds of enzyme and four strains were selected for manufacturing soy sauce. *S. brevicaulis* M2504, M1180, and M0548 show high activity in Protease, Carboxypeptidase, Aspartyl aminopeptidase, and Glutaminase. And the soy sauce made from these strains have a high content of amino acid and total nitrogen. *Penicillium solitum* M0758 shows high activity of Protease and Gamma-glutamyl transferase. The soy sauce made from this strain has a high content of amino acid and total nitrogen. In particular, each *S. brevicaulis* M2504 and *P. solitum* M0758 have an excellent ability to produce taste components. These strains can contribute to the rich taste of soy sauce.

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B010

Pharmaceutical and Personal Care Products Removal and Microbial Community Analysis in a Long-term Operation of Fungal Rotating Bioreactor

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Fungal enzymatic system have attracted rising attention as the novel regulator of pharmaceuticals and personal care products (PPCPs), yet there are still limitations to apply fungal system to continuous treatment. In this study, we operated a rotating fungal reactor for the removal of PPCPs, coupled with solid-state fermentation of ash chips and *Trametes versicolor*. Acetaminophen (ACN), bisphenol A, and carbamazepine were spiked in the synthetic wastewater. Overall, the fungal reactor showed higher removal efficiency compared to the control. ACN was completely removed until day 18, then became be accused and reached half of the removal at day 30. After fermented products were newly replaced, the removal of ACN was retrieved and maintained in the reactor, although laccase decreased faster. Given that reduction of ACN was observed from control, we theorized that later-settled microorganisms played roles in regulating reactor performance. Using amplicon sequencing, we observed the temporal succession of both fungal and bacterial populations. Above 75% of the fungal community from FR30 was maintained with Agaricomycetes (class of *T. versicolor*). NMDS plots and corrplots showed that the structure of communities differed significantly by the properties related to bacteria. This study provides an analysis of microbial dynamics in fungal reactor and warrants further study on transcript sequencing.

[This study was supported by grants from NRF (2017R1D1A3B03029787).]

B011

Phenotypic Response of Antibiotic Treatment in *Staphylococcus aureus* NCTC 8325-4 Using Raman Spectroscopy and Flow Cytometry

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Antibiotic resistance is one of the biggest health problems in the worldwide. To prevent the emergence of multidrug-resistant bacteria, antibiotic misuse should be reduced with accurate prescriptions through rapid diagnosis. Therefore, a need exists for a rapid, label-free technique to identify drug resistant bacterial strains and quickly predict the mode of action of new antibiotics. Antibiotic used in this study included nine antibiotics (seven antibiotics classes and four mode of actions) and treated with minimal inhibitory concentration in *Staphylococcus aureus* NCTC 8325-4. We collected culture samples every six hours for measuring single cells via Raman spectroscopy and flow cytometry. Discriminant analysis of single cell Raman spectra revealed that treatment groups differed from the control after six hours, and that spectral features were well clustered according to the antibiotic types or mode of actions after 12 h. Diversity of phenotype were decreased over time. Our results discovered the characteristics peaks and phenotype responses under antibiotic treatment and provide a database for rapid prediction of mode of action and confirmation of exposure characteristics.

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B012

Carbon Substrates in Resuscitation Process Lead to Phenotypic Plasticity

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Dormant status is a survival strategy, which is widely observed in a subpopulation of bacteria due to insufficient nutrient in environment. These dormant cells can be resuscitated by environmental change and plays critical roles in microbial diversity, soil microbiome assembly and ecosystem functionality. Despite the importance, our understanding of phenotypic plasticity of resuscitated cells remains in its infancy. In this study, we measured the phenotypic change of dormant and awaked *E. coli* K-12 according to the carbon substrate. The phenotypic change was measured using SEM, flow cytometry, Raman microspectroscopy and RNASeq. The result of SEM and flow cytometry showed that the size of awaken cells increased compared to the dormant cells. The viability (FITC/PE) also increased with the concentration of the carbon. As a result of the Raman spectrum, a significant increase in the lipid was observed under the resuscitation conditions excluding sucrose. RNASeq analysis reveals that genes related to transcription were significantly expressed under all resuscitation conditions. Genes related to the iron transport system were significantly expressed in low carbon substrate condition, and genes related to respiration were expressed in the NB. These results suggest that the effects of dormant bacteria on the soil microbiome assembly may vary depending on the type and concentration of the carbon substrate.

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B013

Interaction Analysis System by Co-cultivation: The Difference in Staphylococcus Species Leading to Phenotypic Plasticity of Malassezia

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The skin is colonized by complex microbial communities, most of which are related to various skin diseases. However, few mechanisms of inter-kingdom interactions maintaining the homeostasis of the skin microbiome have been identified. Here, we assess the effect of the inter-kingdom interaction by investigating phenotypic plasticity with Raman spectroscopy, Flow cytometry, and RNASeq. We used the predominant bacterial species *Staphylococcus (aureus and epidermidis)*, and one predominant fungal species *Malassezia restricta*. All strains were cultured in modified Dixon media under axenic and co-culture conditions using Transwell plates. The growth and the viability of *Staphylococcus* strains significantly increased under the presence of *M. restricta*. This shows the effect of chemical interaction with neighboring fungi on bacterial growth and viability. Raman spectroscopy has verified that co-culture is a key factor in determining the phenotypes of *S. epidermidis*, but incubation periods are a key factor of *S. aureus*. Similarly, RNAseq results during co-culture show that *S. epidermidis* had more gene expression changes than *S. aureus*. We also observed the *M. restricta* have followed the phenotypic determinant of co-cultured *Staphylococcus* strains. These findings suggest that *M. restricta* responds passively to chemical interactions with *Staphylococcus* and causes phenotypic plasticity depending on the *Staphylococcus* strains. [Supported by grant from NRF (2019R1A4A1024764).]

B014

Characteristics of *Campylobacter jejuni* Isolates Recovered from Processing Stages of the Chicken Slaughterhouses in South Korea

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Animal and Plant Quarantine Agency

Campylobacter jejuni is a major pathogen of Campylobacteriosis, which causes a gastrointestinal disease in human. In particular, handling and consumption of chicken meat are the major sources for infection. We investigated the prevalence and characteristics (the genetic diversity, antimicrobial resistance, and the distribution of virulence genes) of *C. jejuni* throughout the slaughter process at three slaughterhouses in 2020. At these slaughterhouses (S1, S2, and S3), the prevalence of *C. jejuni* was 80–100% at carcasses after defeathering, chilling water, and final carcasses in S1, 33.3–100% at feces from crates, shackles, carcasses after evisceration, and carcasses after washing in S2, and 10–66.7% at feces from crates, shackles, and final carcasses in S3. In total, 57.1% of the isolates were resistant to quinolone, and all isolates had *cdtA*, *cdtB*, and *cdtC*, while *flaA* only in S2 and S3, and *wlaN* in S3 were detected. Characterization by multi-locus sequence typing indicated that specific genotypes were consistently found during the slaughtering process and some of these also contaminated final chicken carcasses. This study showed that sequential transmissions of *C. jejuni* contamination occurred through the chicken slaughtering line. In conclusion, for *C. jejuni* control, monitoring and sanitization at multiple steps of the slaughtering process should be conducted.

[Supported by grants from Animal and Plant Quarantine Agency.]

B015

Microbial Spatial Footprint Drives the Aerosol Bacterial Diversity in PM_{2.5}

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Geophysical regions are inhabited by unique indigenous microbial communities and the microorganisms can migrate by wind. Given this, spatial variation of the atmospheric microbial community became a rising research topic. With speculating that bacterial taxa also can be an indicator for predicting the spatial history of PM_{2.5}, machine learning classifier was applied. Total of 42 PM_{2.5} samples were collected in Yong-in, South Korea and the bacterial diversity and physicochemical properties were investigated. The transporting pathways of PM_{2.5} were identified by backward trajectory (BT) analysis and were divided into three clusters, North (N), Southwest (S), and Others (O). The N and S were inferred to be transported through the inland and oceanic region, respectively. Chemical properties also seemed to reflect the spatial characteristics of each cluster. Bacterial diversity was significantly higher in the S, and it is considered that this is derived from the heterogeneous origin of S. Among the observed bacterial taxa, the phylum *Planctomycetes* and *Acidobacteria* was predominant in the S and N, respectively. These implied that the bacterial community structure varied in accordance with spatial characteristics of PM_{2.5}. Random forest model further demonstrated that the microbial community is the effective factor to predict BT-clusters, suggesting that the phylogenetic information of the bacterial community can be used as an indicator to deduce the spatial history of the aerosol.

B016

Shift in Benthic Bacterial Communities Associated with Farming Stage and a Microbiological Proxy for Assessing Sediment Conditions Associated with Sulfur Cycles in Fish Farm

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Shift in benthic bacterial communities in accordance with the farming stages was investigated to assess the farming-induced sediment pollution. The sulfate reduction rate (SRR), concentrations of acid volatile sulfide (AVS) and H₂S were significantly higher during mid-and post-stages than at early-stage, which indicated that the impact of aquaculture persists for a while after harvesting. *Firmicutes* and *Bacteroidetes* related to fermenting bacteria, and complete organic carbon oxidizing sulfate-reducing bacteria (CO-SRB) belonging to *Desulfobacteraceae* were prominent during mid-and post-stages, whereas incomplete oxidizing SRB (IO-SRB) affiliated with *Desulfobulbaceae* appeared dominant during early-stage. The results suggest that shift in bacterial communities from IO-SRB to CO-SRB occurs in highly anoxic and sulfidic sediment conditions. *Sulfurovum*-like sulfur-oxidizing bacteria showed highly positive correlation with H₂S (R² = 0.99), AVS (R² = 0.85), and SRR (R² = 0.82) during mid-and post-stages, proving it as a relevant microbiological proxy that reflects the sulfidic conditions of the fish farm.

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B017

Discovery of a Lichen-forming Fungus *Stereocaulon alpinum* Producing Lobaric Acid and Its Antibacterial Activity against Phytopathogenic Bacteria

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Natural products from lichens have been used in folk medicine to treat various diseases and ailments. However, utilization of novel lichen metabolites in agriculture, pharmacy and industry has been hampered by the difficulty in obtaining large amounts of secondary metabolites from the slow-growing lichens. In this study, we profiled the chemical constituents of mycobionts isolated from *Stereocaulon alpinum* thalli. For the first time, we discovered a lichen-forming fungus producing lobaric acid in axenic culture. The lichen-forming fungus was cultured on different growth media to investigate the growth conditions suitable for lobaric acid production. We tested the purified lobaric acid and three other lichen acids – usnic acid, lecanoric acid, and vulpinic acid – for antibacterial activities against four phytopathogenic bacteria. *S. alpinum* cultured in malt extract broth (MEB) for one month produced the greatest amount of lobaric acid. However, exogenous influences such as the change in osmotic pressure, and the addition of trace elements lead to the biosynthesis of various metabolites. The lichen acids except lecanoric acid showed antibacterial activity against *Clavibacter michiganensis* subsp. *michiganensis*, a Gram-positive plant-pathogen that causes tomato canker. Our study confirms that exogenous influences can affect the biosynthesis of lichen metabolites, and that lichen acids can inhibit only Gram-positive bacterial pathogens.

B018

Changes of Methanotrophic Community Structure in Rice Paddy Soil Enrichments Based on Concentrations of Methane and Copper (II)

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Methanotrophs are organisms that consume methane, a potent greenhouse gas, as their sole source of carbon and energy, carrying out indispensable functions in the global carbon cycle. These microorganisms have been widely studied for potential biotechnological applications. Interestingly, copper, as well as methane, plays vital and decisive roles in the physiology and activity of methanotrophs' communities. This study explored their structures through rice paddy soil. The soil is enriched in fed-batch bioreactors supplying six arbitrary conditions. Growth curves, methanotrophic communities, and functional genes of *pmoA* and *mmoX* are monitored using QIIME2 with amplicon sequencing and using Hidden Markov model (HMM) conducted megahit with shotgun sequencing. While the predominant methanotrophic group at low methane level is distributed in alphaproteobacterial genus *Methylocystis* sp. regardless of copper conditions, there are fluctuations at the high concentration. There seem to be differences in the communities due to the changes in the concentration of methane and copper. The isolates from each enrichment become a piece of evidence for their high abundance matched perfectly to the community analysis. Herewith, we demonstrate what kinds of methanotrophs are predominant with sMMO or pMMO in different conditions, what conditions make alphaproteobacteria dominant related copper, and how to even change predominant groups from gammaproteobacteria to alphaproteobacteria or vice versa.

B019

Microbial Production Regulates the Activity of Insect Olfactory Receptor

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The olfactory nervous system recognizes and distinguishes many different chemicals in the general living environment. Insects have evolved a group of odorant-gated ion channels composed of highly-developed olfactory receptors capable of distinguishing and distinguishing between various chemicals with symbolic or evasive specificities. Recently, aphid genomes related to olfaction, including olfactory receptors and proteins, have been identified and olfactory receptors have been reported that are differentially differentiated from *Drosophila*. The genome of the olfactory receptor has a very conservative sequence and a systematic signaling system. A representative receptor, odorant-gated ion channels comprised of a highly conserved co-receptor (Orco) has a homotetramer channel structure with four subunits arranged symmetrically around the central hole. In this study, whole cell voltage clamp recording was performed with cell expression system of OR85b gene, which is a subtype of olfactory neuro-receptor isolated from *Drosophila*. Therefore, it is possible to identify attractant or repellent substance using the olfactory receptor activity regulating system of insects. Through this study, MZ01 shows the attracting phenomenon by activating insect receptor OR85b, the results of the scientific analysis of the performance of the extracts are presented.

B020

Study of Excitatory GABA-gated Channel and Microbiome

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The UNC-49 and EXP-1 receptors are a unique nematode γ -aminobutyric acid (GABA) gated chloride channel that may prove to be a novel target for the development of nematocides. Here we have characterized various charged amino acid residues in and near the agonist binding site of the UNC-49 receptor from the parasitic nematode. Utilizing the *Caenorhabditis elegans* crystal structure as a template, a model was generated and various charged residues were investigated based on their location and conservation. These residues may contribute to structure, function, and molecular interactions with agonists. Results of the mutational analysis and molecular simulations suggest that some residues may be interacting with nematocides by an ionic interaction that may be crucial for general GABA receptor function. Here we show that GABA mediates enteric muscle contraction in the nematode *Caenorhabditis elegans* via the EXP-1 receptor, a cation-selective ligand-gated ion channel. The EXP-1 protein resembles ionotropic GABA receptor subunits in almost all domains. In the pore-forming domain of EXP-1, however, the residues that confer anion selectivity are exchanged for those that specify cation selectivity. We have used the results from this study as well as knowledge of residues involved in GABA receptor binding to identify sequence patterns that may assist in understanding the function of lesser known GABA receptor subunits from nematodes.

B021

Exploring DNA Virome in Fresh Vegetables

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Viruses are the most abundant biological entities on Earth, impacting bacterial evolution and diversity in natural environments such as marine and human gut. Viral infection promotes host fitness, as well as destroys bacterial cells, resulting the alternation on microbial communities. The plants, including fresh vegetables, harbor a large number of bacterial cells on their leaf surfaces, and these phyllosphere microbiota consistently interact with host plants and the surrounding environments. While studies on viruses associated with fresh vegetables have been focused on particular pathogens, attention to viruses as a member of the phyllosphere microbiota of fresh vegetables is largely lacking. Here, we examined diversity and structure of uncultured DNA viral communities on six different types of leafy green vegetables (broccoli, perilla, spinach, green lettuce, red lettuce, and romaine) using virus-like particle enrichment and shotgun metagenome sequencing. We assembled contigs with Illumina sequencing reads of viral metagenomic DNAs, and identified putative viral contigs. The viral contigs were further clustered into viral taxonomic units on the basis of genome similarity. This study demonstrated uncharacterized viral population on fresh vegetables, which expands our knowledge of viral diversity in plant and food ecosystems. [Supported by the National Research Foundation of Korea grant funded by the Ministry of Science and ICT (2019R1C1C1009664).]

B022

Characterization of the DNA Virome in Fermented Vegetables, Kimchi and Sauerkraut

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Bacteriophages hold a dominant position in the microbial world. They possess potential power to modulate the evolution and ecology of host bacteria. Metagenomics made it accessible to characterize the unknown viral world, and a variety of natural environments, including marine and human gut, have begun to be actively studied in recent years. The fermented foods made by bacterial fermentation are considered as a microbial rich environment, of which, Kimchi and Sauerkraut made with cabbage are fermented vegetables popularly consumed in East Asia and Europe, respectively. Compared with bacterial communities, viral communities of the fermented foods have not been fully characterized. Thus, we investigated the ecology of viral communities in two types of fermented vegetables, Kimchi and Sauerkraut. We collected food samples from three types of starter and non-starter Kimchi, and three types of Sauerkraut, and obtained viral metagenomics datasets using virus-like particle enrichment and whole-genome sequencing. Viral communities of the Kimchi were more diverse than those of Sauerkraut. The composition of viral communities was largely different between Kimchi and Sauerkraut, and varied by produce type within each fermented vegetables. Our study provides in-depth knowledge of uncharacterized viral communities in fermented vegetables.

[Supported by the National Research Foundation of Korea grant funded by the Ministry of Science and ICT (2019R1C1C1009664).]

B024

Investigation of Coastal Water Microbiomes during a Spring Algal Bloom for the Inference of Potential Interaction between *Prorocentrum* and Surface Water Microbiome

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Coastal harmful algal blooms (HABs) have undesirable consequences to marine ecosystems and local industries. Nitrogen and/or phosphate is often regarded as the major culprits of increasing frequency and intensity of the coastal HAB; however, fundamental understanding is lacking as to the causes and mechanism of bloom formation. In this study, we interrogated the microbiomes of surface water samples collected at two neighboring segments of East China Sea that contrast greatly in terms of the intensity and frequency of *Prorocentrum*-dominated HAB. A conspicuous feature of the microbiomes at the sites characterized with high trophic state index and eukaryotic algal cell counts was disproportionate proliferation of *Vibrio* spp., and their domination of the functional genes attributable to the dissimilatory nitrate reduction to ammonia (DNRA) pathway significantly enriched at these sites. Co-occurrence network analysis performed with the core prokaryotic microbiome supported that the observed proliferation of *Vibrio* and HAB may be causally associated. The findings of this study suggest an unidentified association between *Vibrio* proliferation and the *Prorocentrum*-dominated HAB in East China Sea, and opens a discussion regarding a theoretically unlikely, but still possible, involvement of *Vibrio*-mediated DNRA in *Vibrio*-*Prorocentrum* symbiosis. Further experimental substantiation of this supposed symbiotic mechanism may prove the potential mechanism between microbiome and algae.

B025

Inhibitory Influence of Nitrous Oxide on Nitrite Reduction in Oxic-to-anoxic Transition

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Recently, dissimilatory nitrate/nitrite reduction to ammonium (DNRA) has attracted attention as a nitrogen retention path to reduce nitrogen loss caused by denitrification. As mitigation of N₂O emission and retention nitrogen source are the one of the major environmental issues, DNRA bacteria possessing *nosZ* have emerged as a key solution of these “nitrogen dilemma”. In this study, NO₂⁻ reduction were mainly monitored in *Bacillus* sp. DNRA2 which had been isolated and had possessed both *nrfA* and *nosZ*. According to the previous study, *Bacillus* sp. DNRA2 had produced nitrous oxide as byproduct which had been observed in acetylene amended culture. To see the influence of nitrous oxide on DNRA, nitrous oxide amended culture and acetylene amended cultures were conducted. 5% of oxygen were added for setting the oxic-to-anoxic transition environment. The rates of NO₂⁻ reduction in nitrous oxide and acetylene amended cultures were 5 times slower and significant amount of nitrous oxide were produced in acetylene amended cultures. Although it was hard to clarify the factor of slower reduction between acetylene and N₂O, acetylene were excluded from the list of possible inhibitor by acetylene control experiment. As oxic-to-anoxic transition were repeated, the nitrite reduction in acetylene and nitrous oxide amended cultures were getting slower. According to the results, we proposed that N₂O inhibit *nrfA* transcription in oxic-to-anoxic transition condition.

[Supported by grants from NRF.]

B026

Biosorption of CR Dye by *Bacillus* sp. SRCM 120569 Isolated from Korean Turbid Rice Wine (Makgeolli)

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In this study, we investigated the biosorption characteristics of SRCM 120569 biomass on anionic congo red (CR) dye. SRCM 120569 strain isolated from Korean turbid rice wine (Makgeolli), which was identified by 16S rRNA sequence analysis as *Bacillus* sp. SRCM 120569 showed superior CR dye biosorption capacity in aqueous solution. Maximum adsorption capacities 61.2 and 133.1 mg/g were obtained at pH 3.41 and 0.01 g/50 ml dried cell dosage, respectively. The CR dye adsorption properties by *Bacillus* sp. SRCM120569 strain were characterized by Fourier transform infrared spectroscopy (FT-IR), point of zero charge (pH_{pzc}) analysis, and phylogenetic analysis. FT-IR analysis result suggest that -OH, amide C=O, secondary amine, S=O, and C-N are involved in CR dye biosorption. The biosorption isotherm and kinetic models are well described with Langmuir adsorption isotherm and pseudo-second-order kinetic models. These results suggest that the *Bacillus* sp. SRCM120569 biomass might be a promising bio-absorbent for the removal CR dye in aqueous solution.

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B027

Overexpression of Putative Acetyltransferases from *Salmonella* Phages

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Bacteriophages infecting host microorganisms control the host metabolism through diverse mechanisms. During marine phage studies a acetyltransferase was found on a *Celeribacter marinus* phage. Screening acetyltransferase genes, acetyltransferase genes from a *Salmonella* Typhimurium-infecting phage SPN3US were cloned and expressed in *E. coli* system. One of unknown type of acetyltransferase were purified and overexpressed for a subsequent crystallization study. The crystals diffracted X-ray to 2.2 Å resolution, revealing that the crystals belong to $P6_122$ or $P6_522$ with unit cell parameters of $a = 68.5$ and $c = 219.0$ Å. We are now raising the selenomethionine-substituted crystals to obtain the phase information. The phase protein structure will provide molecular hints on how the phage hijacks the bacterial host metabolism.

B028

Expanded Kinetics Analysis of Ammonia-oxidizing Microorganisms

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Nitrification is a core process in the biogeochemical nitrogen (N) cycle. The ammonia oxidation is the first step of nitrification which is catalyzed by AOB (ammonia-oxidizing bacteria), AOA (ammonia-oxidizing archaea), and comammox (complete ammonia oxidizers). Substrate kinetics are considered a major niche-differentiating factor between these guilds, but only limited AOA strains have been characterized kinetically. Here, we determined ammonia-oxidizing kinetics of AOA strains from various habitats using microrespirometry system and compared them to the respective parameters of the comammox, and published values of various AOB strains. Our results revealed surprisingly large differences in substrate affinities ($K_{m(app)}$) among AOA, contrasting with previous assumptions that AOA have higher substrate affinities than comammox or AOB counterparts. Therefore, each ammonia oxidizer's different role in various ecosystems is suggested. Furthermore, the substrate affinity of ammonia oxidizers is related to their cell size, especially the surface area to volume ratios. Moreover, the effect of pH on the affinity was similar between all ammonia oxidizers, which could support the hypothesis that ammonia, not ammonium, is the substrate for the ammonia monooxygenase enzyme of all ammonia oxidizers. Together, our results will help understand the different niches of ammonia oxidizers and provide a solid basis for establishing testable hypotheses on competition in each ammonia oxidizer.

B029

Endolichenic Fungal Community Analysis Using Pure Culture Isolation and Metabarcoding: a Case Study from *Parmotrema tinctorum* in South Korea

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Lichen is known as a symbiotic mutualism of mycobiont and photobiont, harboring diverse organisms including endolichenic fungi. The endolichenic fungi (ELF) resemble endophytic fungi and comprise various taxa. Despite the taxonomic and ecological significance of ELF, there has been no investigation of ELF community combining isolation of a pure culture and high-throughput sequencing. Thus, we analyzed the ELF community in *Parmotrema tinctorum* utilizing culture and metabarcoding simultaneously. Overall, alpha diversity of the ELF communities was notably higher in metabarcoding than in culture-derived result. Taxonomic proportion of the ELF communities observed in metabarcoding and culture showed remarkable difference. Sordariomycetes was the dominant fungal class in culture-based analysis, while the Dothideomycetes was the most abundant in metabarcoding analysis. Thirty seven OTUs were commonly observed in two analyses and they showed difference of relative abundance from two approaches. The similarity of the ELF community was different for each lichen segment and thallus in metabarcoding result. Finally, we provide a basic guideline for further metabarcoding study of ELF via a relationship assay between the number of lichen segment and saturation pattern of OTU richness and sample coverage.

B031

Analysis of Bacterial Communities on the Surface of Blood Spots Exposed in Basements and Darkrooms over Time

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Metagenomics DNA recovered from the environment is capable of identification of sample types and geographic locations, as well as post-mortem interval (PMI) identification. A study was conducted to determine whether it is possible to identify the bacterial communities on the blood spot surface exposed to the environment over time. Blood spots of one male and one female were exposed in two places. Bacterial communities were analyzed by next-generation sequencing after 1, 3, 5 days and 1 and 3 months in spring and summer. Taxonomic biomarkers were confirmed by analysis with the Kruskal-Wallis H test between groups based on time lapse. Through the phylum level bacterial communities of all samples, two locations were clearly identified. Although the results of the bacterial communities at the phylum and species level in the time lapse analysis were not clear. The Kruskal-Wallis H test showed specific bacteria strains over time. At the species level, 3 bacteria at 1 day, 7 bacteria at 3 days, 4 bacteria at 5 days, 11 bacteria at 1 months and 11 bacteria at 3 months were identified. These results are limited to two places, and the results of various environments are lacking. However, despite these limitations, it suggests the possibility of time lapse identification with specific bacteria, and the possibility of application as a forensic identification tool was confirmed through analysis of more places.

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B032

Induction of Motility in *Salmonella* Gallinarum Isolated from Chickens in Korea

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Animal and Plant Quarantine Agency

Salmonella Gallinarum causes fowl typhoid in poultry. This organism is a nonmotile and host-adapted serovar of *Salmonella*. However, it has been reported that its motility can be induced under special media with expression of long flagellum-like filaments. In the present study, it was determined if *Salmonella* Gallinarum isolated from chickens in Korea can become motile and express the filaments under a special culture condition. *Salmonella* Gallinarum isolates possessing the *fliC* gene, which encodes the major component of flagellin, were cultured in agar plates of GI medium supplemented with 0.5% dextrose. The isolates showed wavy, diffuse growth over the inoculated site in the medium after 48 h incubation although their migration was limited and occurred only on the medium surface. However, they did not show an obvious migration in the medium without dextrose. Electron micrographs of migrated bacterial cells showed the presence of filament structures extending from the cells. Further studies are needed to elucidate the motility induction in the *in vivo* condition and its implication in the bacterial pathogenesis.

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B034

Geographical Location Influences the Composition of Gut Microbiome in Korean Children

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Theragen Bio Co., Ltd

A wide variety of environmental exposures can influence the bacterial communities in the gut of children. The composition of child's gut microbiome is influenced by several factors including diet, mode of delivery, stress, and antibiotics usage. There are few studies about environmental factor affects gut microbiome of children between urban area and countryside in Korea. Our pilot study shows that geographical location affects the alpha diversity (Shannon index, and Chao-1 richness index) in ninety-one healthy children under twelve years old live in different area. We present the *Bifidobacterium* is enriched in children reside in urban area compared to countryside. *Anaerostipes caccae*, butyrate producing bacteria, is also increased in the gut of children lived in urban area. The overall beta-diversity shows the two group is significantly different from each other. These results demonstrated that the geographical location affect the composition of gut microbiome in Korean children.

B035

Paraoxon Degradation by a Novel Strain of *Paenarthrobacter*

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The organophosphorus (OP)-based nerve agents and pesticides have been applied in the agriculture industry for a long time. However, they were found to have a persistent effect on the environment and also threaten human neuronal system. Biological remediation for OPs utilizing microorganisms and OPs-degrading enzymes is promising due to high efficacy. In this study, an actinobacterial strain designated *Paenarthrobacter* sp. MMS21-TAE1-1 was isolated through enrichment culture, which showed excellent efficacy in the degradation of paraoxon (PO), one of OPs, as a sole carbon source. It took only two days to degrade 200 mg/L of PO by 99%, while no intermediate products could be identified. In the conventional degradation pathway of PO, *p*-nitrophenol (PNP) is known as a main degradation product. However, strain MMS21-TAE1-1 did not degrade PNP as a sole carbon source. The analysis of whole genome identified 71 genes by RASTtk and 99 genes by RAST associated with the metabolism of the aromatic compound within the subsystem. However, no genes could be directly related to the degradation of PO and PNP. Instead, a protein from 'not in the subsystem' was related to paraoxonase and showed 42.2% homology with the phosphotriesterase from *Mycobacterium tuberculosis*. This suggests that degradation may occur in a way other than the conventional degradation pathway for PO.

[Supported by NRF.]

B036

Genome Based Analysis of Antimicrobial Potential of a *Micromonospora* Strain Isolated from Riverside Soil

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The goal of this research is to examine the antimicrobial properties of an actinobacterial strain designated R2-23 belonging to the genus *Micromonospora*, which was isolated from riverside soil. The analysis of 16S rRNA gene sequence indicated the close relationship of the strain with *Micromonospora phytophila* SG15^T (99.29%), *Micromonospora matsumotoense* DSM44100^T (99.23%) and *Micromonospora rifamycinica* AM105^T (99.10%). Using the soft-agar overlay method, the strain was shown to exhibit antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus subtilis*.

The obtained genome data of strain R2-23 consisted of 69 contigs with a total stretch of 7,557,908 base pairs and G + C content of 72.7%. The whole genome contained 6,522 protein-coding genes, 54 tRNA genes, and 3 rRNA genes. Through the analysis of biosynthetic gene clusters (BGCs) for secondary metabolites, BGCs for terpenes, type 1–3 PKS, NRPS, lanthipeptides and siderophores were predicted, thus showing the high potential of the strain as a producer of antimicrobial compounds. Ongoing studies include antimicrobial tests against a broad range of microbes.

B037

Identification of Oxidation Enzymes Involved in Polyethylene Degradation Using a Metagenomics Approach

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Polyethylene (PE) is the one of the widely used plastics in a variety of products due to its chemical and biological inertness. PE has been prevalently used in agricultural sites and its recalcitrant to degradation resulted in the accumulation of its debris in the environment as an emerging pollutant. PE-degrading bacteria have been characterized in some studies. However, although microbiological and chemical evidences, enzymes involved in the degradation have not been elucidated. In this study, we introduced a shotgun metagenomics approach to identify PE-oxidizing enzymes which are responsible for an initial and critical step in the degradation. From various landfills, PE wastes (biofilm) and the surrounding soil samples were collected and community DNAs were extracted and sequenced. Hydroxylases were enriched in the biofilm and the protein classification analysis revealed that a novel phylogenetic clade of group A flavin-dependent monooxygenase might be involved in the PE-oxidation. Furthermore, taxonomic assignment of assembled contigs indicated that the PE-oxidation enzyme was originated from actinobacteria. Here, we elucidated specific a PE-oxidizing enzyme candidate and its related taxa information. The results could provide a critical information for PE-degrading bacteria and enzyme identification. [Supported by a research grant from National Institute of Agricultural Sciences, Rural Development Administration (PJ0149742021).]

B038

Identification of Core Microorganisms in a Full-scale Municipal Wastewater Treatment Plant Using Machine Learning

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While machine learning (ML) modeling using microbiome data has a great potential for advancing our understanding of the diversity and function of microbial communities, little work has been done on biological wastewater treatment at full scale. This study carried out ML modeling, which used activated sludge microbiome data for predicting an operational characteristic (anaerobic/anoxic/aerobic) of a biological unit process in a full-scale municipal wastewater treatment plant (WWTP). Among six ML models used with differential complexity, two linear models such as support vector machine (SVM) and logistic regression (LR) showed high prediction performances, comparable to those of non-linear models such as random forest (RF). Feature importance analysis using the linear ML models identified a list of microbial taxa that were specific for an anoxic process, the majority of which were with ecologically important genomic and phenotypic potentials (nitrate-reducing). Time-series microbial community dynamics demonstrated that the ML-identified taxa were highly dominating and frequently occurring in the anoxic process over time. Overall, the results of this study suggests that application of ML modeling holds a great promise for predictive controls (e.g., performance and early event detection) and identification of key microbial players (biomarkers) governing the system's function and stability in a variety of biological wastewater systems.

B039

Characterization of Microbial Community in Organic Upland Soil of Chives

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Organic farm exerts a very positive effect on the abundance and activity of microorganisms. Several studies have reported a positive effect of organic farm on soil health and quality including microbial community traits. Despite the importance of microbial communities as plant growth and defense, little is known about the bacterial diversity of organic farming soil. Therefore, the objective of this study was to characterize the bacterial community composition in the organic farming soil of chives.

In this study we assessed the microbial community structure using 16S rRNA genes. The results of the bacterial community analysis show that Firmicutes (28.4%) was a predominant phylum. Next on Proteobacteria (28.0%) and Actinobacteria (16.2%) were followed. At the genus level, *Bacillus* was the predominant genera. In principal coordinates analysis based on the weighted Fast UniFrac metric, the bacterial communities were separated by agricultural systems. The numbers of operational taxonomic units were higher in the organic farming soil than in conventional soil. These results show that organic soil harbours an abundant and adaptive microbe which underlies soil functioning. In conclusion, possibility of using the soil bacterial community as an indicator of environmental ecosystem health and soil quality was discussed.

[This study was supported by a research grant from the National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea (PJ01493903).]

B040

Cultivation and Isolation of Anaerobic Human Gut Microbiota Using Culturomics

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A dysbiosis in the human gut microbiota can be one of the leading causes of ulcerative colitis (UC). Accordingly, fecal microbiota transplantation (FMT) has been a promising UC treatment because it showed a high success rate of > 58.6% ($n = 29$). To investigate whether the microbial composition correlates with the severity of UC, we identified fecal-derived anaerobes to compare the microbial community using a culture-based approach between patients and donors examined. Consequently, we identified 237 anaerobes from the fecal samples of ten donors and five patients. We also performed a microbial community analysis using 16S rRNA gene sequence-based approaches compared to the culturable gut microbes. These results supported the notion that the gut microbial communities of donors differ from those of patients. Although both analyses showed similar community compositions at the phylum levels, the culture-based analysis revealed distinct microbial species between donors and patients. Notably, phylogenomic studies of cultured microorganisms indicate that functional dysbiosis significantly affected the efficacy of FMT for UC patients rather than a shift in the microbial ecosystem. Therefore, the present study suggests that culturomics-based functional analysis can complement the conventional community-based microbiome analysis to develop microbiome-aided therapeutics for UC.

B041

Influence of Phenotypic Variation in *Paenibacillus polymyxa* E681 on Growth Promotion in Cucumber

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The present study investigates bacterial adaptation and survival under harsh environmental conditions through optimizing and changing their nature and phenotypes. Here, we report the impact of phenotypic variation in *Paenibacillus polymyxa* E681 (E681), a plant growth-promoting rhizobacterium (PGPR) isolated from winter barley root that possesses various beneficial effects on crop plants. Two types of bacterial cells in E681 were identified in our study. We referred to variant as F-type and wild-type as B-type. In our experimental conditions, the phenotypic variation occurred from the cucumber rhizosphere soil and the surface of the seeds. The F-type variant was transformed from the B-type on tryptic soy agar (TSA) plates; however, F-type was not reversible to B-type. Interestingly, the plant growth promotion test revealed that cucumber seedlings treated with F-type cells showed similar to the non-treated control. Whereas, growth promotion in B-type treated cucumber seedlings varies on temperature conditions. In particular, an increased growth promotion was observed at a low temperature of 20°C. In growth curve analysis of E681, the phenotypic variation in B-type did not occur at 20°C for 6 days, but it occurred on the 4th and 2nd day at 30°C and 37°C, respectively. Thus, phenotypic variation in PGPR strains is a crucial issue to be addressed before its application as a bacterial inoculant for sustainable agriculture.

B042

Changes in the Agricultural Resistome during the Cultivation of Lettuce, Cabbage, and Carrot

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Agricultural products such as raw salad vegetables have been recognized as important sources in transmission of antibiotic resistance genes (ARGs) from the environment to humans. Manure and compost from antibiotic-treated animals have been identified as a major source of ARG transmission in the agricultural environment. In this study, three agricultural products were analyzed for their microbiomes and the antibiotic resistome from various sample types using the Illumina sequencing platform during the cultivation period. Regardless of the cultivation environment, high levels of ARGs against glycopeptide and rifamycin classes were detected in the cultivation soil and agricultural product samples, and ARGs against aminoglycoside and MLSB classes were highly abundant in composted-manure samples. Irrigated water samples displayed different resistome structures from those of other samples. The structure of the antibiotic resistome in agricultural products appeared to be influenced by the resistomes of cultivation soils and irrigated water. Our results revealed the antibiotic resistome structures in various sample types of three different agricultural products and highlight public needs to build antimicrobial resistance monitoring system from agricultural environments to products.

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B043

Demethylation of Vanillate and 4-Methoxybenzoate in *Comamonas testosteroni* Strain P19

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Vanillate and 4-methoxybenzoate are metabolites of lignin which is known as a recalcitrant polymer and demethylation of them to protocatechuate is a key step for biodegradation. It is mediated by vanillate demethylase consisting of oxygenase and oxidoreductase which are encoded by *vanA* and *vanB*, respectively. *Comamonas testosteroni* P19 was capable of utilizing the compounds as a sole carbon and energy source and three *vanA* and *vanB* genes were found in the genome. To elucidate the expression of each *vanAB* genes for each substrate, total RNA and protein were extracted from the harvested cells grown on each compounds and expression profiling was performed by RNA-seq and proteomic analyses. In the genome context of *C. testosterone* P19, locus tag BN2297_RS07910 encoding *vanA* was up-regulated with 26 times of fold change when the cell was cultivated with 4-methoxybenzoate, but there was no significant change with ferulate. On the other hand, BN2297_RS20090 encoding the other *vanA* was up-regulated with 5 times of fold change when the cell was cultivated with ferulate, but there was no significant change in 4-methoxybenzoate. This study represented that strain P19 harbored two different demethylases which had substrate specificities for 4-methoxybenzoate and vanillate individually. It also indicated that enzymatic demethylation occurred depending on the position of the methoxy group. [Supported by grants from NRF (2019R1A2C1087340).]

B044

Quorum Quenching Ability of *Reyranella* sp. Isolated from Riverside Soil

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A novel Gram-negative, rod shaped bacterial strain MMS21-HV4-11 displaying *N*-hexanoyl-L-homoserine lactone (C6-HSL)-degrading potential was isolated from a riverside soil. The strain was found to be closely related to members of the genus *Reyranella* and showed 16S rRNA gene sequence similarities of 98.74, 97.87, and 97.80% with *R. aquatilis* seoho-37^T, *R. soli* KIS14-15^T and *R. massiliensis* 521^T, respectively. *Reyranella* currently contains 5 species, and accordingly the quorum quenching activity of the type strains of 5 known species along with the isolate was tested. All strains of *Reyranella* were capable of degrading C6-HSL, thus showing quorum quenching potential at the genus level. Decomposed C6-HSL showed restoration from some concentrations under acidic conditions. The genome analysis indicated that strain MMS21-HV4-11 was found to have two lactonase and one acylase candidate gene, and phyre2 confirmed the expected protein model. Through in vitro assay using a plant pathogen *Pectobacterium carotovorum* subsp. *Carotovorum*(*Pcc*), the development of soft rot was significantly inhibited by MMS21-HV4-11^T. In addition, the swarming by *Pseudomonas aeruginosa* was also significantly inhibited. Since the isolate did not display antibacterial activity against both species, this inhibition was certainly due to the quorum quenching activity by MMS21-HV4-11. This is the first report on quorum quenching potential from *Reyranella*.

B045

Respiratory Microbiome in Mechanically Ventilated Patients in Healthcare Setting

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Healthcare-associated pneumonia (HCAP) is a heterogeneous disease. We redefined nursing-home and hospital-associated infections (NHAf) group by revising existing HCAP risk factors. Our objective was to determine whether respiratory microbiota profiles were associated with newly defined NHAf group in mechanically ventilated critically ill patients. The 180 endotracheal aspirates (ETAs) from 60 mechanically ventilated ICU patients were collected. The bacterial community profiles of the ETAs were analysed by 16S rRNA gene sequencing. An optimal microbiome-based association test, phylogenetic tree based microbiome association test, generalized linear mixed models, the Wilcoxon test and the reference frame method. The relative abundance of the genus *Corynebacterium* was significantly higher in the pneumonia than in the non-pneumonia group. The microbiome analysis showed significantly lower α -diversity in the NHAf group than in the non-NHAf group. In the β -diversity analysis, the structure of the microbiome also differed significantly between the two groups. The loss of diversity and dysbiosis of the respiratory microbiome were acute in the NHAf group than in the non-NHAf group, which were as a result positively associated with the presence of *Corynebacterium*, and negatively associated with that of *Granulicatella*, *Streptococcus*, *Staphylococcus*, and *Veillonella*. Patients with and without risk factors for NHAf were distinguished by microbiota signature of the ETAs.

B046

Complete Genome Sequence of Diazinon Degrading Bacterium *Paenibacillus tritici* PH55-1

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Organophosphorus pesticides (OPPs) have been extensively used in agricultural practice for more than 40 years. One of them, diazinon has been widely used throughout the world with applications in agriculture and horticulture for controlling insects in crops, ornamentals, lawns, fruits, and vegetables. The residues of persistent pesticides stay in the environment without breaking down for long periods. The microbial degradation is eco-friendly and good strategy that can be used in the field to reduce the occurrence of crops safety problems caused by pesticide residues. We report the whole genome sequence of *P. tritici* strain PH55-1. The sequence analysis revealed that *P. tritici* strain PH55-1 possesses a single 7,708,938 bp circular chromosome with an average DNA G + C content of 51.8%. This chromosome contains 6,450 protein-coding sequences and 30 rRNA and 84 tRNA genes. The *P. tritici* strain PH55-1 contained OPPs degradation related enzyme and diazinon degradation related genes. There are 19 phosphodiesterases, 3 alkaline phosphatases and Metallo- β -hydrolase (GloB, AhID) that are thought to be related to the of diazinon. The isolate PH55-1 may have potential for use in bioremediation of diazinon-contaminated soils. [Supported by grants from RDA.]

B047

Lung Cancer might be Associated with Microbial Composition

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Lung cancer initiation and/or progression might be regulated by the altered microbial composition. Lung cancer is the leading cause of death worldwide. The microbiota has emerged as a key regulator of carcinogenesis but has not yet been precisely identified in lung cancer. We compared the microbial composition of lung cancer with adjacent normal lung tissues to identify histological types and genetic mutations and to investigate the association of the lung microbiome with clinical parameters. We analyzed the microbiome in lung tissue of 162 patients with surgically resected non-small cell lung cancer (NSCLC, 162 cancers, and 54 adjacent normal tissues) from January 2018 to December 2019. We analyzed the lung tissue microbiome of adjacent normal and NSCLC tissues using 16S rRNA amplicon sequencing. The analysis was performed by the QIIME2 pipeline. The NSCLC tissues had significantly lower alpha diversity than normal tissues. Alpha diversity decreased with increasing postoperative lung cancer stage, and microbial community was significantly different with the presence of recurrence. Genus *Stenotrophomonas* was identified to be the most dominant in the recurrence group in NSCLC.

B048

Identification of Novel Antibiotic Resistance Genes in Environmental Bacteria

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Since the introduction of antibiotic resistance concept and One-Health strategy in the field of antimicrobial resistance research, the environment has been considered the origin and reservoir of antibiotic resistance genes (ARGs). Numerous studies have shown the presence of novel ARGs in various environments and some of them are already prevalent in the clinic or ready to mobilize to pathogens via mobile genetic elements, indicating that a thorough understanding of novel ARGs in the environment is essential to mitigate their emergence and transmission in the clinic. In this study, a total of 10 Gram-negative bacterial strains showing distinguished ARG genotypes and phenotypes were isolated from various environments and their complete genome sequences were obtained. Based on sequence similarity and phylogenetic analyses, a total of 44 potential novel ARGs were elucidated in the bacteria. Among those strains, one strain was the closest to the pathogen *Scandinavium goeteborgense* which was recently reported as a novel genus within the family enterobacteriaceae. The strain carries a novel TEM β -lactamase located in a distinct phylogenetic clade of the TEM β -lactamase family, indicating the presence of a novel ARG in an emerging pathogen. Analysis of genetic context and resistance spectra of the novel ARGs will be performed to understand the level of risk of them in the clinic.

[Supported by a research grant from the National Research Foundation of Korea (NRF-2019R111A1A01059574).]

B049

The Differential Skin Microbiome of 20s and 60s Healthy Korean Women

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Recently, several studies examined the diversity and role of the age-related skin microbiome in humans. We recruited healthy Korean women 19–28 years old (Y-group) and 60–63 years old (O-group) and compare their cheek and forehead microbiome in both bacterial and fungal communities. To identify differences in the skin microbiome of two age groups, we examined the microbial and skin physiological indicators for separating the groups. Through the 16S rRNA gene sequencing, we observed a higher alpha-diversity of bacterial and fungal communities in the O-group than in the Y-group. The amplicon sequence variants affiliated with bacteria *Cutibacterium* and *Lactobacillus* and fungi *Malassezia restricta* as microbial indicators showing significant differences between the Y- and O-group. In terms of microbial function and ecology, there were more microbial interactions with a higher network density in the Y-group, and there were more metabolic processes related to skin health too. Skin physiology, including transepidermal water loss and sebum content, differed by two age groups, and the skin metadata, including the frequency in use of mask sheet and skin, had associations with the skin microbes. The biomarker separating two age groups found in this study will be helpful in associating skin microbiome research and skin aging.

[Supported by AmorePacific R&D Center.]

B050

Potential Synergistic Relationship among *Flavobacterium* sp. TCH3-2 and Synthetic Community Members Improved the Growth of Tomato Plants

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Microbial communities in the rhizosphere have a great influence on the plant life. Therefore, understanding the role of these communities is highly essential. Previously, we have isolated a *Flavobacterium* sp. named as TCH3-2 having synergistic interactions with other bacterial species (SynCom). This interaction has shown to enhance the growth of tomato plants, however, relationship among TCH3-2 and each bacterial strain of SynCom has not been understood yet. In this study, we examined the potential relationship among TCH3-2 and SynCom members. We conducted microbial interaction tests using V-shape and top agar methods. The V-shape method was conducted by co-culturing TCH3-2 and SynCom members in known inoculation sites on TSM media. In addition, the top agar method was conducted by inoculating single strain of SynCom on TCH3-2 lawn prepared in TRM media. V-shape method showed that the growth of TCH3-2 was promoted in minimum distance of 1 cm from each member of SynCom. On the other hand, top agar method results indicated that presence of TCH3-2 can stimulate the growth of *Ketobacter* sp. and *Archangium* sp. of SynCom. Taken together, our study suggested a synergistic relationship among TCH3-2 and SynCom members to promote the growth of tomato plants.

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B051

Microbial Production of Recombinant Staphyloferrin A

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The skin surface has various environmental conditions depending on pH, temperature, moisture and sebum production, and consists of various microbial communities. Although strains of *Staphylococcus* sp. normally belong to the normal skin flora, they can opportunistically cause skin diseases. During pathogenesis, siderophores can help pathogenic microbes to acquire extracellular iron. It has been known that purified siderophores can be used for medicinal purposes such as anemia treatment and antimicrobial agent. However, the production and/or purification of siderophores from *Staphylococcus* sp. carries a risk of infection and is not suitable as a method for industrial production. In this study, we attempted to produce staphyloferrin A (SA) derived from *Staphylococcus* sp. in surrogate microbial host. The gene cluster of *sfaABCD* involved in the biosynthesis and export of SA was obtained from the genomic DNAs of *S. epidermidis* and *S. aureus*, and expressed in *E. coli*. The recombinant siderophore production was confirmed by CAS assay. To enhance the production of SA, we eliminated metabolic pathways associated with the reduction of the precursor pool, and also manipulated the relevant transcriptional factors in the cell, which will be helpful for the production of siderophores.

B052

Hydrogen-dependent DNRA of Epsilonproteobacteria (*Arcobacter* and *Sulfurospirillum*) Isolated from Daejeon Wastewater Treatment Plant

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Dissimilatory Nitrate/Nitrite Reduction to Ammonium (DNRA) is a microbial reaction that can offset or reduce nitrogen lost in the form of N_2 or N_2O mainly by denitrification microorganisms in anoxic soil. To date, most studies on DNRA in the field of environmental microbiology have been limited to heterotrophic bacteria and dealt with nitrogen cycle regulated by them. In particular, it is considered orthodoxy among many researchers that the carbon-to-nitrogen (C/N) ratio plays a key role as one of the ecophysiological control factors of the denitrification/DNRA reaction in the environment. In this study, we confirmed the presence of Epsilonproteobacteria performing hydrogen (H_2)-dependent DNRA from Daejeon wastewater treatment plant. The isolated strains were identified as *Arcobacter* and *Sulfurospirillum* by 16S rRNA sequencing. We confirmed that in the minimal salts medium, at least 80% of 2 mM NO_3^- -N added as a final electron acceptor is finally reduced to NH_4^+ -N by these microorganisms under anaerobic conditions in which 5% (v/v) of hydrogen is intermittently supplied. The reductive production of NH_4^+ -N from NO_3^- -N was not observed under conditions in which hydrogen was not supplied in the pure culture of each strain. These characteristics of Epsilonproteobacteria isolated from the environment may reveal a novel regulatory mechanism for the nitrogen cycle by them, which has not yet been elucidated.

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B053

Isolation of the Rhizosphere Bacteria Associated with Bacterial Wilt Resistance in Tomato

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Plant health can be affected by commensal microbiome that inhabits the rhizosphere. In our previous study (Kwak *et al. Nat. Biotechnol.*, 2018), metagenomic analysis was performed to compare the structures of the rhizosphere microorganisms of two cultivars, Hawaii 7996 and Moneymaker, which are resistant and susceptible to bacterial wilt disease, respectively. The results showed that the microbiome in Hawaii 7996 contributes to resistance against bacterial wilt. A novel strain TRM1-10 was isolated from the rhizosphere of the resistant cultivar, which improves disease resistance in tomato. In the current study, we tested the disease suppressive effect of TRM1-10 in other susceptible cultivars. In addition, bacterial wilt resistance-associated bacteria were explored based on the reconstructed metagenomic data. We could find that the genome sequence of a flavobacterium strain was more abundant in Hawaii 7996. We checked the disease suppressive effect using a reference strain, whose genome is similar to that of the flavobacterium resulted from metagenome-assembled genome data. Furthermore, a rhizosphere microbial culture collection was constructed to discover the bacterial strains that could be involved in our disease model system. As a result, 5,131 isolates were collected, of which 1,571 were identified. The results derived from this study will serve as resources to understand the function of plant probiotics and their interaction with the plant as well as the pathogen.

B054

Enrichment Culture for Isolation of the Novel Bacterial Strains from Rhizosphere Soil of Tomato

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Rhizosphere microbiota play an important role in plant immunity, however, it is not clear how the structure of root microbiome is reorganized to improve the health of the host plant. Recent studies proposed that the diverse carbon sources of root exudate can modulate the structure of soil microbiome. Our previous study found that the microbiome fraction (MF) of upland soil supported resistance in a bacterial wilt (BW) resistant tomato cultivar Hawaii 7996 of the BW. In this study, we hypothesized that carbon sources supplementation could induce changes in the rhizosphere microbiome and enrich novel bacterial species. To test this, rhizosphere MF collected from rhizosphere soils of tomato plants treated with upland MF was supplemented with various carbon sources and antibiotics. Microbiome structure was analyzed using enriched culture and attempted to isolate unique bacterial species based on microbiome shift. Here, we isolated 441 bacteria species representing 6 phyla and 74 distinct species, including 23 novel species. Our results provide us with insights into the potential use of carbon-sources and antibiotics as a stimulator for cultivation of novel species in the plant rhizosphere environments. [Supported by National Research Foundation of Korea (NRF) grant funded by the Korea government (No. 2020R1A2C3005453 and 2020R1A6A1A03047729).]

B055

Optimization and Application of Multiplex PCR Method to Detect Genes Involved in Cyanotoxin Synthesis

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Harmful cyanobacterial blooms, caused by cyanobacteria in genera *Microcystis*, *Anabaena*, *Oscillatoria*, and *Aphanizomenon*, have been emerging a global issue because it is routinely occurred in the aquatic environments in every summer worldwide. These harmful cyanobacteria can produce a variety of cyanotoxins, including microcystins, cylindrospermopsins, anatoxins, nodularins, and saxitoxins. The aim of this study was to optimize the multiplex PCR method to detect the genes involved in synthesis of five kinds of cyanotoxins simultaneously, and to verify the applicability of the optimized method in the aquatic environments in Korea. In order to optimize the multiplex PCR the appropriate primers which specific to the five kinds of cyanotoxins were selected or developed, and the PCR conditions such as the annealing temperatures and the concentrations of primers were optimized. In the samples taken from six rivers and seven reservoirs in summer season in Korea, only *mcyE* involved in microcystin synthesis was detected among the five kinds of cyanotoxin synthetase genes, indicating the risk management against microcystins are essential among the these toxins in the Korean freshwaters. The optimized multiplex PCR method in this study can quickly and accurately detect the presence of five kinds of cyanotoxin synthetase genes in the aquatic samples.

B056

Quantification of Microcystins in the Fish Tissues Taken from Korean Freshwaters and Risk Management

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Microcystins (MCs) are cyclic heptapeptide hepatotoxins produced by harmful cyanobacteria. Consumption of fishes living in algal blooming areas is one of the major routes to human exposure to MCs. MCs concentrations were quantitated in the tissues (muscle, kidney, liver, and intestine) of 205 freshwater fishes taken from five sites in the Nakdong and Geum River basins, and the relationship between the toxin concentration and diet (insectivorous, piscivorous and omnivorous) or age (length) was evaluated. MCs concentrations was measured with high-performance liquid chromatography. The mean concentration of MCs in the intestine and liver of the fish was significantly higher than those in other tissues ($p < 0.05$). MCs were detected in the fish muscle of 3 out of 205 (1.46%). MCs concentrations in fishes collected after the end of the cyanobacterial blooming reduced 30% of that collected at the time of cyanobacterial blooming. In case of carp the MCs concentrations in liver and intestine were negatively correlated with the age (body length) ($p < 0.05$). The concentrations of MCs in omnivorous fishes living in the surface layer were the highest. The results can be used to manage the risk through consumption of fishes contaminated with MCs in Korean freshwaters.

B057

Optimization and Application of Multiplex Nested PCR to Detect Free-living Amoeba in Biomass-rich Samples

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Acanthamoeba spp. and *Naegleria fowleri* are opportunistic pathogenic free-living amoebas (FLAs) found in aquatic environment worldwide. According to previous studies, pathogenic FLAs are also founded in freshwaters including sources of tap water in Korea. It is difficult to detect FLAs in the biomass-rich samples using the conventional cultivation methods. In this study, multiplex nested PCR method to detect FLAs simultaneously in the biomass-rich samples was optimized and applied in the samples taken from Korean freshwaters. To optimize the multiplex nested PCR method the appropriate PCR primers targeted 18S rRNA gene of *Acanthamoeba* spp. and *N. fowleri* were selected or designed. It was found that the sensitivity of the optimized method was 100 times higher than that of the conventional PCR method. The optimized method was applied to the samples taken from the Nakdong River and effluents from sewage treatment plants. As a result of analyzing 168 samples from four weirs in the Nakdong River, *Acanthamoeba* spp. was detected in 164 sample (97.6%) and *N. fowleri* was detected in 115 samples (68.5%). In conclusion the method optimized in this study can be used to monitor *Acanthamoeba* spp. and *N. fowleri* in biomass-rich samples. The intensive monitoring is needed to manage the risk from FLAs at the recreational or drinking water sources in Korea.

B058

Transmission of Livestock-derived Antibiotic Resistance Genes into Surrounding Environments

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Antibiotics are widely used in livestock industry and various antibiotic resistance genes (ARGs) are frequently detected in livestock waste around the world. However, publications on the spread and potential contribution of antibiotic resistance from livestock industry is about 10%. Here, we assessed the propagation of ARGs using shotgun metagenome sequencing to determine the distribution of resistome and mobilome in livestock samples, including swine and human feces, swine nasal, and environments. The microbiome and resistome were significantly different according to the livestock environments and the growth state of swines. Tetracycline resistance genes were detected the most in samples on average, followed by aminoglycosides and MLSB resistance genes. In particular, aminoglycoside resistance genes were more detected in the swine feces and livestock environments than human feces, while cephamycin resistance genes were more detected in the human feces samples. Plasmid contigs with ARGs were confirmed to identify a mobile resistance gene. Genes resistant to aminoglycoside, tetracycline and multi-drug were detected in different plasmid contigs, and these plasmid contigs were distributed in all samples. Therefore, it is possible that the ARGs were transferred from one sample to another through the plasmid. This means that ARGs in livestock waste can affect people and the surrounding environment, including the water and soil environment.

[Supported by KCDC.]

B059

Serotypes and Antimicrobial Resistance Patterns of Recent *Salmonella* Isolates from Poultry Production Facilities

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Animal and Plant Quarantine Agency

Salmonella is one of major foodborne pathogens in humans and its most common sources are foods derived from animals, in particular, poultry. Monitoring and control of *Salmonella* has been an important part in poultry production worldwide. The present study was to investigate serotypes and antimicrobial resistance patterns of *Salmonella* recently isolated from poultry production facilities in Korea. *Salmonella* was isolated from poultry and environmental samples collected from poultry farms, hatcheries, and slaughter houses between 2019 and 2020. A total of 355 *Salmonella* isolates were serotyped and tested for their antimicrobial susceptibilities. Thirty serotypes were determined, showing the high prevalence of serovars Bareilly ($n = 76$), Virchow ($n = 33$) and Typhimurium ($n = 32$). The isolates were highly resistant to nalidixic acid (45.1%), followed by ampicillin (17.5%), streptomycin (17.2%) and sulfisoxazole (15.5%). They revealed 42 antimicrobial resistance patterns and 47 of the isolates (13.2%) were resistant to at least five antimicrobials. Consequently, the serovar Bareilly was the most prevalent in recent *Salmonella* isolates from poultry in the present study. This study also showed the distribution of multidrug-resistant *Salmonella* that is resistant to a third-generation cephalosporin as well in poultry, requiring appropriate intervention measures.

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B060

Analysis of Microbial Community Structure Using Next Generation Sequencing Method in the Season of Cyanobacterial Blooming at the Daechung Reservoir

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In Korea, the algal warning system has been operated to supply the drinking water safely at water supply sources. In this study, using the NGS method, the changes in microbial community structure were analyzed at the algal warning system sites in the Daechung Reservoir. The proportion of phylum Cyanobacteria was in the range of 16.5–71.6% (average, 40.3%), accounting for the largest proportion in most samples. The proportion of *Microcystis* was high in areas where blooming occurred, whereas single-cell cyanobacteria such as *Synechococcus* were dominant in areas where blooms did not occur. In the sample with a low percentage of *Microcystis*, the proportion of filamentous cyanobacteria such as *Anabaena* and *Oscillatoria*, which are known as the main producer of odor compounds such as geosmin and 2-MIB, was high. The proportion of phylum Actinobacteria was 2.6–42.2% (average, 20.4%), and the value was depended on the sites and time of sampling. Actinobacteria include soil actinomycetes, such as Actinomycetes. Therefore, it is assumed that the major source of contaminants is the influent from outside of reservoir at a site where the proportion of Actinobacteria is high. In conclusion, the analysis of microbial community structure using NGS method can be used to identify the cause of algal blooming and to establish management strategies in the Daecheong Reservoir.

[This work was supported by Chungbuk National University Korea National University Development Project, 2021.]

B061

Culture-independent Analysis of the Bacterial Community in Chinese Fermented Vegetables and Genomic Analysis of Lactic Acid Bacteria

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World Institute of Kimchi

Six different fermented vegetables were collected from Zhejiang Province, China, to explore the associated bacterial community using a high-throughput sequencing platform. A total of 24 phyla, 274 families and 569 genera were identified from 6 samples. Firmicutes and Proteobacteria were the main phyla in all of the samples. *Brevibacterium* was the major genus in Xiaoshan pickled radish. *Lactobacillus*-related genera and *Vibrio* were the major genera in fermented potherb mustard and its brine. *Enterobacter* and *Cobetia* were the major genera in fermented radish and its brine. *Chromohalobacter* was the major genus in the tuber mustard. These results indicated clear differences were there between the bacterial genera present in Xiaoshan pickled radish, fermented potherb mustard, fermented radish, and tuber mustard. This demonstrated the possible influences of raw materials and manufacturing processes. Furthermore, a large number of lactic acid bacteria were isolated and identified by culture-dependent and 16S rRNA gene sequence analysis, which accounted for more than 68% of all the isolates. In addition, whole-genome analysis of *Levilactobacillus suantsaii*, *Latilactobacillus sakei* subsp. *sakei*, and *Weissella cibaria* showed that they had large numbers of genes associated with carbohydrate metabolism. This may explain why these three bacterial strains can grow in fermented vegetable environments.

[This research was supported by a grant from the World Institute of Kimchi (KE2101-2)]

B062

Isolation and Genomic Characterization of Bacteriophages Infecting the SAR92 Clade, an Abundant Oligotrophic Marine Gammaproteobacterial Group

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Bacteriophages (phages) are the most abundant biological entities in marine environments and have huge effects on bacterial community and biogeochemical cycles. Although the recent deluge of marine viral metagenomics data has shown enormous diversity of marine phages, biological interpretation of those data requires experimental characterization of phage-host systems of abundant marine bacterial groups. In this study, we report on the first isolation of bacteriophages that infect the SAR92 group, an abundant oligotrophic marine gammaproteobacterial group. The host bacterial strain, IMCC15298, was obtained from the East Sea by high-throughput culturing and was found to belong to the SAR92 group of the family *Porticoccaceae*. A mixed culture of bacteriophages infecting IMCC15298 lytically was isolated from a coastal seawater sample collected in the Yellow Sea. Morphological characterization by TEM showed that the culture was dominated by a phage of the family *Siphoviridae*. Three complete genomes, with lengths of ~80, 89, and 59 kb, were obtained by Illumina and Nanopore sequencing. More than 90% of sequencing reads were mapped to the ~80 kb genome, with many polymorphic sites, indicating that this genome sequence represents a major phage population (of the mixed culture) encompassing several very closely related phage strains.

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B063

Dynamics of Bacterial and Viral Communities during Kimchi Fermentation

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Bacteriophages within the viral community in fermented foods can directly affect the fermentation process and food quality through influencing bacterial function and composition. To date, however, limited research has been performed on the overall viral and bacterial communities in fermented foods. In this study, the viral and host bacterial communities during kimchi fermentation were analyzed in triplicate using next-generation sequencing technology. The overall structures of bacterial and viral communities were dominated by lactic acid bacteria in phylum Firmicutes and bacteriophages in order Caudovirales, respectively. After correction for multiple comparisons using false discovery rate (FDR, $P < 0.05$), the relative abundances of 26 bacterial OTUs and 15 bacteriophage strains changed significantly during fermentation. In beta-diversity analysis, bacterial communities were much more clearly differentiated by fermentation period (PERMANOVA pseudo-F = 22.37, $P < 0.001$ in weighted UniFrac PCoA) than viral communities (pseudo-F = 1.25, $P = 0.018$ in Binary Jaccard PCoA). Pearson's correlation analysis showed that some bacteria-phage dynamics during fermentation are consistent with "kill the winner" dynamics, as in other ecosystems. Our results provide insights into the potential impact of bacteriophages as modulators of bacterial communities associated with the fermentation properties of kimchi.

[Supported by grants from World Institute of Kimchi (KE2101-2).]

B064

Microbial Community Structure along a Salinity Gradient and Physiological Characteristics of Culturable Bacteria in the East Siberian Sea

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The rising temperature in the Arctic Ocean causes changes in marine environment such as sea ice fluctuation, primary production, and riverine input and these changes, in turn, impact on benthic ecosystems. To understand how these changes affect benthic ecosystem function, we firstly characterized the microbial community structure of marine sediments of the East Siberian Sea (ESS). In addition, to understand the roles of bacteria in the benthic ecosystems, physiological characteristics of culturable bacteria were investigated. Bacterial community was dominated by the phyla *Proteobacteria* ($28.0 \pm 19.6\%$), *Bacteroidetes* ($12.4 \pm 9.6\%$), and archaeal community was dominated by the phyla *Thaumarchaeota* ($83.5 \pm 22.6\%$). The proportion of *Acidobacteria* and *Euryarchaeota* increased while that of bacterial phylum *Bacteroidetes* and archaeal phyla *Lokiarchaeota* and *Bathyarchaeota* decreased with the increment of salinity. Fifty bacterial isolates were obtained and assigned into 15 phlotypes. Of these, 28 strains produced at least one type of extracellular amylase, protease, polymer and 13 strains were able to hydrolyze hypoxanthine implying that bacterial taxa contribute to nutrient cycling by the hydrolysis of major organic compounds. As the first report on the microbial structure in the ESS, this study provides a fundamental insight on microbial diversity and their putative ecological functions in the ESS that is dynamically affected by global warming.

[Supported by grants from KOPRI.]

B065

***Flavobacterium* sp. Promotes Plant Growth via Collaborating with Native Tomato Rhizosphere Microbiota**

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The rhizosphere microbes affect plant health, such as plant growth and disease resistance. Previously, we indicated that a *Flavobacterium* sp. TCH3-2 strain isolated from tomato rhizosphere soil promotes tomato growth with indigenous microbial community, but not by alone. In this study, we examined the effect of changes in rhizosphere and endosphere microbiome by TCH3-2 on tomato growth. The 16S rRNA gene amplicons were sequenced by MiSeq sequencer and analyzed using QIIME2 pipeline. The microbiome data showed that there was significant difference between the TCH3-2 treated and untreated control group in tomato rhizosphere and endosphere microbiome at 3- and 5-weeks post inoculation. To validate microbiome data, we constructed a native soil mimic-synthetic community (SynCom) comprising several bacterial isolates from tomato rhizosphere soil. Interestingly, soil drenching of SynCom significantly promoted tomato growth with viable TCH3-2 but not with heat-killed one. In addition, using a hydroponics system, we found that plant growth-promoting compound(s) is extracellularly secreted by the combination of SynCom and TCH3-2. Taken together, these data showed that *Flavobacterium* sp. TCH3-2 might improve plant growth by cooperating with indigenous soil microbiota in rhizosphere soil.

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B066

Effects of pH and Antimicrobial Peptides in the *Staphylococcus* Interspecies Co-culture Approach

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Staphylococcus is typical residential bacterial species on the skin. Their interactions provide a baseline for studies that explore the role of bacterial communities on the skin. Among them, *S. aureus* is a representative bacteria causing many skin infections. As *S. aureus* act as a pathogen on the skin, controlling them can be an effective way to alleviate skin diseases. Acne is one of the most common skin diseases, and acne skin has a higher pH than normal healthy skin. Our current focus in their interspecies interaction is how they show the diverse inhibition tendency in different pH environments. 28 strains of *Staphylococcus* spp. were isolated from the human skin by swab. We observed growth modality in both the axenic culture of 28 *Staphylococcus* strains and co-culture of the 28 strains with *S. aureus* USA 300. Red fluorescence protein-expressing pH48 electroporated to *S. aureus* USA300 was used to sort out them. One *S. hominis* (S38) kill *S. aureus* USA 300 at a wide range of pH, but the other *S. hominis* (S39) does not kill the USA 300 at pH 7 but tends to kill the USA 300 as the environment gradually changes to pH 4. They have two unique β -class phenol-soluble modulins. Additionally, there are some *S. aureus* strains that compete with USA 300 at pH 7. We suggested that there are some mechanisms in the group of *Staphylococcus* spp. inhibiting the formation of *S. aureus* biofilm according to the pH.

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B067

Investigation of Microbial Community on Marine Microplastic in Coastal Area of Busan, South Korea

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In recent year, contamination of microplastics (MPs) has become a global environmental concern due to their wide spread in environmental system such as ocean and surface water. Despite important role of biofilm mechanism in MPs, it still remains unknown for their microbial community. In this study, 16S rRNA gene sequencing was used to identify microbial community on MPs from coastal area in Busan. The results indicate that *Rhodobacteraceae*, *Flavobacteriaceae*, *Alteromonadaceae* were dominant in microplastics by coastal samples from Busan. In addition, alpha and beta diversity was significantly different between MPs and sea water samples. These results indicated that MPs is a novel microbial niche and it may increase our understanding of plastisphere ecology.

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B068

Characterization of Spatiotemporal Distribution of Freshwater Bacterioplankton Community of Lake Soyang

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Although the microbial community of freshwater lakes distributed around the world consists of typical and cosmopolitan bacterioplankton lineages, each lake is known to have a unique microbial composition. In addition, lakes in temperate regions have the characteristic that their microbial communities vary according to environmental factors that change dynamically with water depth and season. Here, we applied 16S rRNA gene amplicon sequencing to identify the bacterioplankton community composition of Lake Soyang using samples collected monthly for 2 years. The amplicon sequencing analyses of 120 lake water samples taken from the Soyang Dam station at five water depths revealed that the bacterial community composition was influenced greatly by seasons and depths, and it was especially variable in surface waters. Overall, *Actinobacteria*, *Bacteroidetes*, and *Betaproteobacteria* dominated throughout the water column. The most predominant amplicon sequence variants, affiliated with *Actinobacteria* acl-A7 and B1, showed consistently high abundance in the whole water samples, while those of *Chloroflexi*, *Planctomycetes* (e.g., CL500-11 and CL500-3), and *Nitrosomonadaceae* were abundantly present only at the hypolimnion. This study shows varying patterns of the microbial community of Lake Soyang, which provides information on spatiotemporal and phylogeographic distribution of globally or endemically abundant aquatic microbial lineages.

[Supported by National Research Foundation, Korea.]

B069

Comparison of Fermentative Characteristics between Meju, a Traditional Korean Fermented Soybean Brick, Inoculated with *Leuconostoc mesenteroides* and *Bacillus velezensis* during Fermentation

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Two types of meju (traditional Korean fermented soybean brick) inoculated with *Aspergillus oryzae* were prepared, and *Leuconostoc mesenteroides* and *Bacillus velezensis* were additionally inoculated, respectively, and their fermentative characteristics and metabolites were investigated. The CFU of both *Leuconostoc* and *Bacillus* increased rapidly in early fermentation day, but CFU of *Leuconostoc* and *Bacillus* decreased and increased during fermentation, respectively. The pH values of *Leuconostoc* meju were decreased rapidly in early fermentation day and increased gradually. However, *Bacillus* meju showed constant pH values near 7.0. The concentration of organic acids in *Leuconostoc* meju increased rapidly in early fermentation day and decreased also rapidly, but the concentration of organic acids in *Bacillus* meju increased gradually. Activity of α -amylase, protease in *Leuconostoc* meju increased rapidly from middle fermentation days, and the concentration of free sugars, glycerol, and amino acids in *Leuconostoc* meju also increased rapidly in middle fermentation day and decreased gradually. On the contrary, activities of enzymes in *Bacillus* meju increased gradually, and concentration of free sugar, glycerol, and amino acids also increased gradually. Activity of β -glucosidase in *Leuconostoc* meju was higher than *Bacillus* meju. In addition, concentration of aglycone was also higher in *Leuconostoc* meju, and antioxidant activity using DPPH was higher in *Leuconostoc* meju than *Bacillus* meju.

B070

Dilution-to-Extinction Culturing under Anaerobic Conditions Yielded Diverse Previously-uncultured Bacterial Isolates from Ganghwa Tidal Flat

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Tidal flats are important ecosystems where a large amount of organic matter is cycled through various biogeochemical processes. Sulfate-reducing bacteria is one of the major microbial groups that contribute up to > 30% of microbial assemblages in anaerobic zone of tidal flats. In this study, high-throughput dilution-to-extinction culturing (HTC) was conducted twice using sediment samples collected from a tidal flat in Ganghwa Island. Sediment samples obtained at a depth of 15 cm were homogenized with sterilized seawater. Following the determination of cell counts by flow cytometry, cells were diluted with liquid media and dispensed into multi-well plates. Two kinds of liquid media (each based on natural or artificial seawater) were used for HTC. Sulfate (20 mM) was added to artificial seawater as an electron acceptor and organic acids (such as pyruvate, lactate, and acetate) were added to both media as electron donors. After a month of anaerobic incubation, microbial growth was detected in approximately 33.1% of 3,456 inoculated wells. Phylogenetic analyses of 16S rRNA genes showed that many cultured isolates were affiliated with several previously-uncultured marine anaerobic bacterial groups, such as Sva1033 clade of *Desulfuromonadales*, BD2-2 clade of *Bacteroidales*, IheB3-7 clade of *Ignavibacteriales*, and R76-B128 clade of *Kiritimatiellae*. This study suggests that more diverse anaerobic bacterial groups could be isolated by the application of dilution-to-extinction culturing.

B071

Isolation and Characterization of Geosmin and 2-MIB Producing *Streptomyces* sp. 1PDS1-11

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Actinobacteria were able to produce odours such as geosmin and 2-methylisoborneol (MIB). We isolation of odours-producing Actinobacteria from the Bukhan River, and investigation of geosmin and 2-MIB formation characteristics. Actinobacterial density of sediments were distributed $3.33\sim 2.67 \times 10^5$ CFU/g which was the $3\sim 10^5$ times higher more than waters. We isolated 58 odours-producing Actinobacteria from sediments, geosmin and 2-MIB were identified using GC-MS. *Streptomyces* sp. 1PDS1-11 produced 18.80 ng/L of geosmin and 165.58 ng/L of 2-MIB, was selected as a representative odours-producing Actinobacteria. The effects of different temperatures and phosphorus concentration on the growth and odours production of *Streptomyces* sp. 1PDS1-11 were determined. The relative amounts of extra- and intra-cellular odours were investigated. Under optimum growth conditions, the amounts of extra-cellular odours increased as the growth progressed. Of the different temperatures tested, the maximal cell growth and odours concentration were observed at 25°C while odours productivity was yielded at 10°C. Of the different phosphorus concentration tested, *Streptomyces* sp. PDS1-11 was showed only geosmin production highest, geosmin productivity was observed at without phsphate conditions. It was indicated that low temperature and low nutrient could stimulate geosmin production.

B072

Detection of *Erwinia amylovora* and Analysis of Bacterial Diversity in Buried Fire Blight-diseased Plant and Soil

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Erwinia amylovora is Gram-negative bacteria and causes fire blight-disease. The main affected crops are known as apple and pear trees. When *E. amylovora* infected trees wither flowers, and their branches and leaves turn black as if burned down. Also, the tip of the branch is bent downward to form a hook shape. In principle, *E. amylovora* infected plants are controlled by burial. We recruited burial sites to check how much *E. amylovora* survive in the buried diseased plants and soil. So diseased plants and soil were collected from burial sites in Anseong and Chungju. Thirteen months have passed since Anseong was buried and 15 months have passed since Chungju. The collected diseased plants were subjected to polymerase chain reaction (PCR), and the collected soil was subjected to microbiome taxonomic profile (MTP) analysis of 16SrDNA from bacteria. No *E. amylovora* was detected as a result of PCR. As a result of MTP, 752,153 reading was made in Anseong soil, and 81% of them were Basilli. In Chungju, 446,314 reading was found, and Acinetobacter spp. accounted for the highest ratio among them. As a result, *E. amylovora* was not detected even in buried soil.

B073

The Effects of Cropping Systems on Soil Microorganism Communities under Potato (*Solanum tuberosum* L.) Cropping Slope Field

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Seed potato was mainly produced by Gangwon province of which consists of large amounts of slop field. In this research work, we investigated the soil microorganism community using NGS (next generation sequencing) analysis on four potato cropping systems regarding rotation, monoculture, and cover crop during three years. Soil chemical properties and physical characteristics were also analyzed. Soil chemical properties were affected by potato cropping systems while physical properties were no significant differences among the four treatments. Soil pH, organic matter, available phosphate, and exchangeable cation were increased in cultivation treatment compared to the bared field. PERMANOVA and β -diversity showed distinct bacterial communities depending on climate (year) and four cropping systems, namely bacterial OTUs were influenced by cropping systems. Cover crop cultivation after harvest was decreasing α -diversity of both rotation and monoculture cropping. As a result of correlation analysis, Shannon index which is one of the microbial diversity index, showed negative relationship with most soil physiochemical index including organic matter, soil porosity and exchangeable cation. In conclusion, the potato cropping system could affect soil microbial and physiochemical properties in which interacted with each other. [This research work was supported by grants from Rural development administration (PJ01346004)]

B074

The Gut-lung Axis and Lung Virome Analysis in Asthma Patients

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Asthma is a heterogeneous disease caused by various pathological mechanisms. Viruses are considered one of the factors on the development and exacerbation of asthma, but their roles are not yet clear. The purpose of this study was to investigate the viral microbiome in the airways of asthma patients compared with healthy participants. Sputum samples were collected from 12 healthy and 30 asthma patients and viral particles concentrated using ultrafiltration spin tube. Viral DNA and RNA were extracted and processed with random hexamers. There was clear contrast in airway virus diversity and composition between control and patient group, while 16S rRNA based on bacteriome showed no difference. Herpesviruses were the most abundant type of virus in the asthma group, mainly with cytomegalovirus (CMV) and Epstein-Barr virus (EBV) in contrast to those in the healthy controls. The abundance of CMV and EBV were showed strong correlations with the prevalence, severity, and the exacerbation of the disease as well as lung functions. The results implied that hidden herpesvirus reactivation occurs in the lung of asthma patients. The herpesvirus could be used as a biomarker for upcoming asthma exacerbation.

B075

Sediment Ingestion and Defecation by the Marine Polychaete *Capitella teleta* Shifts Sediment Microbial Community

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Deposit-feeding benthic invertebrates are known to modify sediment structure and impact microbial processes associated with biogeochemical cycles in marine sedimentary environments. Despite this, however, there is limited information on how sediment ingestion and defecation by marine benthos alters microbial community structure and function in sediments. In the current study, we used high-throughput sequencing data of 16S rRNA genes obtained from a previous microcosm study to examine how sediment processing by the marine polychaete *Capitella teleta* specifically affects sediment microbiota. Here we show that both sediment ingestion and defecation by *C. teleta* significantly alters overall microbial community structure and function. Sediment processing by *C. teleta* resulted in significant enrichment of sediment microbial communities involved in sulfur and carbon cycling in worm fecal pellets. Moreover, *C. teleta*'s microbiota was predominantly composed of bacterial functional groups involved in fermentation, relative to microbiota found outside of the host. Collectively, results of this study indicate that *C. teleta* has the ability to alter microbial biogeochemical cycles in the benthic sedimentary environment by altering microbial assemblages in the worm gut, and in the sediment ingested and defecated by worms. In this sense, *C. teleta* plays an important role as an ecosystem engineer and in shaping nutrient cycling in the benthic environment.

B076

Unearthing the Role of Soil Microbiome in the Survival of Climate-sensitive Tree

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Under the influence of global warming, vegetation inhabiting climate-sensitive ecosystems such as alpine and subalpine faces serious threats such as habitat loss and increased extinction potential. *Abies Koreana*, a native species of Korea, is suffering from decreased habitat and population increase dead trees due to the above causes. Various researches have continued to predict the distribution of *Abies Koreana* and characterize the habitat environment using diverse statistical techniques, distribution prediction modeling, geographic information system (GIS) to find the suitable countermeasure to this situation. However, there are still no complete and precise research results on the causes of habitat loss, decline, and death. This study aims to identify the effect of climate change on the survival of *Abies Koreana* by analyzing the rhizosphere microbiomes of *Abies Koreana*, a climate-sensitive species of Mountain Jiri. The collection of rhizosphere soil samples was carried out in dead trees (12), declining trees (21), and healthy trees (20), and analysis of 16S rRNA, ITS, and *nifH* in the DNA of these soil samples were performed. Studies have shown that each tree's rhizosphere microbiome community is different, and the characteristics of each community can be determined. Through the results, it was possible to confirm the correlation between the microbiome change of rhizosphere microbes and the growth, and it became a research opportunity to increase knowledge on related topics.

B077

Genes and a Proposed Metabolic Pathway for the Degradation of n-Alkane in *Pseudomonas taeanensis* MS-3

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Genes responsible for the degradation of n-alkane in *Pseudomonas taeanensis* MS-3 capable of petroleum oil degradation were identified and the metabolic pathway was proposed. Whole genome sequences of which has been known to degrade petroleum oils such as gasoline, diesel and kerosene in a previous report (Lee *et al.*, 2014). It showed that genes encoding enzymes responsible for the degradation of n-alkane compounds such as alkane hydroxylase, short chain dioxygenase, alcohol dehydrogenase, aldehyde dehydrogenase, aldehyde dismutase, nitropropane dioxygenase, esterase, etc. were presented in strain MS-3, suggesting two metabolic pathways for the degradation of n-alkane. It was first proposed that long chain n-alkane was metabolized into primary alcohol by alkane monooxygenase, sequentially into aldehyde by alcohol dehydrogenase, carboxylic acid by aldehyde dehydrogenase, acyl-CoA by acyl-CoA synthetase, and into β -oxidation pathway. Another metabolic pathway for the degradation of long chain n-alkane was metabolized directly into secondary alcohol by alkane monooxygenase, sequentially into ketone by alcohol dehydrogenase, ester by Baeyer-Villiger monooxygenase, primary alcohol or carboxylic acid by esterase.

B078

A Proposed Catabolic Pathway for the Degradation of Naphthalene in *Pseudomonas taeanensis* MS-3

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Pseudomonas taeanensis MS-3 from Taean coastal area contaminated with crude oils has been reported to degrade aromatic hydrocarbons which are major compounds of crude oils in a previous study. The pathway for the degradation of aromatic hydrocarbons could be largely divided into upper pathway involving the conversion of naphthalene to catechol or protocatechuate and a lower pathway for the subsequent degradation of catechol or protocatechuate. Genes encoding enzymes for the degradation of aromatic hydrocarbons identified in strain MS-3 were ring-hydroxylating dioxygenase, xylene monooxygenase, toluate/benzoate 1,2-dioxygenase for the upper pathway, and catechol-1,2-dioxygenase, protocatechuate 3,4-dioxygenase, 3-carboxy muconate cycloisomerase, muconate cycloisomerase, and sequential degradative enzymes for the lower pathway. Other relative enzymes additionally identified were benzoate transporter and several kinds of putative transcriptional regulators such as ICIR family transcriptional regulator, benABC operon transcriptional activator BenR, LysR family transcriptional regulator, and AraC family transcriptional regulator. A naphthalene degradation pathway in strain MS-3 is proposed based on the putative enzymes identified in this study.

B079

Different Rumen pH Changes Rumen Fermentation Characteristics and Microbiome of Dairy Cows

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Sunchon National University

This study evaluated the effects of approximately 5, 6, and 7 rumen pH on the rumen fermentation characteristics and microbiome. Cannulated Holstein cows in 6x3 Latin square design were fed with different proportions Klein grass, concentrate, and total mixed ration to achieve the approximately 5, 6, and 7 rumen pH. Rumen fluids were collected after 14 days of the feeding. As a result, we obtained an average pH of 5.63, 6.19, and 7.16.

Approximately \approx pH 5 rumen fluid resulted in significantly highest acetate, propionate, butyrate, and total volatile fatty acids. Rumen fluid pH is significantly negatively correlated to acetate, butyrate, propionate, and total volatile fatty acids as well as bacteria, Firmicutes, and Actinobacteria relative abundances while the opposite was observed with Bacteroidetes relative abundance. Firmicutes had the highest relative abundance followed by Bacteroidetes. *Christensenella*, *Ihubacter*, *Lutispora*, *Eubacterium*, *Companilactobacillus*, *Howardella*, and *Streptococcus* relative abundances were inversely proportional to rumen pH. *Methanobrevibacter millerae* had the highest relative abundance followed by *M. olleyae*, and both significantly highest in rumen fluid with approximately \approx pH 6. *Methanobrevibacter boviskoreani* and *Methanosphaera stadtmanae* relative abundances were inversely proportional to rumen pH. This study revealed that different rumen pH changes rumen fermentation parameters and microbiome.

[Supported by National Research Foundation of Korea.]

B080

Transcriptome Profiling Analysis of the Sodium-pumping Rhodopsin Bearing Flavobacterium

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Microbial rhodopsins are a light-activated 7-transmembrane protein that functions as an ion pump or sensor. They are commonly found in euphotic zone microorganisms and play important roles in various biogeochemical cycles. *Nonlabens dokdonensis* DSW-6 belongs to the Bacteroidetes phylum, which constitutes a significant proportion of marine bacteria. Interestingly, *N. dokdonensis* DSW-6 contains two rhodopsins that pump out protons and sodium, respectively. Proton-pumping rhodopsin (PR) is known to enhance the production of ATP, a biological energy source, by generating a proton motive force. Besides, the intracellular physiological role of sodium-pumping rhodopsin (NaR) has not yet been identified. The purpose of this study is to investigate NaR-related metabolic processes by comparing the gene expression patterns of a NaR-deletion mutant with those of wild-type. Under the depleted- nutrients, high-salinity, and photic condition, the expression levels of several genes related to the cysteine biosynthesis, secondary metabolite biosynthesis, and type 9 secretion systems were significantly decreased in the NaR mutant. These transcriptome analysis may provide the clues to infer the physiological advantages of NaR to marine microorganisms as well as the ecological significance of NaR.

B081

Diversity of Halophilic and Halotolerant Bacteria in Salt Farm and Mud Flat of the West Coast in South Korea

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In this study, the culturable halophilic and halotolerant bacterial diversity was determined in salt farm and mud flat of the west coast in South Korea. Ten kinds of samples of coastal mud, salt crystals, and salt farm were used to culture them using different media and incubation conditions. Total 352 isolates were grown over 7% NaCl and all isolates were selected for further 16S rDNA gene sequence studies. Based on 16S rRNA gene sequence analyses, the isolates exhibited 97.9–100% sequence similarity to the closest known species of the genera *Marinobacter*, *Roseovarius*, *Halomonas*, *Spiribacter*, *Palleronia*, *Psychroflexus*, *Idiomarina*, and *Chromohalobacter*. Furthermore, 21 isolates showed the lower 16S rRNA gene sequence similarity below 98.7%, which means they are novel genomic species. 331 isolates showed high sequence similarity to validly published species which can be recognized as unrecorded species. These result showed that until now, there have been very a few halophilic bacteria found, and with a little effort, it has shown that a new species can be easily found.

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B082

Lichen-associated Bacterial Communities are Structured according to Mycobiont Type in Polar Regions

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Lichens are symbiotic organisms that are majorly composed of fungi (mycobiont) and algae and/or cyanobacteria. In addition, lichen-associated bacteria (LAB) as an additional and integral component of lichen symbiosis has been proposed and interactions among diverse components have been considered to support a successful survival in extreme environmental conditions. To understand the potential functions of bacteria within the lichens in the subpolar and polar regions, we analyzed the composition and community assembly processes of LAB communities of 182 specimens of the 8 genera. *Alphaproteobacteria* followed by *Acidobacteria* and *Betaproteobacteria* dominated the LAB communities across all samples. The LAB composition was strongly influenced by lichen host followed by the geographical location, photobiont type, and growth type. One ASV with 96.4% 16S rRNA gene similarity with *Acetobacter tropicalis* subsisted in 83 specimens while some ASVs were found only in specific hosts. Stochastic processes largely shaped the assembly of LAB communities across all samples. Functional prediction analysis revealed that the functional patterns related to transporters, photosynthesis proteins, and prokaryotic defense systems were different according to the lichen genus. Our study shows that LAB communities of lichens in subpolar and polar regions are largely structured according to host-related factors and it may, in turn, contribute to the differentiated ecosystem functioning of lichens.

B083

Gut Microbiome Changes in Korea Antarctic Wintering-over Personnel

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It is well known that the gut microbiome is related to the health conditions of the host. In addition, it is affected by diet, age, and environmental conditions. During the summer mission period, the new summer crews stay with the existing crews at each station, suggesting a possibility that induces gut microbial changes in all of the crews. On the other hands, during the long winter mission period, the winter crews at the South Korean Antarctic research stations, King Sejong and Jang Bogo, live in polar conditions after being isolated from their original living conditions. They live without any external contact during the winter season. This environmental change may induce their gut microbiome composition dramatically, which would affect the health of the winter crews. During the summer season, many visiting scientists stay with the station crews, suggesting a possibility of gut microbial changes in all of the crews. In this study, we conducted monthly fecal sampling of all crews before, during and after the winter mission. To investigate the gut microbial changes, we analyze 16S rRNA gene amplicons by computational comparative analysis. The results from this study showed that the composition in microbiome of the winter crews changed and the alpha diversity of them increased during the mission. Due to the lack of gut microbiome studies based on isolated people for a long period, this study will suggest the impact of isolated or polar environments on the human gut microbiome.

B084

Characterization of Two Novel Lytic Bacteriophages to Control *Clostridium perfringens* in Poultry

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Clostridium perfringens cause avian necrotic enteritis (NE) which is one of the significant enteric diseases in terms of economic losses of industrial poultry worldwide. Herein, we characterized two novel lytic bacteriophages infecting *C. perfringens*. Forty-five *C. perfringens* strains collected from chickens with intestinal lesions of NE were used to identify lytic phages. Novel *C. perfringens* phages, CJ_CP_20-11-1 and CJ_CP_20-15, were isolated from sewage samples in livestock farms in South Korea. These phages were characterized by transmission electron microscopy, host range, whole genome sequencing analysis, and pH/thermal stability tests. Two *C. perfringens* phages belonging to the family *Myoviridae* lyse 45 out of 45 *C. perfringens* and are strongly lytic forming clear plaques in a bacterial lawn. The genomes of these phages are linear double-strands of DNA with 51,423 bp and 51,650 bp containing 73 putative open reading frames, which did not detect any virulence, lysogeny, or antibiotic resistance genes respectively. Both phages showed stability at pH 4–10, and retained their activity at 60°C even after 24 h. These results showed that bacteriophage can be considered as a new strategy to control the dissemination of *C. perfringens*; especially, phage CJ_CP_20-11-1 and CJ_CP_20-15 can be used as an alternative feed additive to dietary antibiotics against *C. perfringens* in poultry.

B085

Analysis of Microbiota in Chronic Rhinosinusitis Patients with Nasal Polyps

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Chronic rhinosinusitis (CRS) is a common inflammatory upper airway disease described by persistent symptoms of the nasal cavity and paranasal sinuses for 3 months. The nasal microbiome could contribute to the development of CRS. However, the role of nasal microbiota in CRS remains unclear. In this study, we analyzed the composition of nasal microbiota in CRS according to anatomical location of the sampling site and disease phenotype. Nasal microbiota was analyzed from 108 subjects (95 patients with CRSwNP, 13 control subjects). 95 patients were collected in 3 tissue types. (8 tissue from the uncinate process, 8 tissue from the ethmoid sinus, 79 tissue from nasal polyp). The microbiota was analyzed by Illumina MiSeq based on 16S rRNA gene sequencing. The nasal microbiota were significantly different among anatomical sampling sites. *Cutibacterium*, *Corynebacterium*, and *Staphylococcus* were dominant genera in samples, and their proportions were different among the sampling sites. The microbiota in the NP was clustered within 4 clusters. The predominant genera in each cluster were different (Clusters: 1, *Corynebacterium*; 2, *Cutibacterium*; 3, *Haemophilus*; 4, *Staphylococcus*). We also analyzed the differences of clinical features among 4 clusters. We will analyze the functional genes of nasal microbiome by shotgun metagenome sequences. These results can help to understand the potential role of nasal microbiome in the pathogenesis of CRSwNP.

B086

Investigation of Macrofungal Diversity in Juwangsang National Park

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The Juwangsang was designated as a national park in 1976 and has maintained its well-preserved natural ecosystem. However, the knowledge about macrofungal diversity is still unknown. Thus, the National Institute of Biological Resources (NIBR) has been conducted a project to investigate macrofungal diversity in the national parks from 2018 to 2019 and collected 419 specimens. The mushrooms were identified using morphological observation and DNA work. As a result, 261 species (two phyla, four classes, 17 orders, 52 families, and 125 genera) were identified, including 107 novel species candidates and eight unrecorded species. Here, we provided the information of macrofungal diversity of Juwangsang national park with the description of unrecorded species.

B087

A Bacterial Novel Species Candidate of the Genus *Altererythrobacter* Isolated from a Tidal Flat of Garorim Bay in South Korea

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A yellow coloured bacterium, designated strain JGD-16^T, was isolated from a tidal flat of Janggu-do, Garorim bay, Taean-gun, Chungcheongbuk-do, Republic of Korea. Cells were Gram-staining-negative, aerobic, non-flagellated, and short ovoid to coccoid-shaped bacterium. Growth was observed at temperature 10–37°C (optimum, 30°C), at pH 6.0–9.0 (8.0), and at 1–5% (w/v) NaCl concentration (2%). The 16S rRNA gene sequence analysis indicated that strain JGD-16^T was closely related to *A. xiamenensis* LY02^T (97.1%), *A. aurantiacus* O30^T (96.3%), *A. ishigakiensis* JPCCMB0017^T (95.8%), *A. epoxidivorans* JCS350^T (95.7%), and *A. insulae* BPTF-M16^T (95.3%). Phylogenomic analysis using Maximum-likelihood algorithm showed that strain JGD-16^T formed a clade with *A. ishigakiensis* NBRC 107699^T (CP015963), *A. insulae* BPTF-M16^T (QURJ00000000), *A. xiamenensis* CGMCC1.12494^T (FXWG00000000) and *A. epoxidivorans* CGMCC 1.7731^T (CP012669). The genomic DNA G+C content was 57.8%. The predominant respiratory quinone was ubiquinone-10. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, sphingoglycolipid, an unidentified glycolipid, and an unidentified lipid. The major fatty acids were C_{18:1} ω7c (31.5%) and C_{18:3} ω6c (19.6%). On the basis of phylogenomic, physiological, and chemotaxonomical characteristics, strain JGD-16^T represents a novel species within the genus *Altererythrobacter*, for which the name *Altererythrobacter* sp. JGD-16^T sp. nov. is proposed. [This research was funded by the Ministry of Oceans and Fisheries of Korea (MOF) [20170318 and 20170325], and a Korea University Grant.]

B088

Comparison of Mock Communities Based on Probiotics Using Various Regions and Five NGS Platforms

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The analysis of microbial profiling based on the 16S rRNA gene has been used to investigate microbiome composition in food, environment, and various fields. Many studies like microbial community analysis were rapidly performed by the development of the next-generation sequencing (NGS). Although NGS technology has been developed, microbial profiling results are biased by different library preparation methods, various NGS equipment, bioinformatics tools, or analysis methods. In this study, eight strains (*Lactobacillus acidophilus* KCTC 3164^T, *Lactobacillus fermentum* KCTC 3112^T, *Lactobacillus gasseri* KCTC 3163^T, *Lactobacillus paracasei* subsp. *paracasei* KCTC 3510^T, *Lactobacillus reuteri* KCTC 3594^T, *Bifidobacterium animalis* subsp. *lactis* KCTC 5854^T, and *Bifidobacterium breve* KCTC 3220^T, and *Lactococcus lactis* subsp. *lactis* KCTC 3769^T) were selected among 19 probiotics announced by the Korean Ministry of Food and Drug Safety. In addition, extracted genomic DNAs from selected probiotics were pooled to eight mock communities according to the results of copy number from Droplet digital PCR (ddPCR). All mock communities were amplified to V1-V2, V3, V4, V1-V3, and V1-V9 regions for comprising bias. Also, Miseq (Illumina), IonTorrent (ThermoFisher Scientific), MGISEQ-2000 (BGI), Sequel II (Pacific Biosciences), and MinION (Oxford Nanopore) NGS platforms performed to sequencing. As a result, the microbial profiling of the V1-V2 and V3 regions showed similar to the original ratio of the in a comparison of primer-dependent bias. By contrast, the V1-V3 region showed relatively biased. In a comparison of platform-dependent bias, the NGS sequencer based on short-read (Miseq, IonTorrent, and MGISEQ-2000) were lower biased while those based on long-read (Sequel II and MinION) were relatively biased in mock A, C, E, G, and H. In the results of all-region and platforms, *L. acidophilus* showed greatly underrepresented. By contrast, *Lactococcus lactis* subsp. *lactis* showed greatly overrepresented. In the results of PCoA and Bias Index (BI), the relatively biased samples showed separated from other samples with original in PCA plots and were calculated to high BI value. According to the results of many studies, the regions or whole genomic DNA containing AT or GC rich can lead to sequencing bias. However, we confirmed that only low-GC contents of the whole genomic DNA caused bias to microbial profiling in this study. We suggest that a new internal standard should be developed and corrected to minimize the bias of microbial profiling.

C001

Screening and Identification of Bioactive Peptides Derived from Collagen Using *Lactobacillus* Species

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Bioactive peptides (BPs) are chains of covalently linked amino acids that play a significant role in human physiology and metabolism. In recent years, food-derived BPs have received considerable attention as post-biotics for human gut health. However, conventional hit-and-miss approaches for screening BPs are time-consuming and labor-intensive. Herein we developed a bioinformatics-aided strategy to screen collagen BPs using *Lactobacillus* strains for binding to gastrointestinal G-protein coupled receptor (GPCR). *In silico* protein digestion revealed numerous BPs with a wide range of biological functions embedded in fish collagen. To release collagen BPs, bacterial substrate utilization experiments integrated with comparative genomic analyses were performed to choose three *Lactobacillus* strains as potential collagen BP producers. The collagen hydrolysates obtained by anaerobic digestion with *Lactobacillus* strains were fractionated further using stepwise ultrafiltration (i.e., > 10 kDa, 1–10 kDa, and < 1 kDa). Remarkably, < 10 kDa fractions exhibited binding activities to GPR35 and GLP2, GPCRs highly expressed in the gastrointestinal tract. In addition, several putative collagen BPs were identified using nano-scale liquid chromatography-mass spectrometry. These results suggest that such a rational approach enabled us to screen low molecular weight collagen peptides as signaling molecules interacting with gastrointestinal GPCRs.

C002

Evaluation of Functional Verification on *Abeliophyllum distichum* Nakai at Antibacterial Effect and Cell Proliferation

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Interest in the study of various medicinal plants has increased globally during last few decades, may be due to their antibacterial and antioxidant activities. This study was to verify the antioxidant effect, antibacterial activity, collagen synthesis and cell viability of adipocytes by *Abeliophyllum distichum* Nakai. Antibacterial activity was measured through the Disc diffusion method to compare the growth ability of pathogenic microorganisms (*E.coli*, *Salmonella*). Measuring the absorbance at 560 nm to calculate the active oxygen scavenging ability. Fibroblasts were dispensed in a 96 well plate at a density of 1×10^5 cells/well. The amount of procollagen was measured by procollagen type 1 C-peptide EIA KIT (MK101, TaKaRa). The cytotoxicity of AdN extract against animal adipocytes (Hanwoo backfat cell) was determined using the MTS Assay, a method that measures the conversion of MTS to Formazan by Mitochondrial Dehydrogenases. The concentration of the sample was made to be 0.0125, 0.025, 0.05, 0.1, 0.5% completely absorbed into the disc in an incubator at 37°C for 24 to 36 h. The 0.125 mg/disc of *Abeliophyllum distichum* Nakai effect on antioxidant effect, antibacterial activity, and cell viability of adipocytes. However, *Abeliophyllum distichum* Nakai was no effect on collagen synthesis. Therefore, this result suggests that *Abeliophyllum distichum* Nakai extracts could be useful for prevention or treatment of obesity.

C003

Correlation between the Gut Microbiome Composition and Body Weight of Pigs: Longitudinal Study

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Gastrointestinal microbiota composition has been known as a crucial factor in the swine industry. In this study, the association between the gut microbial community and body weights of pigs were investigated across different growth stages from pre-weaning to growing at 2, 8, and 14 weeks of age. A total of 48 fecal samples from heavy (n = 8) and light (n = 8) weight pigs were selected for cross-sectional and longitudinal studies of intestinal microbial communities. Total DNA was extracted from fresh fecal samples and the V5 to V6 hypervariable regions of the 16S rRNA genes were amplified and sequenced using the Illumina MiSeq platform. Beta-diversity analysis revealed that microbial community structures were significantly different among the three age groups. Distinct differences in fecal microbial composition were observed at the phylum and genus levels. At the genus level, at week 2, population of *Corynebacterium* was elevated in the light group than in the heavy group. In contrast, *Faecalibacterium* in heavy group was higher; at week 8, higher abundance *Prevotella* was observed in the heavy group than in the light group; at the week 14, *Roseburia* was observed as significantly high in heavy group than in the light group. The pig gut microbiota may be associated with the differences in body weight at different growth stages. Our results may provide insights into swine gut microbiome succession related with body weight gain at different growth stages.

[Supported by grants from NRF.]

C004

Effects of Saponin (Garlic Extract) Supplement on the Gut Microbial Compositions and *Lawsonia intracellularis* Fecal Sheddings in Piglets

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Saponin (garlic extract) is one of the plant extract diets, and it is a feasible feed additive to control post-weaning infections by modulation of the gut microbiota. Also, garlic saponins are known to have various beneficial activities such as antitumor and antimicrobial effects. This study was conducted to determine effect of garlic extract on the intestinal microbiota and *Lawsonia intracellularis* fecal shedding of weaned piglets. A total of 12 Landrace-Yorkshire-Duroc (LYD) pigs at 4 weeks of age were randomly assigned to 2 dietary treatments (2 pigs/pen; 3 pens/treatment). The dietary treatments were 1) control diet based on corn and soybean meal (CON) and 2) garlic group (CON + 300 ppm garlic extract). Fecal samples were collected at 0 and 6 weeks of treatments. Total DNA extracted from fresh fecal samples were used to amplify the 16S rRNA gene for Illumina MiSeq sequencing. Sequencing data was analyzed using QIIME software. Assessment of *L. intracellularis* in feces was conducted using quantitative real-time PCR (qPCR). The fecal microbiome of the garlic fed piglets showed high relative abundance of bacteria in the genus *Parabacteroides*, *Methanobrevibacter*, *Oscillospira* and *Ruminococcus*. In addition, fecal shedding of *L. intracellularis* was significantly low in garlic extract-fed piglets. In conclusion, supplementation with garlicon reduced the fecal shedding of *L. intracellularis* and caused intestinal microbial shifts in weaned piglets. [Supported by grants from RDA.]

C005

Microbiome Regulate the Depression in the Intestine and Brain

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The human intestinal microflora were used to assess whether individuals with a depression phenotype can be identified from healthy reference subjects. The gastrointestinal tract is stimulated by exogenous neurons that connect with its intestinal and central nervous systems. Nerve distribution detects and responds to various stimuli in the gastrointestinal tract, which not only regulates motility and secretion, but also affects physiology, behavior and immunity. The basic mechanisms of gastrointestinal neuron formation have begun to emerge. Research on the things that keep plastics over the life of the organism still remains.

C006

Molecular Study of Microbiome-regulated Insect Odorant Receptors

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The highly specialized olfactory receptor neurons (ORNs) on the antennae of male moths can recognize blends of several pheromone components. In present study, a total of six candidate pheromone compounds are found and functionally characterized in the electrophysiological study. In here, we report on novel candidate pheromone compounds in the same species. The olfactory receptor is analyzed revealed that finding compounds are specifically affect in on olfactory receptor. *In silico* study revealed that odorant-gated ion channels comprised of a highly conserved co-receptor and our chemicals are binding at extra cellular site. Functional analyses on the odorant-gated ion channels comprised of a highly conserved co-receptor were then performed using the heterologous expression system of *Xenopus* oocytes. pheromone components did not respond to any tested pheromone components and analogs. These results may contribute to clarifying how pheromone detection works in odorant-gated ion channels comprised of a highly conserved co-receptor.

C007

Isolation of *Lactobacillus brevis* SCML 432 Having Antioxidant Activity and Medium Optimization for Improving Biomass by Response Surface Methodology

Ji Won Seo, Hee-Jong Yang, Su-Ji Jeong, Myeong Seon Ryu, and Do-Youn Jeong*

Microbial Institute for Fermentation Industry (MIFI)

We selected, and measured its enzyme activity and antibiotic resistance. Next, we investigated its cell growth, showed maximum biomass of 3.5 g/L after 28 h of culture. The ingredients of the medium to improve biomass were selected using the Plackett-Burman design (PBD) and central composite design (CCD). The results obtained using PBD revealed molasses, yeast extract, and maltose to be significant factors determining the biomass of the *L. brevis* SCML 432 strain. The CCD was then applied with three variables found from PBD and the optimum values were predicted to be 5.5% molasses, 1.5% yeast extract, and 2.0% maltose, and the maximum biomass was predicted to be 11.2 g/L. Through model verification, we confirmed that the predicted and actual results were similar, with about 3.2-fold improvement in the biomass from 3.5 g/L to 11.3 g/L when compared to that obtained in basal medium. These results suggest that SCML 432 has high potential in the food industry as a starter.

[This work was supported by a grant from the Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA)].

C008

Potential Probiotic Properties *Bifidobacterium breve* JSRL-01 Isolated from Fecal Materials of Neonates

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This study was carried out to isolate new *Bifidobacteridum* that can be used as probiotic material from fecal of neonates. Various physiological experiments such as extracellular enzyme, haemolytic, and antioxidant activities were conducted to select the probiotic strains, and five species of microorganisms were selected. JSRL-01 was finally selected with superior physiological activities, and identified as *Bifidobacterium breve* JSRL-01 by 16S rRNA sequencing analysis. JSRL-01 showed a high survival rate of more than 62% at pH 3.0 and 72% at 1.0% oxgall. In particular, JSRL-01 had a survival rate of more than 93% above 80°C, and also was resistant to a variety of antibiotics, which is believed to be highly to survive in the various environment including human intestines. This research is expected to be highly utilized in the probiotics industry for industrial application as well as screening *Bifidobacterium breve* JSRL-01 as probiotic resource for health funtional foods.

[This work was supported by a grant from the Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA)].

C009

Study of Diversity Using Pyrosequencing Analysis of Characterizing Microbial Community in Livestock Feces

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This study was conducted to evaluate distribution of microbial population in 78 fecal samples from swine and cows. Microbial communities were analyzed using next generation sequencing. Total of 7,214,020 sequences were used to microbial community analysis. The operational taxonomic units (OTUs) of 622-2371 feces assessed by analysis of CD-HIT. In this result, The 7,214,020 sequences were investigated to 5 phyla, of which Firmicutes was the most predominant phylum, according to 53.97% in cows and 69.43% in swine of all sequence. As microbial distribution was examined at the genus level considering two animals basically. Especially, it was only detected to *Clostridium* sp. (16.48%), *Prevotella* sp. (11.41%), and *Lactobacillus* sp. (6.77%) in swine feces. Also, microbial distribution was showed the specific difference in species level. The rate of predominant species was investigated to PAC001136 (*Clostridium*) was occupied 8.66% in swine fecal and 6.56% *Solibacillus sivestris* has portion of cows feces. Therefore, these results showed significant differences in fecal microbiota between types of source animals, suggesting that there are many diversity about fecal bacteria species.

[This work was supported by a grant from the Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA)].

C010

Characterization of Lactic Acid Bacteria Having Antibacterial and Potential Probiotics Activity Isolated from Traditional Fermented Foods

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Lactic acid bacteria isolated from traditional fermented foods (kimchi and soybean pastes) were selected on the basis of their broad spectrum antibacterial activity, safety, cell adhesion as well as resistance to acid, bile, temperature and examined for availability as a probiotic material. In order to estimate the potential risk of food poisoning, we investigated for antimicrobial activities of against foodborne pathogens (*Bacillus cereus*, *Escherichia coli*, *Listeria ivanovii*, *Listeria monocytogenes*, *Micrococcus luteus*, *Salmonella* Typhimurium, *Shigella sonnei*, *Shigella flexneri*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and the production of harmful substance including indole, phenylpyruvic acid, β -glucuronidase, urease. Three strains selected based on antimicrobial activities results also showed notable cell adhesion activity. Selected strains identified *Lactobacillus sakei* JSRL329, *Lactobacillus paraplantarum* JSRL388, *Lactobacillus pentosus* JSRL398 by the phylogenetic analysis based on 16S rRNA gene sequencing. Thus, the three *Lactobacillus* strains could be considered as potential antimicrobial probiotic strains against human pathogens and should be further studied for their human health benefits. [This work was supported by a grant from the Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA)].

C011

Blocking Phytosphingosine Synthesis by the *SUR2* Deletion Increased the Cell Surface-associated Production of Dihydrosphingosine and Glucosylceramides in *Yarrowia lipolytica*

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Sphingolipids are essential membrane components found in mammalian cells as well as in plants and microbes. In this study, to block the PHS-based sphingolipid biosynthetic pathways in the oleaginous yeast *Yarrowia lipolytica*, a mutant strain with the deletion of the *SUR2* gene, encoding sphinganine C4-hydroxylase, was constructed and functionally characterized. The resultant *Ylsur2Δ* exhibited a retarded growth with increased pseudohyphal formation and displayed the increased sensitivity to high temperature, osmotic, and cell wall stresses compared to the wild-type strain. The treatment with myriocin or exogenous addition of PHS did not rescue the growth defect of the *Ylsur2Δ* mutant, suggesting that neither the accumulation of DHS or the deficiency of PHS is associated with the growth defect. Notably, the *Ylsur2Δ* mutant showed increased production of DHS, which was mostly secreted to the cell surface or extracellular medium even without acetylation. Furthermore, the *Ylsur2Δ* mutant showed the remarkably increased synthesis of glucosylceramides in the form of sphingosine-based ceramides, which are also mostly detected at the cell surface. Taken together, our results suggest the potential of *Ylsur2Δ* strain as a production host for cell surface-associated sphingosine-based ceramides, as well as a sphingosine precursor, DHS, which are useful ingredient for cosmeceutical or nutraceutical formulations.

C012

Screening of Antagonistic Bacteria Having Antifungal Activity against Phytopathogenic Fungi of *Panax ginseng*

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Ginseng root rot, damping-off disease, and *Alternaria* blight of *Panax ginseng* are caused by fungal pathogens such as *Pythium ultimum*, *Cylindrocarpon destructans*, *Rhizoctonia solani* AG-2-2 (IV), and *Alternaria panax* threatening the production of *Panax ginseng*. To search biological control agents (BCAs) of phytopathogenic fungi, five *Bacillus* isolates were selected from field soil of Sunchang in Korea. The selected isolates were characterized by production of enzyme, siderophore and indole-3-acetic acid (IAA) activities. Also, antifungal activity against four of the phytopathogenic fungi that frequently occur in *Panax ginseng* was tested. Among them, PBS-69 strain had the most excellent antifungal activity. Physiological characteristics of PBS-69 strain also confirmed by analysis of carbohydrate fermentation patterns and enzyme production ability. Based on the experimental results, PBS-69 strain was finally selected as a candidate for BCA. BLASTn search of the 16S rRNA gene sequence via NCBI database indicated that the isolate, PBS-69 strain matched *Bacillus amyloliquefaciens* (NR112685) with similarity values of 99.53%. Based on the above results, *Bacillus amyloliquefaciens* PBS-69 strain is expected to be used as a BCA for the *Panax ginseng* pathogenic fungi.

[This work was supported by a grant from the Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA)].

C013

Selection and Genetic Analysis of *Bacillus* Strains with Potential Probiotics Activity from Traditionally Fermented Gochujang

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As the diseases caused by westernized diets such as obesity, gastrointestinal disorders, probiotic market is rapidly growing, requiring the development of new probiotic materials. We have focused on *Bacillus* strains that can be utilized as probiotic material isolated from gochujang for confirmed that isolates have potential value as probiotic materials. First, non-hemolytic bacterial strains that do not produce harmful substances were analyzed to confirm safety to select the main bacterial strains as a probiotic. Next, the analysis of the basic characteristics of the selected 5 strains, including enzyme activity, and resistance to acid, bile, and heat. There was a need to identify the rationale behind the excellent characteristics of the five selected strains of *Bacillus* through genomic analysis. Therefore, the PacBio RSII platform was used to analyze whole genome sequence. A comparative analysis of the functional genes revealed that the five selected strains had the highest number of genes in gene groups related to amino acid transport and metabolism, after the group of genes with unknown functions. Additionally, among 1,474 genes found unique to each strain, 1,014 genes were in a poorly characterized gene group, suggesting various changes in genes in the gene pool.

[This work was supported by a grant from the Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA)].

C014

Evaluation of Probiotic Potential of *Pediococcus acidilactici* FFC 224 Isolated from Young Radish Kimchi

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Lactic acid bacteria (LAB) are a kind of bacteria that can convert saccharides to metabolites like lactic acid, diacetyl and bacteriocin. Traditionally, LAB have been used to make various fermented foods and it was recognized as generally recognized as safe microorganism. LAB have several probiotic properties in human body such as improvement of the intestinal environment because of their diverse physiological activity including bile salt hydrolase (BSH) activity and antibacterial activity. In this study, LABs were isolated from kimchi and its probiotic properties were determined. All isolates were tested in antibacterial activity, BSH activity, and DPPH scavenging activity. Among the isolates, biogenic amine production and thrombolytic activity of 3 LAB with the superior antibacterial activity were conducted to investigate food safety. The FFC 224 strain showed a higher antibacterial and BSH activity, however, BA production ability and thrombolytic activity were not detected. Finally, FFC 224 strain was selected for further experiments and it was identified as *Pediococcus acidilactici* by 16S rRNA gene sequencing. These results indicated superior potential ability of *Pediococcus acidilactici* FFC 224 as a probiotic strain.

[This work was supported by a grant from the Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA)].

C015

Primary Sample Tracking by Using Microbial Profiling of Next Generation Sequencing

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Evidences at the crime scenes are found in a variety forms like solids to liquids and gases. Available sample is biological evidence related to human identification, but visually indistinguishable samples are found indiscriminately. Various methods for identification body fluids have already been studied, but each has advantages and disadvantages. They depend on the condition of the sample and sufficient results may not be obtained. This study for carry out the purpose of proposing a microbial analysis method to determine effective identify results. In this study, 16S rRNA bacterial profiling was performed with Next Generation Sequencing (NGS) method and collected samples from gargles, urine, fingertips, and mobile phones in 10 participants. A list was prepared by collecting abundant microorganisms (metagenome seq), specific microorganisms (LEfSe), and reported microbiome strains for each part shown in the analysis results. With the list, gargle matched of all 10 strains, and about 60% of urine and more than 80% of fingers and mobile phones were able to infer the origin of each sample. These results suggest that not only human DNA but also bacterial DNA profiling can help for forensic identification of body related evidences.

C016

Comparison of Microbial Community Profiling on Korean Salting Foods, Kimchi and Jeotgal, Using Next Generation Sequencing

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In order to identify and characterize the microbial communities in two types of Korean salting foods, Kimchi and Jeotgal, microbial communities were analyzed using next generation sequencing. 40 Kimchi and Jeotgal samples for analysis were purchased randomly from all over the country, regardless of ingredients. According to the taxonomic results, the overall microbial composition was then compared between the groups. *Firmicutes* was the most common phylum in both groups, representing 96.04%, 67.92% in Kimchi and Jeotgal groups, respectively. *Proteobacteria* was the second dominant phylum, accounting for 3.16%, 25.94% in the both groups, respectively. The most dominant genus of the microbiota of the both Kimchi and Jeotgal was *Lactobacillus*. *Weissella* and *Leuconostoc* were significantly more abundant in Kimchi than in Jeotgal, whereas halophile and alkaliphilic bacterium such as *Tetragenococcus* and *Halanaerobium* is significantly more abundant in Jeotgal than in Kimchi. Especially, The *Firmicutes* to *Bacteroidetes* ratio correlated with obesity and other diseases was significantly higher in Kimchi, with a value more than seven times that of Jeotgal. As microbiological research data are still limited, this study could be useful as a microbiological study of Korean salting foods.

[This work was supported by a grant from the Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA).].

C017

Killing Effect of Deinoxanthins on Cyanobloom-forming *Microcystis aeruginosa*: Eco-friendly Production and Specific Activity of Deinoxanthins

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Cyanobacterial blooms caused mainly by *Microcystis aeruginosa* could be controlled using chemical and biological agents. Little is known about the possible toxic effects of bacterial membrane pigments on *M. aeruginosa* cells. *Deinococcus metallilatus* MA1002 cultured under light increased the production of several carotenoid-like compounds by upregulating two deinoxanthin biosynthesis genes: *crtO* and *cruC*. The deinoxanthin compounds were identified using thin-layer chromatography, high-performance liquid chromatography, and liquid chromatography-mass spectrometry. *Deinococcus metallilatus* was cultured with agricultural by-products under light to produce the deinoxanthin compounds. Soybean meal was selected as the single factor for making an economical medium to produce deinoxanthin compounds. The growth of axenic *M. aeruginosa* PCC7806, as well as other four xenic cyanobacteria, were inhibited by the deinoxanthin compounds. Scanning electron microscopic images showed the complete collapse of *M. aeruginosa* cells under deinoxanthin treatment. Deinoxanthins appeared to be nontoxic to other bacteria such as *Acinetobacter*, *Pseudomonas*, and *Bacillus* species, suggesting that it can be a novel candidate for preventing cyanobacterial blooms through its specific activity against cyanobacteria.

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C018

Development of New Compression-type Bacterial Agents for Self-healing Concrete

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Concrete crack repair using microbial-induced calcium carbonate precipitation (MICP) could be applicable for healing damaged concrete structures inaccessible to humans. A novel bacterial carrier using microcrystalline cellulose (MCC)-tablet having methylcellulose, silicon dioxide, and magnesium stearate were employed to encapsulate spores, which could improve self-healing efficiency of bacterial agents by assuring bacterial viability. Compressed MCC-tablet with 5 mm × 1 mm harbored $3.0 \pm 0.5 \times 10^{11}$ spores per dried gram of the bacterial carriers. The MCC-tablets were incorporated into three types of mortar specimens to evaluate their self-healing efficiency and mechanical properties with crack healing rates, water permeability, and compressive strength at different curing ages (0, 7, 14, and 28 days) compared with the reference group. The cracks with 0.3–0.4 mm width could be sealed in 7 days by MICP-capable bacteria in MCC-tablets, whereas control specimens were not repaired until 14 days. The water permeability of mortar specimens mixed with MCC-tablets was steadily reduced with increases of curing ages, healing cracks up to 91.1% at 28 days. Calcium carbonate formed on cracks was analyzed using scanning electron microscope, energy dispersive spectrometer, and X-ray diffraction to verify MICP and bacterial viability. Our study will provide that the new MCC-tablet is expected to introduce bacterial agents for self-healing concrete.

C019

Biotransformation of Saikosaponins of *Radix Bupleuri* Using Recombinant Enzymes, Cloned and Expressed in *Escherichia coli*

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Saikosaponins, saponin of *Radix Bupleuri*, are the major responsible component for the pharmacological and biological activities. Absorption of major saikosaponins from the gastrointestinal tract is extremely low, when *Radix Bupleuri* is orally administered. In order to improve absorption and biological activity, biotransformation of high-molecule saikosaponins into low-molecule saikosaponins (prosaikogenins) is required. Here, we applied four recombinant glycoside hydrolases that has glycoside cleavage activity of saikosaponins. Four enzymes, BglBX10, BglPm, BglL.kor, Bgl3082 are cloned from *Flavobacterium johnsoniae*, *Paenibacillus mucilaginosus*, *Lactobacillus koreensis*, *Terrabacter ginsenosidimutans*, respectively. The enzymes exhibited a good activity at between 30–37°C and pH 6.5–7.0. The major components, saikosaponin a and saikosaponin d, were purified and obtained from the crude *Radix Bupleuri* extract using Prep-HPLC system. Both were converted into saikogenin F via prosaikogenin F and saikogenin G via prosaikogenin G, respectively using enzyme transformation with high β -glycosidase activity. After reaction, four compounds were purified with 98% purity using Prep-HPLC system. This result showed little amount of saponin metabolites of *Radix Bupleuri* would be produced massively using enzyme technology, which will lead various efficacy test possibly.

C020

Anaerobic Expression of Equol Biotransformation Enzymes

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Equol is a kind of isoflavone mainly obtained from soybeans. It is biotransformed from diadzein, a precursor, and is known to have antioxidant and anti-inflammatory activities. Equol is not synthesized in the human, but is produced through the action of reductases of specific bacteria in gut microbiome. Microbiome-derived microorganisms known to produce equol are generally anaerobic bacteria, including *Adlercreutzia equolifaciens*, *Eggerthella* sp., and *Slackia isoflavoniconvertens*. In this study, we expressed equol biotransformation enzymes in *E. coli*, a facultative anaerobic bacterium, and we tried to compare the expression levels of the enzymes and the production of equol under aerobic and anaerobic conditions. From the genomic information of equol producing microbial strains, the sequences of the clusters of genes encoding the enzymes daidzein reductase (DZNR), dihydrodaidzein reductase (DHDR), and tetrahydrodaidzein reductase (THDR) involved in equol biotransformation were compared. The genes encoding DZNR, DHDR, and THDR from *A. equolifaciens* and *S. isoflavoniconvertens* were cloned in *E. coli*. When *E. coli* cells carrying equol biotransformation enzymes were grown aerobically and anaerobically in a culture medium containing soymilk, the expression levels of the enzymes and equol production were higher under anaerobic conditions. Consequently, anaerobic expression of equol biotransformation enzymes is important for the microbial production of equol.

C021

Screening Microbes for the Antagonistic Effect against *Fusobacterium nucleatum*

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Fusobacterium nucleatum is a Gram-negative, anaerobic bacillus that inhabits the human mouth and gastrointestinal tract and is likely to be associated with various diseases such as periodontitis, inflammatory bowel disease, and colon cancer. Many researchers have observed the presence of *F. nucleatum* in colorectal tumor specimens and also found at high relative abundance in gastric cancer patients. Thus, *F. nucleatum* can be an important target to prevent gastrointestinal diseases. This study found bacteria with an antagonistic effect against *F. nucleatum* by screening human gastric culture collection from stomach biopsies established in our lab and identified them by 16S rRNA gene sequencing. We performed the screening by agar overlay and cross streak methods with 913 isolates to observe zone of inhibition suggesting that the bacteria have potential antagonism against *F. nucleatum*. The results of screening turned out some candidate bacteria that belong to the *Streptococcus* genus. By co-culturing *F. nucleatum* with the supernatant of each *Streptococcus* bacteria, *F. nucleatum* showed a lower OD value than mono-culture that suggests a growth suppression. Based on the co-culture and acid tolerance tests, *Streptococcus salivarius* was ultimately turned out to have the most antagonistic effect among *Streptococcus* bacteria. To characterize and identify the antagonistic material, further experiments such as supernatant concentration, cloning, and genetic analysis could be required.

C022

Assemblages of Root Endophytic Fungi in Three Different Forest Plants and Antagonism against Major Forest Pathogens *In Vitro*

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Root endophytic fungi were isolated from *Machilus thunbergii*, *Dendropanax trifidus*, and *Quercus acuta* plants in Wando Island, Jeonnam province, Korea in 2020. A total of 34 fungal isolates were recovered in which 19 isolates were from *M. thunbergii* followed by 9 isolates from *D. trifidus* and 6 isolates from *Q. acuta*. All the isolates were sequenced with the internal transcribed spacer (ITS) gene and BLASTN search analysis revealed that *Illynectria* sp. was the most abundant and diverse fungal taxa. The other fungal taxa were *Biocogniauxia*, *Chaetomium*, *Cylindrocarpon*, *Fusarium*, *Hyphxalon*, *Lachnam*, *Mycena*, *Mortierella*, *Neonectria*, *Pezicula*, *Phanerochaete*, *Phlebia*, and *Trichoderma*. Furthermore, all the endophytic fungi were tested against five major forest fungal and oomycete pathogens: *Ceratocystis fimbriata*, *Fusarium oxysporum*, *Pythium ultimum*, *Rhizoctonia solani*, and *Sclerotinia sclerotium*. Twenty-six endophytic fungi showed inhibition against at least one pathogenic fungus. Based on the *in vitro* antifungal assay, two *Trichoderma* isolates HU10-2 and HW4 from *M. thunbergii* and *D. trifidus*, respectively were selected and cell-free culture filtrate of these isolates were tested against all the pathogens. The result revealed that the isolate HU10-2 significantly inhibited the growth of the pathogens compare to the isolate HW4. Therefore, further study is in progress to confirm the isolate HU10-2 as a biocontrol agent against forest diseases.

C023

Effects of Feeding System on Ruminal Fermentation and Microbial Population of Hanwoo Steer during the Growing Stage

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National Institute of Animal Science

This study investigated the effect of feeding system on feed in 9 month-old Hanwoo (native Korean cattle) steers. Thirty one steers were randomly assigned to three groups: (CON) Italian ryegrass and concentrate (1.6% BW) until 14 month of age, (G1) pasture grazing 101 days without concentrate, (G2) pasture grazing 101 days and concentrate (1.6% BW). The treatments were rumen fluid collected from stomach tube at 10 and 15 month-old. In rumen microbiome composition, *Firmicutes* (10 month old: 26–33%; 15 month old: 40–50%) representing the most dominant phylum, followed by *Bacteroidetes* (10 month old: 40–50%; 15 month old: 27–33%) at 10 and 15 month-old. However, there were no significant differences among the three sample groups in rumen microbiome composition. The VFA concentration did no differ among the three sample groups. The beta diversity PCoA plot indicated that the rumen microbiomes of the treatments were separated. It means that rumen microbial diversity in Hanwoo steers is affected by different feeding system.

C024

Characteristic of Microbiome in Scalp Skin of Healthy and Alopecia Subjects

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Diverse microbes exist in the human scalp skin. The skin microbiota plays important roles in maintaining skin homeostasis, and microbiota dysbiosis is associated with the onset and progression of several skin diseases. Alopecia is the most common form of hair loss, is influenced by multifactorial conditions involving genetic predisposition, hormonal change and scalp microorganisms. However, difference of microbiome in the scalp ecosystem between subjects with alopecia and non-alopecia. Here, we analyzed the scalp skin microbiome in subjects with and without alopecia (alopecia, n = 60; non-alopecia, n = 60) in different seasons (summer and winter). Clinical features, such as corneum, Trans-Epidermal Water Loss (TEWL), moisture, sebum, were obtained in subjects, too. The bacterial microbiota significantly differed by sex (P -value = 0.041), and the functional genes in scalp skin microbiome were clearly distinguished within two groups (P -value = 0.001, $R^2 = 0.7116$). The fungal microbiota were divided into three clusters. Results in this study can help to understand the role of scalp microbiome (bacteria and fungi) in alopecia.

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C025

Characterization of Halophilic Microorganisms Isolated from the Fermented Seafood

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To identify the diversity of halophilic bacteria from fermented seafoods, 86 strains were isolated and phylogenetic analysis was carried out based on the result of 16S rRNA gene sequencing. The isolated strains were divided into 3 phyla, 7 families, 9 genera, 24 species. Bacilli class, the main phyletic group, comprised 84.9% with 4 families, 6 genera and 19 species of *Bacillaceae*, *Planococcaceae*, *Staphylococcaceae*, and *Enterococcaceae*. The strains were tested for amylolytic, cellulolytic, lipolytic, proteolytic activity and 55 strains showed at least one enzyme activity. Furthermore, auxin activity was determined in two strains. These results indicated that isolated strains have the possibility of the application for the food and feed industries and importance of genetic resource in Korea.

C026

Bacterial Wilt Resistance in Tomato Plant Supported by Root Microbiome

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Plant microbiomes are important for plant disease resistance. Previously, we found that the upland microbiome fraction (MF) application fully assisted the resistance to bacterial wilt (BW) in a BW-resistant tomato cultivar Hawaii 7996, but forest soil MF transplant dramatically abolished the BW resistance in Hawaii 7996. We aimed to find out which parts of the microbiome affect BW-resistance in tomato plants. In this study, rhizosphere and endosphere microbiomes extracted from the roots of upland MF-transplanted plants were transplanted separately and BW resistance was assessed in the tomato plants. BW-resistance in Hawaii 7996 plants were only enhanced when the rhizosphere fraction was transplanted into the Hawaii 7996. The MF effect was reproduced by adopting synthetic community (SynCom) consisting of bacteria from rhizosphere soil of upland MF-transplanted tomato. After 14 days post inoculation of *Ralstonia solanacearum*, treatment of SynCom reduced the BW occurrence in tomato by 1.6-fold, compared to control plant. In addition, bacteria of SynCom did not show antagonistic activity against *R. solanacearum* on agar medium. Overall, our results suggest that the rhizosphere microbiome by upland MF transplant can fully modulate the expression of BW-resistance in the tomato plant.

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C027

Effects of Bacterial Inoculum on the Decomposition and Bacterial Community Composition in Decomposing Poultry Carcasses

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Microbes are key players in decomposition as they produce enzymes that helps in the breakdown of complex biomolecules in animal carcasses. Additional inoculants can facilitate the decomposition process. This study investigated the effects of two bacterial inoculants on the decomposition and structure of bacterial communities in decomposing poultry carcasses. Two bacterial strains, *Bacillus pseudomycooides* and *Paenibacillus cellulosilyticus*, were inoculated into the poultry carcass decomposing tanks and decomposed for 100 days. Taxonomic characterization of microbial community was analyzed using Illumina metagenomic sequencing technology. Poultry carcass decomposing tanks inoculated with *P. cellulosilyticus* have high number of observed OTUs and Chao1, while Shannon diversity was low. Meanwhile, beta-diversity analysis revealed that bacterial community composition varied between the decomposing tanks at different time points. The sequencing results showed that the dominant phyla were *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*, and the dominant genera were *Bacteroides* and *Ignatzschineria*. This study demonstrated that the inoculants affected the decomposition progress of poultry carcasses and altered the structure of bacterial communities in decomposing poultry carcasses.

[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET), funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (118099-03).]

C028

Diversity and Bioprospecting of Cold Adapted Fungi and Actinomycetes Isolated from the Arctic Norwegian Region

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We studied the diversity of microorganisms isolated from samples collected in Arctic Norway and their ability to produce bioactive compounds. Sample diluted by 10 fold dilution method after homogenization, were inoculated on NA(nutrient medium), R2A, PDA (Potato Dextrose Agar), and ISP 4 (Inorganic Salts Starch Agar) agar plate and then incubated at 10°C for 7–30 days. Fifty-four fungal and bacterial colonies were isolated and stored in 15% (v/v) glycerol solution. isolates were tested for their ability to grow at temperatures (10 and 25°C). As a result, the 28 isolates were mesophile and 16 isolates were psychrotolerant. Fungal and bacterial extracts were incubated at 15°C for 10 to 15 days and then extracted with ethyl acetate. As a result of the PTP1B inhibition assay using the extract, 15 strains showed strong inhibitory activity. From the results of identification using the internal transcription spacer (ITS) and 16S rRNA sequence, the fungal strain consisted of 49 taxa in 19 genera and the bacterial strain consisted of 4 taxa in 2 genera. These results suggest that fungi and actinomycetes isolated from the Arctic Norwegian region might be valuable resources for screening of bioactive compounds.

C029

Effect of Feeding Crude Fiber Level on Intestinal Microbiota in Growing Pigs under Heat Stress

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This study investigated the effect of Feeding Crude fiber level on Intestinal Microbiota in Growing Pigs under Heat Stress. A total of seventy five crossbred pigs (initial body weight 49.79 ± 3.52 kg) were randomly assigned to three groups (T1) feeding crude fiber 2.8%; (T2) feeding crude fiber 3.8%; (T3) feeding crude fiber 4.8%. This study was carried out for 4 weeks. At start and end of the study, fecal samples were collected from five pig for each treatment and stored at -70°C until DNA extraction was performed. The average temperature was 32.08°C , the Rh was 76.68% and average THI score was 85.71 during the study. There were no significant differences among the three sample groups in feces microbiome composition from the start and finish. In feces microbiome composition, *Bacteroidetes* (40–42%) representing the most dominant phylum, followed by *Firmicutes* (37–39%) at the start. At the finishing point, the relative abundance of *Firmicutes* (41–46%) phyla increased and *Bacteroidetes* (34–35%) phyla decreased in three groups. The beta diversity PCoA plot showed that the feces microbiomes were spread by heat stress level. [This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project title: A set of studies to evaluate the effects of different nutritional strategies in order to improve the nutrient efficiency in sows under heat stress, Project No. PJ014796)” Rural Development Administration, Republic of Korea.]

C030

Investigation of Tomato Seed Endophytes

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Bacterial seed endophytes have been known for their ability in promoting plant growth and defense against biotic and abiotic stress. To analyze the bacterial endophytes inhabiting the tomato seeds, we isolated DNA from the resistant (Hawaii 7996) and susceptible (Moneymaker and BonnyBest) tomato cultivar after removing microbiota on the surface of them. After that, 16S rRNA gene-based taxonomic profile was performed. The comparison of bacterial seed endophytes among the cultivars showed that *Proteobacteria* was significantly more abundant in the resistant cultivar than in the susceptible cultivar. Moreover, the relative abundance of *Firmicutes* was significantly higher in the susceptible cultivar compared to in the resistant cultivar. To construct the culture collection of tomato seed endophytes, the surface-sterilized tomato seeds were ground using the sterilized mortar and pestle. Nine endophytic bacteria were isolated. Among them, *Paenibacillus* sp. and *Bacillus* sp. displayed the effect of growth promoting on the tomato plant and antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* causes Fusarium wilt disease in the tomato plant. In conclusion, the endophytic bacterial community between susceptible and resistant cultivar of tomato seed was significantly different and the isolated endophytic bacteria showed a beneficial effect on the tomato plant.

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C031

Analysis of Bumble Bee Gut Microbiome

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One-third of the food humans consume requires pollination of insects such as bees. However, bee populations continue to decline for several reasons: exposure to chemicals, habitat loss, and pests and pathogen. In the recent research, bee gut microbiota play an important role of pathogen defense. We hypothesized that the bee (*Bombus terrestris*) gut microbiome between resistant (RH and RR) and susceptible (RL) to specific entomopathogen may be different. Total 14 gut microbes were cultured. Among them, 4 isolates displayed the antifungal activity against *Ascosphaera apis* causes chalkbrood disease in honeybees. To compare the gut bacterial community between the resistant and susceptible bee, alpha-diversity and beta-diversity analysis was performed. The analysis of alpha-diversity revealed that RH was significantly higher in Shannon diversity than RL was. The analysis of beta-diversity showed that the RH gut bacterial community was significantly different as compared to that of RL. Total 4 bacterial phyla were identified, and *Proteobacteria* was the most abundant across all samples. Especially, three OTUs belong to *Pasteurellales*, *Burkholderiales*, and *Pseudomonadales* were solely identified in RL. In conclusion, the gut bacterial community between resistant and susceptible bee was significantly different and this difference may contribute to disease resistant.

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C032

Isolation and Enzymatic Characterization of *Janthinobacterium* sp. 4CPA Secreting Extracellular Lipase

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Bacterial enzyme are versatile enzymes, and produced the attention of the several industrial processes. Lipase can catalyze the hydrolysis of ester bonds of triglycerides and besides hydrolysis activity they display interesterification, esterification, aminolysis activity. We isolated lipase-producing bacterium strain 4CPA. On the basis of 16S rRNA gene sequence, strain 4CPA was shown to belong to the genus *Janthinobacterium* and most closely related to *Janthinobacterium lividum* DSM 1522^T (99.58%) and *Janthinobacterium rivuli* FT68W^T (99.65%). The *Janthinobacterium* sp. 4CPA was optimal growth at 25°C, 0–1.5% NaCl and pH 5.5–8.5. The highest lipase activity was showed at 20°C, when it reached 216 U/ml. This lipase had a relatively high stability of more than 80% between 4–15°C. Furthermore, *Janthinobacterium* sp. 4CPA was produced esterolytic enzymes such as esterase and esterase lipase. Our results obtained that lipase/esterase-producing bacterium active at low temperatures of potential use in industrial processes.

C033

Biological Control of Potato Common Scab by Antagonistic Bacteria Isolated from Roots of Resistant Cultivar

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Potato common scab disease is generally caused by *Streptomyces scabiei* pathogen. This disease decreases the quality of potatoes and results in economic damage to farmers. The pathogen is soil-borne which extremely difficult to control the disease. Although many researchers tried to develop the control technique last few decades, effective techniques are yet undeveloped even chemical control. The use of genetically resistant cultivars is the best way to control the disease. In this research work, we tried to elucidate the mechanism of resistant cultivar with a comparison between susceptible-Daeji- and resistant-Seohong- cultivar and find promising sources to control the disease. In the pot experiment, the cultivation of Seohong reduced the pathogen density and bacterial and fungal communities were significantly affected by cultivar with NGS (Next Generation Sequencing) analysis. Though root exudates gained with water culture showed no significant antagonistic activity against *S. scabiei*, some bacterial isolates from root of resistant cultivar showed clear antagonistic activity against *S. scabiei*. In conclusion, there were promising bacteria that inhabit the root of resistant cultivar and they support the resistibility of resistant cultivar on potato common scab disease.

[This research work was supported by grants from Rural development administration (PJ01486603).]

C034

Characterization and Genomic Analysis of Two Novel Eubacteriaceae Bacterial Strains ES2 & ES3

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An anaerobic, mesophilic, chemolithoautotrophic, and acetogenic bacterial strains ES2 and ES3 were isolated from sediment of Eulsukdo, a delta island in South Korea. Both strains clustered in coherent branch and were phylogenetically located between the genera *Acetobacterium* and *Eubacterium*. Considering phylogenetic distance over 16S rRNA and ANI values with neighboring genera, ES strains appear to be novel at the genus level. The genome size of the strains ES2 and ES3 was 3.76 Mbp and 3.39 Mbp, respectively and the DNA G + C contents of two strains ranged 39.6–40.7 mol%. Genomic evidence for the Wood-Ljungdahl pathway in ES strains reflects chemolithoautotrophic growth. Both strains could grow with CO₂/H₂ or CO as the sole sources of carbon and energy. ES2 expected to utilize methanol as an intermediate of WLP via genomic analysis and it was proved by batch culture. In addition, the genome of the strain contained a group of genes involved in lactic acid, 1-propanol, 2,3-BDO, and ethanol metabolism. A comparative genome analysis of ES strains with *Acetobacterium woodii* and *Eubacterium limosum* was conducted on key clusters of WLP. Synteny analysis revealed that genome arrangement of two novel strains were close to *Acetobacterium woodii* than *Eubacterium limosum*. In this study we aim to isolate industrially valuable acetogen strains from the marine environment. As a result we isolated ES strains and identified their genomic and metabolic characteristics.

[Supported by KIOST.]

C035

Screening of Exopolysaccharide Producing Lactic Acid Bacteria Derived from Seaweed in East Coast

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Exopolysaccharide (EPS) are polysaccharides secreted from the bacterial cell, or produced on the outer cell by extracellular enzyme. Among the various EPS producing bacteria, Lactic acid bacteria (LAB) have gained special attention because of their capability to produce EPS having wide diversity of structures and biological activities including immune stimulation, anti-oxidant and anti-bacterial without health risk, therefore, generally recognized as safe (GRAS). A total of 323 strains of bacteria were isolated from marine samples (seaweeds and surface water) collected from the East Coast in July 2019. Among them, 54 strains of lactic acid bacteria were isolated from BCP-added MRS medium. As a result of screening in MRS solid medium containing 10% sucrose, 15 strains secreting a transparent viscous substance were selected. Based on 16S rRNA sequence analysis, it was found to be similar to *Lactobacillus* sp. and *Leuconostoc* sp. After culturing for 24 h, only the EPS in the supernatant were recovered by ethanol precipitation, and the crude EPS were quantified by the phenol-sulfuric acid method. As a result, it was reached up to 6.7 g/L of EPS. Finally, 4 strains were selected as strains that produced a large amount of EPS, and the biological activities of these EPS are expected.

[This study is the result of the research and development project supported by Gyeongsangbuk-do.]

C036

Safety Evaluation of Probiotics Strains Isolated from Korean Traditional Fermented Foods

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Four probiotic strains (three *Lactiplantibacillus plantarum* and one *Bifidobacterium bifidus*) were isolated from three Korean traditional fermented foods. The safety of these strains was evaluated by genotype and phenotype tests.

By searching Comprehensive Antibiotic Resistance Database (CARD) and Virulence Factor Database (VFDB), their whole genome sequences were used to detect virulence factor and antibiotic resistance gene. Antibiotic resistance gene and virulence factor should not be found on their genome sequence. These may have been transferred through horizontal gene transfer from the other bacteria.

As a result, there was no acquired antibiotics resistance gene or virulence factor. This indicates that they are safe *in silico*. Hemolysis test was performed by checking hemolysis zone. There were no beta-hemolytic traits.

Furthermore, cell cytotoxicity assay conducted via LDH assay kit showed that all tested probiotics strains had no cytotoxicity on Caco-2 cell. Minimum inhibitory concentration assay (MIC) was performed on nine antibiotics with ETEST® strip to compare their MIC values with European Food Safety Authority (EFSA)'s cut-offs.

In conclusion, all four probiotic strains used in the experiments are safe in accordance with international standards. [This research was supported by the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ0158652021)" Rural Development Administration, Republic of Korea.]

C037

The Effect of Storage Condition and Characterization of *Protaetia brevitarsis* Fermented with Lactic Acid Bacteria

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One of the edible insect, *Protaetia brevitarsis* (Pb) has been temporarily registered as a food material by the Ministry of Food and Drug Safety of Korea (MFDS) but not yet ensured of safety for storage and distribution. In order to initiate and provide controlled and predictable as food, the production of fermented foods is based on the use of lactic acid bacteria (LAB) as starter cultures. This study first selected LAB suitable for fermentation of edible insect and then investigated change and characterization in physiochemical properties of LAB cultures (HePb-LAB) in hot water extract from Pb (HePb). Fermentation test of HePb conducted with 2% dextrose and some carbon source for cultivating LAB, lyophilized, and then counted the viable LAB (10^9 – 10^{11} CFU/mg). Our results showed that antioxidant, antibacterial, and potential enzyme activity were higher in HePb-LAB than in HePb, but not in the non-fermented Pb. These results provide basic or first data for the development of fermented food with edible insect. [This work was supported by IPET through Agri-Bio industry Technology Development Project, funded by MAFRA (121047-2 and 119027-3).]

C038

Enzymatic Activities of Marine-derived *Trichoderma* Species

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Microorganisms serve as a sustainable resource of natural products. In particular, *Trichoderma* species are considered as producers of many extracellular enzymes. National Marine Biodiversity Institute of Korea (MABIK) collects, preserves, and studies marine-derived fungi in Korea. Among the culture collections, we selected *Trichoderma* species to elucidate enzyme activities. Based on translation elongation factor 1 α (TEF-1 α) sequences, 6 strains of marine-derived *Trichoderma* were identified as *T. hamatum*, *T. koningiopsis*, *T. citrinoviride*, *T. afroharzianum*, *T. xixiacum*, and *T. asperellum*. Activities of 4 widely used enzymes including amylase, lipase, protease and chitinase were examined in these *Trichoderma* spp. on plate assay methods. All tested *Trichoderma* isolates displayed chitinase and lipase activities. Enzyme assay of chitinase indicated strong cell-wall degrading enzyme activities of these *Trichoderma* isolates. In addition, we examined activity of L-asparaginase which is utilized to treat acute lymphoblastic leukemia. As a result, all six strains showed L-asparaginase production. Overall, enzyme assays showed that *Trichoderma* spp. not only demonstrate ecological roles but also have great potential in industrial applications.

[Supported by a grant from National Marine Biodiversity Institute of Korea (MABIK, 2021M00500).]

C039

Anti-obesity Potential of Selected Medicinal Plants via Pancreatic Lipase Inhibition

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Obesity is a global public health concern associated with the increased risk of several comorbidities. In this study, we examined the anti-obesity potential of fermented and non-fermented four medicinal plants (glasswort [*Salicornia europaea*], omija [*Schisandra chinensis*], annual wormwood [*Artemisia annua*], and turmeric [*Curcuma longa*]) by measuring the inhibitory activity against pancreatic lipase. Indigenous microorganisms were isolated from these plants. *Enterococcus faecium* was used as a starter culture for the fermentation of turmeric and glasswort. *Lactobacillus plantarum* and *Wickerhamomyces anomalus* were used as starter cultures toward the fermentation of annual wormwood and omija, respectively. All the fermentation were carried out for 16 h and then hot water extracts were prepared for measuring the enzyme inhibitory activity. The fermented extract of annual wormwood significantly inhibited the activity of pancreatic lipase as compared with that of the non-fermented extract ($p < 0.05$). On the other hand, the non-fermented glasswort extract inhibited the activity of pancreatic lipase, however, there was no significant differences in the inhibition of pancreatic lipase activity before and after fermentation in case of turmeric and omija extracts. These results suggest that the fermented annual wormwood may have anti-obesity potential.

[Supported by grants from Rural Development Administration.]

C040

Characteristic Study and Optimization of Culture Conditions for *Bacillus* sp. Strain as Probiotic Materials for Companion Animal

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Microbial Institute for Fermentation Industry (MIFI)

The purpose of this study was to investigate the probiotic characteristics of selected *Bacillus* strains as a probiotic material for companion animal feed and supplements. Finally selected strain, SRCM 100731 was isolated from traditional soy sauces, and that have safety and probiotics activity (cell surface hydrophobicity and antibiotic sensitivity). SRCM 100731 carried out optimization of cell growth for industrial applications such as pet food and feed. The effects of 14 different components on cell growth were investigated and three significant positive factors, molasses, sodium chloride, and potassium chloride, were selected as the main factors for improving cell growth based on a Plackett-Burman design (PBD). In order to find out optimal concentration on each constituent, we carried out central composite design. The predicted optimized concentrations were 7% molasses, 0.5% potassium chloride, and 1.1% sodium chloride. Finally, an overall approximate 7 fold increase in dry cell weight yield was achieved using the optimized medium compared with the non-optimized medium. This research is expected to be highly utilized in the growing the pet industry by establishing cultivation conditions for industrial application and screening of probiotic resource.

[This work was supported by a grant from Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA).]

C041

Comparison of Microbial Community of Rhizosphere in *Capsicum annuum* and *Allium sativum* Using Next Generation Sequencing

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Understanding the microbial community and function are crucial knowledge for crop management. To profile the taxonomical compositions of rhizosphere microbial communities of two types of upland field crops, *Capsicum annuum* (pepper, PE) and *Allium sativum* (garlic, GA) reported to possess excellent antioxidant properties and compare the taxonomical differences, we analyzed the V3-V4 region of bacteria from rhizosphere samples by using Illumina sequencing. Bacterial community relative abundances and diversity index values were compared for different crops. Alpha-diversity result showed that index values indicating bacterial community abundances and diversity were statistically not significant. Linear discriminant analysis (LDA) effect size (LEfSe) analysis was performed to reveal the significant ranking of abundant modules in different cultivars. A size-effect threshold of 2.0 on the logarithmic LDA score was used for discriminative functional biomarkers. The phyla with significant differences in relative abundance between PE and GA was *Acidobacteria* ($p = 0.0156$). On genus level, *Tepidisphaera*, PAC001874_g (*Vicinamibacter_f*), PAC001869_g (*Vicinamibacter_f*), *Pseudolabrys* were the dominant genera. In top 10 genera in relative abundance, the genera with significant differences in relative abundance between PE and GA were PAC001869_g ($p = 0.0065$).

C042

Study of Microbial Community Profiling on Livestock Feces, Swine and Cow Using Next Generation Sequencing

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Microbial Institute for Fermentation Industry (MIFI)

This study was conducted to evaluate distribution of microbial population in 78 fecal samples from swine and cows. Microbial communities were analyzed using next generation sequencing. Total of 7,214,020 sequences were used to microbial community analysis. The operational taxonomic units (OTUs) of 622–2371 feces assessed by analysis of CD-HIT. In this result, the 7,214,020 sequences were investigated to 5 phyla, of which Firmicutes was the most predominant phylum, according to 53.97% in cows and 69.43% in swine of all sequence. As microbial distribution was examined at the genus level considering two animals basically. Especially, it was only detected to *Clostridium* sp. (16.48%), *Prevotella* sp. (11.41%) and *Lactobacillus* sp. (6.77%) in swine feces. also, microbial distribution was showed the specific difference in species level. The rate of predominant species was investigated to PAC001136 (*Clostridium*) was occupied 8.66% in swine fecal and 6.56% *Solibacillus sivestris* has portion of cows feces. Therefore, these results showed significant differences in fecal microbiota between types of source animals, suggesting that there are many diversity about fecal bacteria species.

[This work was supported by a grant from Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA).]

C043

Potential Probiotic Properties *Bifidobacterium longum* JSRL-02 Isolated from Fecal Materials of Neonates

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This study was carried out to isolate new *Bifidobacterium* that can be used as probiotic material from fecal of neonates. Various physiological experiments such as haemolytic, bile salt hydrolase and antibacterial activities were conducted to select the probiotic strains, and five species of microorganisms were selected. JSRL-02 was finally selected with superior physiological activities, and identified as *Bifidobacterium longum* JSRL-02 by 16S rRNA sequencing analysis. JSRL-02 showed a high survival rate of more than 61% at pH 3.0 and 89% at 1.0% ox gall. In particular, JSRL-02 had a survival rate of more than 100% above 80°C, and also was resistant to a variety of antibiotics, which is believed to be highly to survive in the various environment including human intestines. This research is expected to be highly utilized in the probiotics industry for industrial application as well as screening *Bifidobacterium longum* JSRL-02 as probiotic resource for health functional foods.

[This work was supported by a grant from Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA)]

C044

Effect of Fermentation on the Antioxidant Properties of Black Ginger (*Kaempferia parviflora*)

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Black ginger (BG) has been used as a folk medicine in Thailand for its antioxidant, anti-inflammatory, cardioprotective, and anti-obesity properties. In this study, we examined the effect of fermentation on the antioxidant properties of fermented and non-fermented BG by *Enterococcus faecium*. For fermentation, two kinds of media were used namely Bacillus minimal medium (BMM) and 1/5th of de Man Rogosa Sharpe (MRS) medium. In both the media, the viable cell counts of *E. faecium* increased from their initial values (~6.6–6.8 log CFU/ml) to ~8.5–8.8 log CFU/ml after 8 h of fermentation, and then remained virtually constant. However, the pH was much lower in MRS medium, which may be due to higher lactic acid production. To analyze antioxidant properties, we used hot water and ethanol (70%, v/v) extracts of fermented and non-fermented BG. Total polyphenols were decreased in the hot water extracts of BG after fermentation while total flavonoids were decreased only in the MRS fermented hot water extracts ($p < 0.05$). In the results of antioxidant assays, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity was increased in MRS fermented BG ethanol extract while 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity was decreased in MRS fermented BG hot water extract ($p < 0.05$). Overall, BG can be successfully fermented without much altering its antioxidant properties.

[Supported by grants from Rural Development Administration]

C045

Characterization of Novel *Myoviral* Bacteriophage Eco_20-4 that Lyses *Enterotoxigenic Escherichia coli*

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Enterotoxigenic Escherichia coli (ETEC), the major pathogenic *E. coli*, cause diarrheal illness and sepsis in humans and animals, Especially, ETEC causes porcine post-weaning diarrhea and lead to severe economic losses in the swine industry worldwide. Bacteriophages, bacterial viruses, have been reviewed recently as a potential therapeutic agent for controlling bacterial diseases. In this study, ETEC strains and phages were isolated from sewage water and faeces in livestock farms, South Korea. Novel ETEC phage, Eco_20-4, of the family *Myoviridae* showed host range of 10 out of 45 ETEC strains including the virulence gene (Stx2e and F18) which causes porcine edema disease and strong host cell lytic activities at MOI = 0.1. This phage exhibited high stability in a broad pH range of pH3–10 and retained lytic activity even 24 h at 60°C. Phage Eco_20-4 has the genome of 170,632 bp linear ds DNA (GC%, 39.6) and 276 putative ORFs, and known lysogeny, antibiotic resistance, or toxin-related genes were not identified *in silico*. Recently, the administration of many antibiotics to prevent and treat ETEC in swine has been leading to increasing antibiotic resistance worldwide. Therefore, this study strongly suggests that phage could be a biological alternative antibacterial agent for prophylaxis and therapy to control ETEC infection.

C046

Antagonistic Effect of *Bacillus amyloliquefaciens* KJB-1 Isolated from Soybean Rhizosphere

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In this present study, we isolated a bacterium from soybean rhizosphere and assessed its antifungal ability against eight most problematic soybean pathogens including *Phytophthora sojae* KACC 40412 and one serious rice pathogen, *Pyricularia oryzae* 16-111 etc. First, by combined sequence analysis using 16S rRNA and *gyrA* sequences, strain was identified as *Bacillus amyloliquefaciens*. Also, we examined whether *B. amyloliquefaciens* KJB-1 can produce hydrolytic enzymes (cellulase and protease) and iron-chelating siderophores. As a result of testing of antifungal activity against eight soybean fungal pathogens, 75.6%, a percentage of inhibition (%), on *Colletotrichum gloeosporioides* 15-126 was observed, 69.8% on *Diaporthe longicolla* 15-145, 59.6% on *Fusarium graminearum* sb14-2, 56.4% on *Fusarium oxysporum* 15-047, 34.9% on *Fusarium commune* 16-28, and 29.7% on *Fusarium solani* sb14-6. Especially, *B. amyloliquefaciens* KJB-1 showed the highest inhibition rate (80.8%) against *Phytophthora sojae* KACC 40412 among the soybean pathogens and 97.3% on *Pyricularia oryzae* 16-111. These results showed that *B. amyloliquefaciens* KJB-1 have good potential as a biocontrol agent against above nine pathogens, soybean and rice.

C047

Enzymatic Activity as Useful Tools in Marine Bacteria

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Marine bacteria producing extracellular hydrolytic enzymes are important for various biotechnological applications. In this study, we investigated the diversity of industrial enzyme producing marine bacteria from some of culture collections deposited at the National Marine Biodiversity Institute of Korea (MABIK). In 778 MABIK isolates, 483 isolates (62%) exhibited extracellular enzyme activities such as amylase, asparaginase, chitinase, glutaminase, lipase, and protease by the plate diffusion test, while 295 isolates (38%) did not exhibit any enzyme activity. Among these 483 isolates, 44% of isolates produced at least one of the six enzymes, and 5% of isolates were able to produce more than four enzymes. A highest proportion of six enzyme activities was observed in the protease (68%, 330 isolates), followed by lipase (41%, 199 isolates), amylase (36%, 176 isolates), asparaginase (23%, 109 isolates), glutaminase (6%, 28 isolates), and chitinase (2%, 12 isolates). Based on 16S rRNA gene sequences, 483 isolates belonging to 4 phyla, 7 classes, 22 orders, 43 families, and 99 genera were identified. The genus *Bacillus* showed the highest proportion in enzymic activities including protease (29%), amylase (24%), and asparaginase (34%). Our result appears that marine bacteria are useful biological source of industrial enzymes for possible commercial. [Supported by a grant from National Marine Biodiversity Institute of Korea (MABIK, 2021M00500).]

D001

A Consortia of Commensal Microbes Alleviates Diseased Symptoms by Persistent Intestinal Colonization

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Recently, FMT (Fecal Microbiota Transplantation) has gained popularity in clinics as attempts to restore the eubiotic gut microbiome. While FMT has yielded impressive treatment outcomes against conditions such as rCDI (recurrent *C. difficile* infection), its safety still remains elusive, due to the diversity of components - many of which with yet unidentified effects - present in human stool.

In this context, to alleviate gut microbial dysbiosis and potentially reduce inflammation in the gut, while minimizing the risk associated with FMT, we have established an artificial consortium of 12 bacterial species. The selected 12 species are frequently found in the guts of Korean population. We hereinafter call this "MGMK (Major Gut Microbes among Koreans)."

Interestingly, our preliminary study of transplanting MGMK into GF (Germ Free) mice revealed 3 species with notably stronger colonizing capabilities than the rest. These 3 species, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, and *Ruminococcus faecis*, referred to as "SIC3 (Strong Intestinal Colonizers 3)", predominate in the mouse gut at day 33 post-transplantation.

Furthermore, we observed that SIC3 treatment ameliorates DSS-induced colitis in mice, as reflected in the decreased DAI scores (Disease Activity Index) and reverses the antibiotic-triggered dysbiotic gut microbiome to eubiosis. Detailed experimental results are described below.

D002

Meat Quality Affected by the Foodborne Pathogen Contamination in Livestock Products

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Foodborne pathogen contamination during the food supply process has been a food safety issue worldwide including South Korea. Foodborne pathogens cause diarrhea, edema, and sepsis in livestock depending on age, which reduces overall productivity. Disease symptoms such as dehydration and stress increase incidence of abnormal meat, and negatively affect nutritional value and sensory qualities due to the sterilization measures during the meat process. This study was conducted to evaluate the prevalence of foodborne pathogens in livestock of 83 samples (43 pigs; 23 cattle; 14 chickens; 2 ducks; 1 sheep) in South Korea using polymerase chain reaction (PCR) targeting virulence genes. The prevalence of *Escherichia coli* (*E. coli*) and *Salmonella* spp. were investigated and a total of 31 foodborne pathogens were detected. As a result of PCR targeting virulence genes in 83 samples, 3 were detected as ETEC and EHEC, and 25 were detected as *InvA*, resulting in more prevalence of *Salmonella* than *E. coli*. *InvA* was detected in all animals, but ETEC was detected only in pigs, and EHEC was detected only in cattle. The detection of foodborne pathogens in livestock products indicates that foodborne pathogens can decrease the overall meat quality due to disease outbreaks in livestock farms. Therefore, monitoring contamination levels of foodborne pathogens can help to improve the meat quality by controlling the outbreak of diseases caused by foodborne pathogens.

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D003

Epigallocatechin Gallate Inhibits the Replication of SARS-CoV-2 by Targeting the Uridylate-specific Endoribonuclease Nsp15

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SARS-CoV-2, the coronavirus strain that initiated the COVID-19 pandemic, and its subsequent variants present challenges to vaccine development and treatment. As the coronavirus evades the host innate immune response at the initial stage of infection, the disease can have a long nonsymptomatic period. The uridylate-specific endoribonuclease Nsp15 processes the viral genome for replication and cleaves the polyU sequence in the viral RNA to interfere with the host immune system. This study screened natural compounds *in vitro* to identify inhibitors against Nsp15 from SARS-CoV-2. Three natural compounds, epigallocatechin gallate (EGCG), baicalin, and quercetin, were identified as potential inhibitors. Potent antiviral activity of EGCG was confirmed in plaque reduction neutralization tests with a SARS-CoV-2 strain (PRNT₅₀ = 0.20 μM). Because the compound has been used as a functional food ingredient due to its beneficial health effects, we theorize that this natural compound may help inhibit viral replication while minimizing safety issues.

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D004

***Mycobacterium tuberculosis* MPT63-derived Peptide Regulates Mycobacteria Survival by Innate Immune Response**

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Mycobacterium tuberculosis (MTB) causes tuberculosis (TB), appears to have co-evolved with humans over at least 70,000 years and is estimated to latently infect about a quarter of the global population. Secretory proteins of MTB are the major antigens that modulate host immune response. Once phagocytized, MTB resists killing by macrophages, replicates inside them and leads to their death, releasing MTB antigens that can infect other cells. MPT63 is a 16kDa secreted immunogenic protein that had highly expression level in active TB. MPT63 induces cell death of macrophages and enhances phagocytic activity by regulating the secretion of inflammatory cytokines in murine peritoneal macrophages. However, the functions of MPT63 through interaction with host protein are unclear. Here, we demonstrated that MPT63 binds to TBK1 and p47phox. Specifically, we constructed a peptide using MPT63 domain that interacts with p47phox, which interacts with p22phox and p67phox in a dose-dependent manner. Furthermore, p47phox peptide increased pro-inflammatory cytokines such as TNF- α , IL-6 and regulated the level of cellular ROS in MTB-infected macrophages. Moreover, intracellular MTB was decreased with higher concentration of p47phox peptide. In summary, our result suggested that p47phox peptide increased inflammation and killed mycobacteria via enhancing the immune system of macrophages.

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D005

Anti-inflammatory Effects of the Ginsenosides by Regulating Inflammatory Signaling in Macrophages

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Inflammation is a complex process for the immune system to defend against harmful agents including pathogens, tissue injuries and damaged cells. However, a drastic or chronic inflammatory response causes various inflammatory diseases such as sepsis, inflammatory bowel diseases, and autoimmune diseases. Korean ginseng (*Panax ginseng*) is the traditional medicinal herb and used for therapeutic benefits. Ginsenosides are the main constituents of *Panax ginseng* and have the biological activities for therapeutic effects such as antioxidant, anticancer, and anti-inflammatory activity. However, the anti-inflammatory mechanism of ginsenosides has been clearly unknown. We investigated the anti-inflammatory effect of ginsenosides and how ginsenosides suppress the production of pro-inflammatory cytokines in LPS-stimulated macrophages. Ginsenosides regulated the cytokines such as TNF- α , IL-6, IL-1 β , and IL-10. The regulation of NF- κ B and MAPK signaling and NLRP3 inflammasomes activation was found as the mechanism of ginsenosides for anti-inflammation effects. Taken together, these results demonstrate that ginsenosides may exhibit anti-inflammatory effects through the regulation of NF- κ B and MAPK, suggesting ginsenosides are the potential compounds to prevent and treat inflammatory disease.

[This work was supported by the NRF grant funded by the Korea government (MSIP) (2019R111A2A01064237).]

D006

***Mycobacterium tuberculosis*-infected Macrophage-specific Delivery Peptide Derived by MPT64**

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Mycobacterium tuberculosis (MTB) infects macrophages and evades the host immune system by secreting immunodominant antigens. MPT64 highly expressed in active TB is released during MTB infection and interacts with host proteins for bacterial survival and proliferation. However, little is known about the role of MPT64 in the mechanisms regulating host proteins. In this study, we found that MPT64 is associated with several proteins including HK2 and TBK1. As previous studies showed that HK2 is important for the immune response via modulation of glycolysis metabolism in TB and accumulates within an inflammatory environment to activate an immune response, we examined the interacted domains in MPT64 with HK2 and constructed a MPT64-derived peptide that can be associated with HK2 (HK2 peptide). Furthermore, we treated the HK2 peptide in MTB-infected macrophages to evaluate the effects of HK2 peptide in MTB infection. Although no significant effects in controlling the MTB load were shown, it specifically targeted the MTB-infected macrophages but not in other immune cells. Taken together, the present study demonstrated that MPT64 bound directly with HK2 and MPT64-derived HK2 peptide was able to target macrophages in MTB infection, suggesting HK2 peptide has a role in signal peptide that can be utilized as a component of therapeutic protein against MTB.

[This work was supported by the NRF grant funded by the Korea government (MSIP) (2019R111A2A01064237).]

D007

Host-mimicking Systems to Identify BBB-migration Mechanisms of Fungal Meningitis Pathogen

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Cryptococcus neoformans is a major fungal pathogen causes meningoencephalitis. However, there are limitations in studying the mechanism of fungal brain infection in biological models. In this study, we constructed two *in vitro* BBB monitoring systems to identify the roles in brain infection-related signaling components including kinases and transcription factors (TFs). We used *in vivo* murine model to screen the brain infectivity with the signature-tagged mutagenesis (STM)-based libraries of kinases and TFs deletion mutants. To dissect the roles in brain infection processes including BBB adhesion and BBB crossing, we screened the brain infection-related signaling components via *in vitro* BBB screening system. Furthermore, we monitored the brain infection process on a human neurovascular unit chip designed by reconstituting necessary cellular and extracellular components in microfluidic devices. We analyzed the neurotropic behaviors and BBB penetration process of *C. neoformans*. As a result, we showed the signaling components which role in brain infection processes of *C. neoformans* including BBB adhesion and penetration. These signaling components will be a putative drug target to control the brain infection of *C. neoformans*.

D008

Signaling Networks in the Sexual Reproduction Cycle of *Cryptococcus neoformans*

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Cryptococcus neoformans causes cryptococcosis by the inhalation of infectious spores generated by unisexual or bisexual reproduction. To understand complex signaling networks modulating the developmental process, a complete understanding of genome-scale transcription factors (TFs) and kinases is needed. Previously we reported that 37 TFs and 42 kinase mutants constructed in *C. neoformans* MAT α 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 α isogenic strain of H99 strain—to perform an analysis of their function in the developmental process. We constructed 54 gene-deletion strains representing 27 TFs and 50 gene-deletion strains representing 25 kinases and are currently constructing gene-deletion strains for the remaining genes. For confirmed mutant strains, we are examining mating phenotypes during bilateral 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*.

D009

Crosstalk among Cpk1, Mpk1 and Hog1 MAPK Pathways Regulate the Thermosensitivity in *Cryptococcus neoformans*

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Cryptococcus neoformans causes meningoencephalitis regardless of immune system disruption and is responsible for approximately 600,000 deaths annually. Mitogen-activated protein kinases (MAPK), the pivotal kinases of eukaryotes, play major roles in metabolism and stress response. There are three major MAPK pathways including Cpk1, Mpk1 and Hog1 pathways in *C. neoformans*. The research concerning the crosstalk among three MAPK pathways have yet to be elucidated. We constructed the double and triple MAPK deletion mutants and verified characterizing how MAPK crosstalk regulate the downstream factors. We discovered all three MAPKs have roles in thermosensitivity with phenotypic analysis. In our previous studies, it is known that Hog1 of *C. neoformans* serotype A H99 was highly phosphorylated at basal condition in wild type. When moved up to 37°C, Hog1 was dramatically dephosphorylated. As a wild type, *cpk1Δ* and *mpk1Δ* showed dephosphorylation of Hog1 at 37°C. However, *mpk1Δ cpk1Δ* showed more severe dephosphorylation of Hog1 in response to 37°C. In addition, using qRT-PCR, we screened the downstream transcription factors (TFs) which are essential or heat-related. We discovered that MAPKs regulate the expression of TFs: Hxl1, Hsf1, Crz1, Sre1, Pzf1, and Mbs2. Hog1 mainly regulates the mRNA expression level and Mpk1 and Cpk1 regulate minor. Therefore, we aim to provide insight into the regulation of thermal response by complex MAPK crosstalk.

D010

Systematic Study of Host-derived Cues for the Regulation of Pathogenicity-related Transcription Factors in *Cryptococcus neoformans*

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Cryptococcus neoformans is a causative agent of fungal meningoencephalitis, which results in more than 180,000 deaths annually. Nevertheless, its treatment option is limited mainly due to a lack of complete understanding of how the pathogen interacts with the host during infection and disease progression. Although a number of signaling pathways involved in the pathogenicity of *C. neoformans* have been characterized in past years, it remains elusive how complex signaling pathways are coordinated and regulated during the whole infection process. Previously we performed NanoString-based *in vivo* transcription profiling of 183 kinases, 178 transcription factors, and 139 phosphatases during the whole infection process of *C. neoformans*. Here we focused on 12 transcription factors, including *PDR802*, *BZP4*, *HOB5*, *ZNF2*, *FZC39*, *FZC30*, *SRE1*, *HLH1*, *STB4*, *MLN1*, *MET32*, and *GAT201* of which *in vivo* expression were highly induced during host infection but did not exhibit evident *in vitro* phenotypes. The expression level of the 12 genes were measured in host mimic condition (HMC). We found that all of them were highly induced by HMC. Next, we dissected the HMC signals that trigger the induction of the 12 transcription factors. We found that *PDR802* was highly induced by body temperature and carbon starvation. Also, *MLN1* deleted mutants shown growth defects when they have maltose. In conclusion, we provided insight into signaling pathways modulating the pathogenicity of *C. neoformans*.

D011

Genome-wide Analysis of Putative WD40 Repeat-containing Proteins Involved in Pathogenicity of *Cryptococcus neoformans*

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Cryptococcus neoformans is the major human fungal pathogens which causes death by meningoencephalitis. The cell signaling regulation in various environments and host conditions is known to be important for the survival and virulence of *C. neoformans*. Among the cell signaling regulation, protein-protein interaction (PPI) is known as an essential phenomenon. Though the WD40 domain is one of the most common domains related to PPIs, the role of WD40 domain-containing proteins remains elusive. We focused on 132 WD40 repeat-containing genes, constructed knockout mutants, and examined *in vitro* phenotypic traits under 30 different growth conditions. As a result, we discovered Rav1, which is known as a subunit of the regulator of ATPase of vacuoles and endosomes (RAVE) complex in the model yeast, was related to cellular growth on various temperature and stress conditions and the production of virulence factors in *C. neoformans*. Doa1, which is involved in protein degradation, was related to oxidative stress response and antifungal drug susceptibility. Interestingly, *doa1Δ* mutants were highly susceptible to fluconazole, but resistant to fludioxonil. Swd1, known as a histone methyltransferase, was related to DNA damage response. Through further study, we can dissect the roles of WD40 proteins and PPI partners on various stress responses and host interaction of *C. neoformans*, and these findings will provide comprehensive insight to develop the PPI inhibitors as novel antifungal agents.

D012

Discovering the Mechanism and Function of *CEX1*

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Cryptococcus neoformans is an opportunistic fungal pathogen causes meningoencephalitis in immunocompromised individuals. The cell adhesion ability and the power to pass through Blood-Brain-Barrier (BBB) play a critical role in the development of meningitis. Previous study revealed the correlation of TF and kinase with BBB adhesion and crossing. We focused on *CEX1* which is a kinase gene involved in both BBB adhesion and crossing. We tagged the RFP on *CEX1* to check localization on basal and ER stress and check the phenotype for *cex1Δ* strains. *HXL1* splicing was conducted for ER stress sensitivity, the most obvious phenotype of *cex1Δ*, and it was found that the phenotype by ER stress of *cex1Δ* strains was not *HXL1* dependent. We performed the GFP chromosomal tagging on secretion proteins which are co-work with Cex1p in *Saccharomyces cerevisiae* as a COP1 complex. We performed GFP chromosome tagging on secretive proteins operating with Cex1p in *Saccharomyces cerevisiae* as a COP1 complex in *Cryptococcus neoformans* to verify localization relationships through fluorescence microscopy and conduct western blot and co-IP experiments. Through these studies, we can find out if *CEX1* contributes to tRNA trafficking by doing COP1 complex in *Cryptococcus neoformans* as in *Saccharomyces cerevisiae*.

D013

Adenylyl Cyclase and Protein Kinase A Play Critical and Individual Roles in Antifungal Drug Resistance and Pathogenicity of *Candida auris*

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Candida auris is an emerging multidrug-resistant fungal pathogen causing invasive human infection and associated with high fatality diseases in immunocompromised patients. Accordingly, the importance of research on *Candida auris* is increasing. In this study, we characterized cyclic adenosine monophosphate pathway of *Candida auris* which has been considered to be one of the most important signal transduction pathways of pathogenic fungal species. Among the various genes associated with the cAMP pathway, we constructed knockout strains for the adenylyl cyclase *CYR1*, PKA gene catalytic subunit *TPK1*, *TPK2*, and regulatory subunit *BCY1*. Then we conducted a phenotypic analysis of each mutant to find out what stress these genes are involved in. In addition, we confirmed differences in biofilm formation between wild-type and mutants, and we found that *BCY1* and *TPK1* served as positive regulators. We also found that the expression level of multi-drug resistance related genes, stress response related genes, and hyphae formation related genes are controlled by cAMP pathway genes. Furthermore, we demonstrated that PKA genes are also involved in the pathogenicity and ploidy change of *C. auris*. Consequently, these results will indicate that targeting cAMP pathway genes in *Candida auris* could serve as an effective alternative to antifungal therapy against emerging multidrug-resistant fungal pathogen *Candida auris*.

D014

Determination of Antigenic Relatedness between Foot-and-Mouth Disease (FMD) Serotype O Vaccine Strains and Currently Circulating Serotype O Viruses in Southern East Asia

Seo Young Moon, Min Young Kim, Seon Woo Kim, Ha-Hyun Kim, Seung Heon Lee, Jong-Hyeon Park, and Jae Jo Kim*

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The antigenic variability between and within serotypes of foot-and-mouth disease virus (FMDV) can make difficulty in applying the appropriate vaccine in the field. The vaccine matching test by two-dimensional virus neutralization test (2D-VNT) is used to evaluate the vaccine suitability by measuring the antigenic relationship (r_1 -value) between vaccine and field strains using bovine post-vaccinal sera. In this study, six FMD serotype O vaccines were assessed by 2D-VNT whether they were antigenically matched with the field FMDVs circulating in Southern East Asia. In our results, seven O/ME-SA/PanAsia lineage FMDVs exhibited a good match ($r_1 > 0.3$) to O₁/Manisa and O/3039 vaccine (100% and 85.7%, respectively). Additionally, they were 100% matched to O/BE/SKR/2017 vaccine. However, the seven FMDVs exhibited lower cross-reactivity with O/Primorsky, O₁/Campos and Om-O/PanAsia2 vaccine (28.6%, 28.6% and 42.9% respectively). This indicates that O₁/Manisa, O/3039 and O/BE/SKR/2017 could be the priority vaccine against possible future outbreak caused by the PanAsia lineage FMDV. It has been known that the Cathay topotype of FMDVs exhibited low antigenic match to the most of vaccines. Our results showed an O/VN/30/2017 virus (O/Cathay) was a good matched to O₁ Manisa and O₁ Campos vaccine ($r_1 \geq 0.49$). Therefore, further *in vivo* study will be needed to clarify the cross-reactive potential of these two vaccines against the O/VN/30/2017 virus. [Supported by a research grant from the APQA]

D015

Assessment of Cross-reactivity between Foot-and-Mouth Disease (FMD) Serotype A Vaccine Strains and Currently Circulating A/Asia/Sea-97 Lineage FMD Viruses in Southern East Asia

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The vaccine matching test by two-dimensional virus neutralization test (2D-VNT) is used for evaluation of the foot-and-mouth disease (FMD) vaccine suitability. The assay measures the cross-reactivity of a bovine post-vaccinal sera with the field virus in question, and the results are expressed as antigenic relationship (r_1 -value). In this study, the r_1 -value of bovine post-vaccinal serum pools raised against the FMD serotype A vaccines, A₂₂/Iraq, A₂₄/Cruzeiro and A/Zabaikalsky/2013, were measured by 2D-VNT with five A/Asia/Sea-97 lineage field FMD viruses (FMDV) isolated from Southern East Asia (SEA). In our results, three of five viruses reacted well ($r_1 > 0.3$) with A₂₂/Iraq and A/Zabaikalsky/2013 antisera, but not to A₂₄/Cruzeiro. Interestingly, three responsive viruses, A/VN18/2015, A/CAM16/2019 and A/LA/22, were belonging to Sea-97/G2 lineage indicating that A₂₂/Iraq and A/Zabaikalsky/2013 are more cross-reactive to Sea-97/G2 than A₂₄/Cruzeiro and might provide protection against the incursion of Sea-97/G2 lineage FMDVs. However, two Sea-97/G1 lineage FMDVs, namely A/VN/2017 and A/VN/2015 exhibited lower r_1 -value ($r_1 < 0.3$) with all antisera indicating that the Sea-97/G1 FMDVs might not be covered by vaccines used in this assay. Therefore, close monitoring of FMD outbreaks in neighboring countries of South Korea along with regular vaccine matching studies is essential to select appropriate vaccine strains for use in FMD control. [Supported by a research grant from the APQA]

D016

Post-vaccination Monitoring: The Foot-and-Mouth Disease (FMD) Antibody Profiles in Pigs Following the Heterologous Prime-boost Vaccination with Commercial Vaccines Used for the Disease Control in South Korea

Min Young Kim, Seo Young Moon, Seon Woo Kim, Seung Heon Lee, Ha-Hyun Kim, Jong-Hyeon Park, and Jae Jo Kim*

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In Korea, three commercial foot-and-mouth disease (FMD) bivalent vaccines were allowed for use in routine vaccination. In field, there are concerns that heterologous prime-boost vaccinations may have a negative effect on immune persistence in vaccinated animals. In this study, neutralizing antibody (NA) titers in fattening pigs were measured during a twenty-four-week period following homologous- or heterologous prime-boost vaccination with three different commercial vaccines (A vaccine contains O₁/Manisa and O/3039, B contains O/Primorsky, and C contains O₁/Campos). The experimental groups consisted of six heterologous (HTV groups: A+B, A+C, B+A, B+C, C+A, and C+B) and three homologous prime-boost vaccination groups (HLV groups: A+A, B+B, and C+C) and twenty-two pigs were assigned to each group. In our results, there was no immune interference by heterologous booster vaccination during the study period. Even, the NA titers of HTV groups were higher than or equal as compared to HLV groups until the end. In addition, at twenty-four weeks, all groups exhibited mean log₁₀ NA titers against homologues vaccine strains were 1.65 or slightly higher. This indicates that both heterologous- and homologous prime-boost vaccination can maintain protective NA level until the time of pig slaughter. Taken together, it can be concluded that the heterologous prime-boost vaccination could be used as the strategic method for FMD vaccination program.

[Supported by a research grant from the APQA]

D017

Characteristics of High-level Aminoglycoside-resistant *Enterococcus faecalis* Isolated from Bulk Tank Milk in Korea

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Enterococci, which are considered environmental mastitis-causing pathogens, have easily acquired aminoglycoside-resistant genes that encode various aminoglycoside-modifying enzymes (AME). Therefore, this study was conducted to compare the distribution of high-level aminoglycoside-resistant (HLAR) and multidrug-resistant (MDR) *Enterococcus faecalis* (*E. faecalis*) bacteria isolated from bulk tank milk in four dairy companies in Korea. Moreover, it analyzed the characteristics of their antimicrobial resistance genes and virulence factors. Among 301 *E. faecalis* isolates, 185 (61.5%) showed HLAR without significant differences among the dairy companies and 129 (69.7%) of the 185 HLAR *E. faecalis* showed MDR without significant differences. In contrast, HLAR *E. faecalis* from companies A, B, and C were significantly higher in resistance to the four classes than those in company D, which had the highest MDR against the three antimicrobial classes ($p < 0.05$). Regarding the distribution of AME genes, 72 (38.9%) and 36 (19.5%) of the isolates carried both *aac(6')Ie-aph(2'')-Ia* and *ant(6)-Ia* genes, and the *ant(6)-Ia* gene alone, respectively, with significant differences among the companies ($p < 0.05$). As for the virulence genes, the *ace* (99.5%), *efaA* (98.9%), and *cad1* (98.4%) genes were significantly prevalent ($p < 0.05$). Thus, our results support that an advanced management program by companies is required to minimize the dissemination of antimicrobial resistance and virulence factors.

D018

Genetic Characteristics of a Novel Reassortant Low Pathogenic Avian Influenza H7N6 Virus Isolated in Cambodia in 2019

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H7 subtype influenza A viruses, found mainly in birds, cause outbreaks in poultry and, occasionally, zoonotic infections. The first human case of zoonotic H7N4 avian influenza virus (AIV) infection was reported in early 2018 in China. Two months after this case, novel H7N4 viruses phylogenetically related to Jiangsu isolate were emerged in ducks from LBMs in Cambodia. During active surveillance in Cambodia, a novel H7N6 reassortant of the zoonotic H7N4 low pathogenic AIV (LPAIV) was detected from domestic ducks at a slaughterhouse. We analyzed the genetic characteristics of the H7N6 isolate. The Cambodian H7N6 isolates harboured PELPKGR/GLF sequences at the HA cleavage site, indicating a low-pathogenicity phenotype in chickens. The amino acids at positions 226 and 228 of the receptor-binding sites were glutamine and glycine, respectively, indicating a preference for the avian-like receptor rather than the human-like receptor. Similar to the H7N4 viruses isolated from poultry, we did not detect well-known mammalian-adaptive markers, such as E627K and D701N in PB2, in any of the H7N6 isolates. Complete genome sequencing and phylogenetic analysis showed that the Cambodian H7N6 virus is a reassortant, in which four gene segments originated from Cambodia H7N4 viruses and four gene segments originated from LPAIVs in Eurasia. Our study reports the emergence of a new reassortant of zoonotic A(H7N4) AIVs and emphasizes the need for ongoing surveillance of avian-origin A (H7Nx) viruses.

D019

Isolation and Characterization of H10N7 Low Pathogenic Avian Influenza Viruses in South Korea

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Animal and Plant Quarantine Agency

Subtype H10 avian influenza viruses (AIVs) have been detected from wild waterfowl and caused sporadic infections in mammals. During 2014-2017, we isolated seven H10N7 viruses from wild bird habitats through national active surveillance in South Korea. Whole-genome sequencing showed that the isolates had 92.5-98.1% nucleotide-sequence identity to the seal viruses. Moreover, phylogenetic analysis revealed that H10 genes of the isolates were distinct from those of the seal viruses and divided into three different subgroups. Gene-constellation analysis showed that the H10N7 viruses constituted 3 distinct genotypes consistent with HA subgroup. Furthermore, we investigated the known mammalian-adapting substitutions present in the sequences of the H10N7 viruses. The amino acids at positions 226, and 228 of the receptor-binding sites of the HA1 proteins indicated a preference for α 2,3 rather than α 2,6-linked sialic acid receptors. Interestingly, five of seven H10N7 isolates carried the V292I mutation in the PB2 protein, which has been shown to promote virus replication in mammalian hosts. We did not find any well-known mammalian adaptive markers, such as E627K and D701N in PB2. Nonetheless, given that the seal viruses did not possess any mammalian adaptive markers, it is important to evaluate the zoonotic potential of genetically distinct subgroups of H10N7 viruses. Therefore, further studies to determine the pathogenicity of the viruses in mammalian models of influenza are warranted.

D020

***Toxoplasma gondii* GRA9 Regulates the Activation of NLRP3 Inflammasome to Exert Anti-septic Effects in Mice**

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Dense granule proteins (GRAs) are essential components in *Toxoplasma gondii*, which are suggested to be promising serodiagnostic markers in toxoplasmosis. In this study, we investigated the function of GRA9 in host response and the associated regulatory mechanism, which were unknown. We found that GRA9 interacts with NLR family pyrin domain containing 3 (NLRP3) involved in inflammation by forming the NLRP3 inflammasome. The C-terminal of GRA9 (GRA9C) is essential for GRA9-NLRP3 interaction by disrupting the NLRP3 inflammasome through blocking the binding of apoptotic speck-containing (ASC)-NLRP3. Notably, Q200 of GRA9C is essential for the interaction of NLRP3 and blocking the conjugation of ASC. Recombinant GRA9C (rGRA9C) showed an anti-inflammatory effect and the elimination of bacteria by converting M1 to M2 macrophages. In vivo, rGRA9C increased the anti-inflammatory and bactericidal effects and subsequent anti-septic activity in CLP- and *E. coli*- or *P. aeruginosa*-induced sepsis model mice by increasing M2 polarization. Taken together, our findings defined a role of *T. gondii* GRA9 associated with NLRP3 in host macrophages, suggesting its potential as a new candidate therapeutic agent for sepsis.

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D021

Uncovering the Role of C₂H₂ Zinc-finger Transcription Factor Zfc2 Involved in the Pathogenicity and Virulence of *Cryptococcus neoformans*

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Cryptococcus neoformans is a fungal pathogen that causes meningoencephalitis and over 600,000 deaths every year. *ZFC2* is a pathogenicity-related transcription factor of *C. neoformans*, which has no ortholog in other known fungal transcription factors, does not display the typical pathogenicity-related phenotypes of *C. neoformans*, and exhibits attenuated virulence and reduced lung infectivity. In spot assays, *zfc2Δ* showed increased resistance against tunicamycin (TM). Under basal conditions, Zfc2 was enriched in the nucleus, but after 30 min of TM treatment, Zfc2 translocated into the cytoplasm and re-located back into the nucleus after 90 min. Additionally, splicing levels of *HXL1* mRNA in *zfc2Δ* were similar to that of the WT strain with or without TM treatment. When transcriptome analysis of *zfc2Δ* was performed, a total of 782 genes were differentially regulated in the *zfc2Δ* mutant compared to the WT under basal conditions, and the similar level of genes upregulated and downregulated suggests that Zfc2 could serve as both transcriptional activator and repressor. However, under ER stress, total of 170 genes were differently regulated in *zfc2Δ* compared to the WT. Moreover, electron microscope imaging showed that capsule formation was notably increased in *zfc2Δ* compared to the WT, but there were changes in *N* and *O*-glycosylation compared to the wild type. Overall, we aim to provide an extensive understanding of a unique pathogenic transcription factor of *C. neoformans*.

D022

Comparative Analysis of Serological Estimate the Protective Immunity against Challenge with the Foot-and-Mouth Disease Virus in Vaccinated Pigs

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In this study, two pairs of avidity and isotype subclass ratio (IgG₁/IgG₂) ELISA assays were developed to measure specific antibodies against the foot-and-mouth disease (FMD) O/SKR/Jincheon/2014 virus (O/Jincheon, O/SEA/Mya-98 lineage) and A/SKR/Yeoncheon/2017 virus (A/Yeoncheon, A/ASIA/Sea-97 lineage), respectively. One-hundred-two porcine sera from type O FMD vaccine efficacy test and thirty-three porcine sera from type A FMD vaccine efficacy test were evaluated to correlate the results of two ELISA assays with in vivo protection against the FMD virus. According to the comparison results of the avidity ELISA with in vivo protection, the avidity index (%) of sera has significant correlation with in vivo protection and the cut-off values were 55% and 71.8% with the O/Jincheon and the A/Yeoncheon viruses, respectively. According to the isotype subclass ratio ELISA assays, the correlation between isotype subclass ratio and protection was demonstrated and the cut-off value was 1.5 with O/Jincheon virus, but not with the A/Yeoncheon virus. As the results of this serological study, it might be concluded that the avidity assays could be used as the alternative serological tests to screen the potent vaccine against the O/Jincheon and the A/Yeoncheon viruses. But the reason of the discrete results by isotype assays need to be explored.
[Supported by a research grant from the APQA]

D023

Commensal Gut Bacteria Predict Recurrence of Colorectal Cancer

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Trillions of bacteria inhabit the surface of human body affecting many aspects of host biology such as energy harvest, metabolisms, immunity. Although growing evidence solidify the oncogenic properties of certain pathogens, protective role of commensal bacteria on the colorectal cancer (CRC) remains largely unanswered. Here, by sequencing stools of 160 colorectal CRC patients, we found that preoperative level of typical gut bacteria predicted recurrence of CRC after curative resection. Gut microbiotas of the cancer patients are grouped into three enterotypes represented by *Prevotella*, *Bacteroides*, and *Faecalibacterium* respectively. After 3 years follow-up, we identified that *Prevotella* and *Faecalibacterium* separate medical outcomes of CRC patients ($P_{Prevotella} = 0.014$; $P_{Faecalibacterium} = 0.18$). Then, we designed a simple *Prevotella*-*Faecalibacterium* score that dichotomizes samples by the levels of the genera. Of note, the score precisely discriminated high risk patients to regain cancer, outperforming predefined inflammatory indexes and the other circulating metabolite biomarkers. To our knowledge, this is the first finding that showed the prognostic value of typical bacteria, and we anticipated that on-going study with the related genera would provide important insights into involvement of gut microbiota in CRC.

D024

***Cryptococcus neoformans* Utilizes Ferritin as an Iron Source**

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Ferritin is a cytosolic protein found in almost all organisms, and it stores and releases iron in response to iron deficiency and overload respectively. However, upon infection of the vertebrate host, several pathogenic microbes utilize ferritin as a sole iron source. In this study, we investigated whether the human fungal pathogen *Cryptococcus neoformans* is able to use ferritin as a sole iron source and analyzed the interaction between the fungus and ferritin to identify the underlying mechanism of ferritin utilization in the fungus. We found that *C. neoformans* grew well in the presence of ferritin as a sole iron source in the medium, and our results obtained by fluorescence microscopy showed that the physical interaction between ferritin and a fungal cell is critical. Unlike other pathogenic microbes, *C. neoformans* utilizes ferritin in a protease-independent manner but requires Cfo1 and Cft1, which encode ferrioxidase and iron permease, respectively, for high-affinity iron uptake at the cell membrane. Comprehensive transcriptome analysis is currently being conducted to identify the underlying molecular mechanisms of ferritin utilization in *C. neoformans*, and the results will be presented.

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D025

Genetic and Biochemical Equivalent Characterization of *Candida albicans* NCCP Resources as Reference Strains

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The National Culture Collection for Pathogens (NCCP) is conducting research to replace the reference *Candida albicans* strain (ATCC 10231) in the Korean Pharmacopoeia with a suitable strain from 49 domestic strains. The strains were identified using MALDI-TOF MS and internal transcribed spacer (ITS) region sequencing. Multilocus sequence typing (MLST) analysis and antifungal susceptibility tests were performed for characterization. Genetic homologies were assessed using the ITS region sequencing and MLST analysis results. Usefulness evaluation was conducted using the test method to determine the replacement potential.

MLST analysis indicated strain ATCC 10231 as ST 402; however, no such NCCP strain was known. The reference strain exhibited sensitivity to all antifungal agents; the 46 strains from NCCP and ATCC 10231 exhibited equal sensitivities. ITS region sequencing revealed that nine NCCP strains were identical. MLST analysis showed that NCCP 31538 was closest to the reference strain. Its usability was evaluated by applying the Microbial Limit Test and Sterility Test with two other strains, and the same results were obtained.

Accordingly, we selected NCCP 31538, a domestic isolate, to replace the imported reference strain; we intend to introduce a parallel notation in the Korean Pharmacopoeia.

[This study is supported by the National Institute of Health.]

D026

Characterization of 46 *Vibrio parahaemolyticus* Strains Registered in the National Culture Collection for Pathogens (NCCP)

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Vibrio parahaemolyticus is a marine bacterium widely detected in seawater and aquatic products and is the leading cause of bacterial food poisoning.

In the National Culture Collection for Pathogens (NCCP), 46 strain of *V. parahaemolyticus* are registered with minimum species identification and information on culture conditions. Herein, we aimed to characterize these *V. parahaemolyticus* strains.

Antibiotic susceptibility, toxin type, and serotype and multilocus sequence typing (MLST) of *V. parahaemolyticus* strains were performed. Antibiotic susceptibility analysis performed using the MIC method according to the guidelines of the CLSI indicated that all strains were susceptible to the ten tested antibiotics. MLST analysis was performed using PubMLST (<https://PubMLST.org>); the strains were classified into 14 serotypes (STs); ST 3 was the most common (52.2%). Toxin type analysis of the toxin-associated genes *tdh*, *trh*, and *tlh* was performed using PCR; *V. parahaemolyticus* possesses *tdh* (93.5%), *trh* (19.6%), and *tlh* (100%). Serotype analysis was performed using the antisera O and K type commercial kit (Denka, JPN). Twenty-two STs were identified; the most common being O3:K6 (50%), followed by O3:K untype (6.5%).

We believe that our data on the characterization of *V. parahaemolyticus* strains and quality assurance would be useful for the development of diagnostic and therapeutic tools against *V. parahaemolyticus* infection.

[This study is funded by the National Institute of Health]

D027

Application of MALDI-TOF MS for Gram-negative Bacterial Identification

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National culture collection for pathogens (NCCP) possess a collection of domestic pathogenic bacteria, and species identification technology is very important for the development of bacterial resources. In recent years, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) technology emerged as a fast and reliable tool for bacterial identification. We identified gram-negative bacteria using MALDI-TOF MS to evaluate its applicability.

In this study, we used 645 frozen bacterial strains for the identification analysis. Bacterial identification was preferentially confirmed through 16S rRNA sequencing, and the results of MALDI-TOF MS were comparatively analyzed. For 16S rRNA identification, when a strain exhibited more than 99% similarity, and the MALDI-TOF MS score value was 1.7 or more, it was confirmed as a reliable identification.

Among the 645 gram-negative bacterial strains, the results of 16S rRNA identification using MALDI-TOF MS was consistent for 640 (99.23%) strains at the genus level, and 5 (0.77%) strains remained unidentified.

Conclusively, our results indicated that MALDI-TOF MS is a useful tool with high matching rates for bacterial identification. As a nationally responsible agency, the NCCP is expected to improve the credibility of data through providing useful information regarding pathogens to the health and medical researchers.

[Supported by grants from NIH]

D028

Severe Fever with Thrombocytopenia Syndrome Virus in Companion Animals

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Severe fever with thrombocytopenia syndrome virus (SFTSV) is a novel tick-borne *Dabie bandavirus* belonging to the *Phenuiviridae* family. Human SFTS has been reported in China, Republic of Korea (ROK), Japan, and Taiwan. In clinical cases of SFTS, the most common symptoms presented were severe fever, myalgia, and diarrhea. Laboratory analyses of blood samples suggested leukopenia and thrombocytopenia, besides increased values in liver function test. This study showed the human-like SFTS in companion dog with fever, anorexia, and the scar of tick-bite in the region around his left armpit, after being bitten by tick. After the disease onset, viral loads surged in 2 days and reduced in 7 days. However, viral RNA was not detected in 13 days. IgG antibody against SFTSV was not detected in serum on day 2, whereas it was on days 7 and 12 by IFA. Moreover, to determine the antibodies specific to nucleocapsid protein (NP) of SFTSV in companion dog, indirect Enzyme-linked immunosorbent assays (ELISA) was performed. The 10 (2.6%) of 382 sera was positive for IgG antibody against SFTSV. The present study suggests that SFTSV has been circulating in companion animals. Although in the present study dog-to-human transmission has not been identified, the status of SFTSV infection in companion animals is very important with respect to public health, and veterinarians should especially be alert since there is no available guideline for SFTS infection in animals.

D029

Prevalence of Severe Fever with Thrombocytopenia Syndrome Virus in Feral and House Cats

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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne zoonosis in China, Japan, Taiwan, and the Republic of Korea (ROK). Recently, human SFTS-like clinical symptoms in cats and cheetahs have been reported in Japan. Moreover, the case of cat to human transmission was reported. Therefore, the prevalence of the SFTSV gene or antibody in cats is important for public health as well as veterinary medicine. Feline blood was collected from 101 feral and 100 house cats in the ROK in 2017. Viral RNA was extracted from sera and one-step RT-nested PCR was performed to amplify the S segment of SFTSV. Anti-SFTSV antibody was detected by ELISA and IFA. Eight (4.0%), nine (4.5%), fourteen (6.7%) of 201 cat sera were found to be positive for the RT-PCR, ELISA, and IFA, respectively. Specifically, 5.9% feral and 2.0% house cats were positive for the SFTSV gene, and 6.9% feral and 2.0% house cats were positive for anti-SFTSV NP specific antibodies. Moreover, 9.9% feral and 4.0% house cats were positive for anti-SFTSV antibody. All obtained sequences of the SFTSV S segment were clustered in the B-2 genotype. Evidence of SFTSV in companion animals indicates that SFTSV can circulate in homes and that more intensive precautions and education measures are needed for companion animal guardians and veterinarians. [This study was supported by grants from the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education.]

D030

Long-term Immunogenicity of a Recombinant Subunit Severe Fever with Thrombocytopenia Syndrome Virus Vaccine

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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease caused by *Dabie bandavirus* (formally called as SFTS virus (SFTSV)) infection in China, Japan, Taiwan, and Korea. Despite a gradual increase of SFTS cases and high mortality in endemic regions, no specific viral therapy nor vaccine is available. The recombinant proteins of SFTSV nucleocapsid protein (NP), Glycoprotein N (Gn), and Glycoprotein C (Gc) were purified for vaccine study. Each 10 µg recombinant protein was mixed with aluminum hydroxide for immunization to C57BL6 mice. Total three times immunization has been conducted with 2 weeks interval for up to 1 year. Humoral immunity was determined by enzyme-linked immunosorbent assay and virus neutralization assay. Mice immunized with NP did not show neutralizing activity against SFTSV. On the other hand, Mice immunized with Gn and Gc did show strong neutralizing activity against SFTSV. Total IgG titer in all mice has been increased until about 20 weeks, but slightly decreased after 24 weeks. The results showed that the humoral immunity has been sustained until 1 year in all groups and suggest that recombinant protein vaccine could provide long term immunogenicity for SFTSV.

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D031

Novel Isolates of *Lactobacillus plantarum* and *Pediococcus pentosaceus* Strain Resistant to Reactive Nitrogen Species

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Appropriate level of Reactive nitrogen species (RNS) exerts an immense function on maintaining microbiota homeostasis and regulating IBD. But excessive RNS have critical side effects on the surrounding tissues and lead to dysfunction of the intestinal barrier, intestinal dysmotility and nutrient absorption disorders. Furthermore, RNS form by-products that act as terminal electron acceptors to support the growth of facultative anaerobes, resulting in gut microbiota dysbiosis. In this study, series of bacteria were tested against nitric oxide (NO) which has free radical activity that can cause cellular damages and participates in inflammatory process. Here, *Lactobacillus plantarum* (LP) and *Pediococcus pentosaceus* were isolated from human feces and tested for their resistance to NO. The results showed that *L. plantarum* #7 and *P. pentosaceus* #6 were able to survive significantly better in each different concentration of sodium nitrite. Especially, *L. plantarum* #7 and reference strain (KCTC 3108), also *L. plantarum*, are elongated when they are exposed to acidified nitrite, but elongation was more extreme in the reference strain. To better understand the resistance phenotype of this new isolate, we performed a whole genome sequencing analysis. The result demonstrated that the gene of the *L. plantarum* #7 has synchronized with the gene of the reference strain to 91%. According to functional genotyping, 300 functional genes of *L. plantarum* #7 were different from the reference strain. Among these 300 genes, 94 genes have been identified and 6 of them are presumably related to nitric oxide detoxification. The findings indicated that *L. plantarum* #7 with NO resistant properties could be an effective probiotic candidate for human gut health against myriads of inflammatory damages.

D032

LAMMER Kinase Affects PAMP Expression and Virulence in *Aspergillus fumigatus*

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Invasive aspergillosis is a life-threatening disease in immunosuppressed patient caused by *Aspergillus fumigatus*. Previous studies in our laboratory have been reported that LAMMER kinase is involved in various fungal developmental processes. However, its function in virulence is relatively unknown. Therefore, we analyzed changes in cell wall composition of wild-type and LAMMER kinase deletion strain ($\Delta lkhA$) and studied immune response against each strain with murine alveolar macrophage (AM). Deletion of *lkhA* gene significantly increased β -1,3-glucan and expression of β -1,3-glucan synthase gene than wild-type strain. *In vitro* experiments revealed that $\Delta lkhA$ conidia was more susceptible to AM phagocytosis and that extracellular signal-regulated kinase (ERK) in AM was activated earlier in response to $\Delta lkhA$ than wild-type conidia. An invasive fungal infection assay using T-cell deficient zebrafish model also revealed a reduced virulence by infection with $\Delta lkhA$ conidia. These results indicated that LAMMER kinase plays critical roles in pathogen-associated molecular pattern (PAMP) expression, interaction with AM, and thus in virulence of *A. fumigatus* at the organismal level.

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D033

LAMMER Kinase is Regulator of Virulence Factors in *Aspergillus fumigatus*

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Aspergillus fumigatus is a causative agent of invasive aspergillosis, respiratory disease in immunocompromised patients. LAMMER kinases are dual-specificity kinases and exist in all eukaryotic organisms. Previously, LAMMER kinases are known to be involved in multiple cellular processes, however, its role in the production of virulence factors has not been studied. In this study, we investigated the relationship between LAMMER kinase (LkhA) and production of virulence factors in *A. fumigatus*. LkhA-deficient strain increased gliotoxin biosynthesis compared to the wild-type. These results were consistent with increased expression level of genes for gliotoxin biosynthesis, *gliP*, *gliZ* and *gliA*. And LkhA-deficient strain increased the biosynthesis of alkaline protease and expression level of *alp1*, a gene related to biosynthesis of alkaline protease. The amount of biosynthesis of trehalose acting as substance that can prevent damage to cells was increased in the LkhA-deficient strain. These results coincided with increased expression level of *tpsA* and *tpsB* essential for trehalose biosynthesis. However, the amount of reduced glutathione acting as an antioxidant was reduced. In conclusion, our results indicated that LAMMER kinase plays important role in pathogenicity of *A. fumigatus* by regulating production of virulence factors.

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D034

Extended *O*-Mannosylation is Critical for Host-pathogen Interaction in *Cryptococcus neoformans* Infection

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The human fungal pathogen *Cryptococcus neoformans* assembles glycans on its cell surface glycoproteins. To investigate the roles of *O*-glycan extension in the interaction with host cells, the *C. neoformans* mutant strains defective in the addition of second mannose residue in α 1,2 linkage to major- *O*-glycans (*ktr3* Δ), in α 1,3 linkage to minor *O*-glycans (*cap6* Δ), or to both *O*-glycans (*ktr3* Δ *cap6* Δ) were constructed. The *C. neoformans* *ktr3* Δ single and the *ktr3* Δ *cap6* Δ double mutants showed the greatly reduced adhesion to A549 lung epithelial cells, indicating that the truncated *O*-glycan structure affect the interaction of fungal cells with lung epithelial cells. Despite comparable phagocytosis efficiency by macrophage-like cells (J774A.1) between the wild-type cells and the mutant cells, the *ktr3* Δ *cap6* Δ mutant displayed the most reduced survival ability in macrophages. Moreover, the *ktr3* Δ and *ktr3* Δ *cap6* Δ mutants were highly defective in blood brain barrier (BBB) traversal while no significant change in the adhesion efficiency to human brain microvascular endothelial cells (hBMECs), indicating that extension of *O*-glycans is required for mediating the transcytosis of *C. neoformans* through BBB. In conclusion, the results demonstrate that the extension of major *O*-glycans in *C. neoformans* plays critical roles in modulating the interactions with host cells in various stages of the infection, including adherence to lung epithelial cells, survival in macrophages, and BBB transmigration.

D035

Elucidating the Roles of the Casein Kinase 2 in the Pathogenicity of the Human Fungal Meningitis Pathogen *Cryptococcus neoformans*

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The opportunistic human fungal pathogen *Cryptococcus neoformans* causes fatal meningoencephalitis both in immunocompromised patients and immunocompetent individuals. However, the therapeutic options for treatment of cryptococcosis are currently highly limited. As a potential antifungal drug target, kinases have been considered to be good candidates and play important regulatory roles in cellular mechanisms and virulence of fungal pathogens. In previous studies, we found Cka1, a serine/threonine eukaryotic kinase, is involved in regulation of cell cycle, cellular morphology and pathogenicity of *C. neoformans*. In this study, we aim to figure out the regulatory mechanism of Cka1. We found one catalytic subunit Cka1 and two regulatory subunits which are Ckb1 and Ckb2 as a putative complex subunit. We identified the physical interactions between each subunit of casein kinase 2 using co-IP. To find out that CK2 is related with cell cycle, we performed FACS analysis, and the knock out mutants show cell cycle disruption. Also, the knock out mutants shows abnormal and swollen cell morphology in SEM and TEM analysis. These data support that CK2 acts as a cell cycle regulator and each subunit plays distinct roles. As a result, Cka1 plays major roles and Ckb1/Ckb2 have minor roles in casein kinase 2 complex. This study will provide a comprehensive Cka1 cellular mechanism to develop an antifungal drug.

D036

Establishment of a Gastric Culture Collection and Screening Microbes for Antagonistic Activity against *Helicobacter pylori*

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Gastric cancer is the third leading cause of death globally with higher incidences in Korea. *Helicobacter pylori* is considered as the most severe risk factor of gastric cancer, since over 60% gastric diseases are caused by *H. pylori* infection. It is Gram-negative, spiral shaped, and grows under the microaerophilic condition. Additionally, the Cag pathogenicity island and the type IV secretion system promote the gastric cancer development by disrupting host signaling system. Therefore, eradication of this bacterium in the stomach is prerequisite to prevent the cancer development. Despite well-established treatment regimens, the emergence of antibiotic resistance hinders proper treatments. To overcome this, microbial interaction-mediated eradication is proposed for the removal of *H. pylori* in this study. To this end, the bacterial culture collection from stomach biopsies was established, and over 900 isolates were collected followed by taxonomic identification. Subsequently, we performed antagonistic tests against *H. pylori* by screening out 904 isolates. Antagonistic effects from 12 isolates and further co-culture assay confirmed the growth suppression effects from two *Streptococcus* species. The whole genome sequences of the two species provided the factors that are responsible for antagonistic effects. In conclusion, potent candidates for the removal of *H. pylori* have been identified, and further safety issues and *in vivo* validation of the antagonistic effects are planned.

D037

Functional Characterization of the Adenylyl Cyclase, *Cac1* in *Cryptococcus neoformans*

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Cryptococcus neoformans causes fatal cryptococcal meningoencephalitis mainly in immunocompromised patients, which leads to an average of 223,100 infections and 181,100 deaths annually in worldwide. Despite its clinical importance, comprehensive understanding of its therapeutic options for treatment of systemic cryptococcosis are highly limited. For the fungal pathogens, adaptation to external stresses caused by environmental change is important to survive and proliferate in the host. *Cryptococcus neoformans* has various signaling pathway to adapt to external stresses including cyclic AMP/protein kinase A (cAMP/PKA) signaling pathway. Cryptococcal adenylyl cyclase (*Cac1*), a major component of cAMP/PKA signaling pathway that catalyzes production of cAMP. Adenylyl cyclase consists of conserved five domains: adenylyl cyclase catalytic domain, phosphatase domain, adenylyl cyclase G-alpha binding domain, ras associating domain, and leucine rich repeats. To functionally analyze the *Cac1* in *C. neoformans*, we constructed domain deletion strains of *Cac1*. Phosphatase domain and adenylyl cyclase catalytic domain deletion strains were constructed and their phenotypic traits were tested. *Cac1* allele deleted of the Adenylyl cyclase catalytic domain could not completely non-functional essential for the function of *Cac1*. But phosphatase domain is required for some stress responses, capsule and melanin production of *Cac1*. This study will provide functional role of each domains in *Cac1*.

D038

Deciphering the Role of Pseudouridine Synthases in *Cryptococcus neoformans*

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Cryptococcus neoformans is a basidiomycetous fungal pathogen that causes systemic cryptococcosis and meningoencephalitis in immunocompromised individuals. Due to its clinical importance, revealing the factors that can affect its life cycle is critical for development of antifungal and anticryptococcal drugs. Among the various factors, pseudouridylation of RNA is the most abundant type of post-transcriptional modification. Pseudouridylases isomerize uridine into pseudouridine, which subsequently affects the stability of RNA structure. In *Saccharomyces cerevisiae*, eight proteins exist as stand-alone pseudouridylases, and each protein has specific catalytic sites and roles. To unravel the biological functions of the enzymes in *C. neoformans*, we identified six putative pseudouridylases in *C. neoformans* by using BLAST search in the FungiDB database with protein sequences of the known pseudouridylase genes. To characterize the function of pseudouridylases, we constructed more than two independent strains for 5 putative pseudouridylase genes and examined their phenotypic traits under various conditions. *CBF1*, which is essential gene in *S. cerevisiae*, is also suspected to be essential in *C. neoformans*. Among the proteins, *DEG1* and *PUS7* seemed to have major roles in stress responses and virulence of *C. neoformans*. By using pseudouridylation RNA-sequencing, we will identify pseudouridylated mRNA transcripts and characterize their role in pathogenicity of *C. neoformans*.

D039

Degree of Lipid A Modification Linked to Polymyxin Resistance in Clinical *Acinetobacter baumannii* Strains

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Acinetobacter baumannii is a nosocomial opportunistic pathogen that is considered a threat to human health due to the increasingly frequent isolation of its multi-drug resistant (MDR) strains. Among 40 *A. baumannii* clinical isolates, the 14 MDR strains were obtained based on minimum inhibitory concentration test using 5 different antibiotic classes compared to the EUCAST. As a result of multi-locus sequence typing for MDR strains, 5 strain types (STs) were identified. A total of nine MDR-strains [4 resistant strains, PMB^R; 5 susceptible strains, PMB^S] were chosen for further lipid A analyses based on STs and the level of polymyxin B (PMB) resistance. PCR-based sequence analyses for the *pmrCAB* operon, involved in modifying the charge of lipid A to which PMB binds, showed that PmrB variant was only found in the clinical PMB^R strains compared to the wild type 17978 strain. The higher levels (74.3~153.4 folds) of *pmrC* expression possibly by PmrB variant were measured by quantitative real-time PCR, which could not be seen in the PMB^S strains under PMB conditions. Matrix-assisted laser desorption ionization-time of flight mass spectrometry proved that *pmrB* mutation might play an important role in adding more frequent phosphoethanolamine to lipid A by inducing the *pmrC* gene, which resulted in high PMB resistant phenotypes in clinical MDR strains, but not in MDR PMB^S strains.

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D040

Two *Streptococcus* Species Show Antagonistic Effects against *Helicobacter pylori* and Their Genome Sequences Reveal Possible Mechanisms

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Gastric cancer is a buildup of abnormal cells that form a mass in the stomach. It can develop in any part of the stomach, and its incidence is high in Japan and Korea. More than 60% of the gastric cancer cases are due to *Helicobacter pylori* infection. Upon the colonization of the bacterium in the stomach, cancer development is induced by major virulence factors such as the type IV secretion system encoded in the Cag pathogenicity island. Therefore, to prevent carcinogenesis the removal of *H. pylori* is important. To meet the demand, we screened our in-house gastric culture collection to identify bacteria antagonistic against *Helicobacter* by interfering with the infection of *H. pylori* or suppressing its growth. We first established a culture collection, Bank of Gastric Microbiota, from gastric biopsy samples, and successfully recovered over 900 bacterial isolates. Screening tests found two *Streptococcus* species showing antagonistic effects against *H. pylori*. In addition, further liquid co-culture assay implied that the antagonistic activities of two isolates function through a contact-independent mechanism. Subsequently, whole genome sequencing of two bacteria was performed to investigate the factors associated with antagonistic effects using a hybrid sequencing strategy that employed PacBio and Illumina NovaSeq 6000. Initial sequence analyses raised a possibility that the antagonistic effects against *H. pylori* could be mediated by bacteriocins or secondary metabolites.

D041

Development of Real-Time PCR Method for Rapid Determination of *E. coli* Serotypes

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O-antigens are part of the lipopolysaccharide which constitutes the outer layer of Gram-negative bacteria that are responsible for classifying *Escherichia coli* strains and determining O-serotype. Although *E. coli* serotypes are important for epidemiological studies, conventional serotypes have been performed based on agglutination reactions using antisera, a delicate, difficult, time-consuming and costly procedure. Therefore, the purpose of this study was to develop a fast, accurate, and economic serotype identification method using Real-Time PCR (RT-PCR). Eleven *E. coli* serotypes (O145,157,26,103,111,6,25,78,55,104 and 124) were selected according to the frequency of foodborne outbreak. As a result of pan-genome analysis based on the selected serotype genome, it was confirmed that the homology of the *wzy* gene was significantly different. Based on these results, 11 different RT-PCR primer sets targeting the *wzy* gene were designed and confirmed by singleplex, cross-check, and multiplex PCR methods. The validated RT-PCR primer set does not differ from the results of other serotyping assays (antisera and *in silico* serotyping). Subsequent food application tests using this RT-PCR method revealed that this method detected 10¹ CFU/sample. Therefore, RT-PCR methods for rapid *E. coli* serotyping provided the rapid, accurate, and robust serotyping compared to the existing method.

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D042

Screening of Bat Guano Samples Collected in Korea between 2018 and 2020 through Real Time RT-PCR with Sarbecovirus-specific Primers and SARS-CoV-2-specific Probe

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SARS-CoV-2 has been thought to be associated with several bat species, but there has not been a confirmative report on that. In this regard, we tried to survey SARS-CoV-2 and its related viruses from bat guano samples in Korea. First, we designed primers and probes that can specifically detect SARS-CoV-2. The S1/S2 Cleavage site was targeted at the SARS-CoV-2 Spike Protein sequence to design primers and probes. The primer was designed to detect Sarbecovirus and the probe was designed to detect only SARS-CoV-2. RNAs from a bat guano sample that was previously positive for coronavirus, MERS-CoV, and SARS-CoV-2 were tested with the real-time PCR. No signal was observed in the RNA from bat guano and MERS-CoV. A detection limit of the real-time PCR was 2.5×10^{-4} ng/ μ l to 1.0×10^{-3} ng/ μ l of plasmid. A total of 364 bat guano samples collected from 2018 to 2020 were screened with the real-time PCR. When primers and probe were used, there were no positive samples. However, when primers without probe were used, 1 sample was positive. Sequencing data from that positive sample showed that it was a previously known bat SARS-related coronavirus. Thus, there was no SARS-CoV-2 and its closely related SARS-CoV-2 in bat guano samples collected between 2018 and 2020 in Korea.

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D043

α -2,3 Linked Sialic Acids are the Potential Receptor for Shaan Virus Infection in MARC-145 Cells

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Shaan virus, a novel species of family *Paramyxoviridae* was isolated from an insectivore bat (*Miniopterus schreibersii*) in Korea, 2016. Through sequence analyses, the isolated virus was found to have haemagglutinin-neuraminidase (HN) protein as one of the structural proteins. HN protein is known to perform the critical function of primary attachment of the paramyxoviruses to the target cell through interactions with sialic acids on the cell surface. In order to observe possible interaction between Shaan virus and sialic acids, neuraminidase inhibition assay was conducted using the neuraminidase inhibitor. We also analyzed the sialyl linkage specificity of Shaan virus using either α -2,3 or α -2,6 specific sialidases. The effect of neuraminidase inhibitor and sialidases to the viral replication were evaluated by measuring the virus-induced cytopathic effect (CPE) in MARC-145 cells and the viral antigenome production through quantitative PCR (qPCR). In addition, we analyzed the sialyl linkage pattern through a glycan array. The treatment of DANA in the maintenance medium reduced the Shaan virus specific CPEs in MARC145 cells. In the sialidase treatment assay, α -2,3 specific sialidases reduced the viral replication more. The glycan array results also indicated that Shaan virus prefers α -2,3 linked sialic acid. In conclusion, α -2,3 linked Sialic acids are the potential receptor for Shaan virus infection.

[This research was supported by NRF-2020R1C1C1010440.]

D044

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

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Vibrio vulnificus is a pathogen and has been subdivided into three biotypes. Biotype 1 is responsible for the majority of human infections. Also, biotype 1 is composed of two genotypes: a clinical genotype, and an environmental genotype. To identify their virulence factors and clinical characteristics, complete genome sequences of clinical genotype and environmental type were compared using pan-genome approach. This revealed that clinical genotype has a putative *fimZ* gene, but environmental genotype doesn't. The putative *fimZ* gene deficient mutant showed a colonial morphology change from opaque form to translucent form. Moreover, the mutant type formed significantly more biofilm than wild type. Previous studies have shown that the colonial morphology change and biofilm increase are due to a reduction of capsular polysaccharide (CPS). A virulent opaque form produces CPS and a translucent phenotype produces little or no CPS. According to the result of qRT-PCR, the mutant type displayed highly reduced expression of CPS associated genes. In addition, mutant type showed low swimming motility and low viability in macrophage. These results suggest that the putative *fimZ* gene affect the CPS production of clinical strains and indicate that this gene may be associated with virulence factor of clinical strains. [This research was supported by a grant (19162MFDS037) from Ministry of Food and Drug Safety in 2021]

D045

Characterization and Application of a New *Klebsiella pneumoniae*-infecting Phage KPP20 for Food Safety

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Klebsiella pneumoniae is an opportunistic human pathogen causing chronic pulmonary obstruction and diabetes mellitus. Due to abuse of various antibiotics, multidrug-resistant *Klebsiella* strains have been reported to be increasing, so bacteriophage has been considered as a novel biocontrol agent to control this pathogen. A novel lytic bacteriophage KPP20 was isolated from a sewage sample. Morphological observation using TEM showed that this phage belongs to the family *Siphoviridae*. And host range assay showed that KPP20 specifically infects *Klebsiella pneumoniae*. Stability test showed that KPP20 is highly stable under various temperature (-20 to 60°C) and pH (pH 3 to 11) conditions and useful for various applications. In addition, challenge assay showed 3.52 log reduction of *Klebsiella pneumoniae* in 2 h. The complete genome of KPP20 was analyzed and showed that the double-stranded DNA chromosome consists of 49,044 bp containing 95 ORFs with a GC content of 54.33%. Subsequent bioinformatics annotation revealed that KPP20 doesn't contain any toxin gene, virulence factor and antibiotics resistance. Therefore, this research suggests that KPP20 is safe for food applications.

D046

ERR α (Estrogen Related Receptor Alpha) is an Important Regulator of Intestinal Homeostasis through Modulation of Gut Microbiota and Activation of Autophagic Flux

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The ERR α is an orphan nuclear receptor and the roles of ERR α in intestinal functions remain unclear. Herein we identified that ERR α acts as a key regulator of intestinal homeostasis by amelioration of colonic inflammation through alteration of the gut microbiota and activation of autophagic flux. ERR α deficient mice presented with increased susceptibility to DSS-induced colitis with aggravation of intestinal inflammation. Also, ERR α deficient mice showed distinct gut microbiota composition and significantly higher microbial diversity than wild-type mice. Cohousing or fecal microbiota transplantation from WT mice to ERR α -deficient mice ameliorated DSS-induced colitis. In addition, ERR α deficient mice had decreased expression of AMPK and TFEB, and accumulation of SQSTM1/p62 with defective mitochondria in intestinal tissues. Importantly, patients with ulcerative colitis had significantly decreased ERR α expression in intestinal mucosal tissues that correlated with disease activity, suggesting clinical relevance of ERR α in UC. Taken together, our results show that ERR α contributes to intestinal homeostasis through modulation of gut microbiota and autophagy activation to protect the host from detrimental inflammation and dysfunctional mitochondria.

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D047

In Vitro* Combination Studies of Respiration Inhibitor against *Mycobacterium tuberculosis

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Respiration inhibitor is a new class of drug candidates to combat tuberculosis that inhibit an essential enzyme involved in oxygen respiration. Using the checkerboard method combined with traditional CFU determination assay, we have studied the interaction profiles of respiration inhibitor (RI), with several anti-tuberculosis drugs or drug candidates against *Mycobacterium tuberculosis*. Some antagonism was found between RI and the tested compounds, and most of the interactions were generally additive. Data clearly indicate that RI acts synergistically with decaprenyl phospho-ribose 2'-epimerase inhibitor (DNB), with a fractional inhibitory concentration index of 0.5. We therefore hypothesize that sub-MICs of DNB weaken the bacterial cell wall and allow improved entrance of compound X to reach its target. The synergy between two new antimycobacterial compounds offers a new tuberculosis regimen.

D048

Increased NK Immune-surveillance toward NLRP3-deficient Hepatocellular Carcinoma (HCC) through MICA/NKG2D Interaction

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Natural killer (NK) cell which is innate lymphoid cell has an important role in cancer development and metastasis. Human hepatocellular carcinoma (HCC) is common and poor at the prognosis because they are accompanied with other liver diseases such as cirrhosis. NLRP3 inflammasome is implicated in innate immune system but that could limit cancer treatments, which result to all stage of cancer development. To date, role of NLRP3 in various cancers have been reported but is not well studied how NLRP3 in HCC influences that function of NK immune-surveillance. Firstly, we found that NLRP3 was specifically expressed in human HCC SK-hep1 but not in human NK cell line NK-92. Then, we blocked the expression of NLRP3 in HCC SK-Hep1 using lentiviral CRISPR Lenti-CRISPR-NLRP3/Cas9 system. Subsequently, we identified that NK cytotoxicity was higher in a co-culture of NLRP3-deficient HCC than in a co-culture of NLRP3-expressing HCC, and also the expression of activating receptors on NK-92. This results connected to the expression of MICA on a surface of HCC cells. Lastly, we verified that mice implanted with NLRP3-deficient HCC cells was substantially inhibited in tumor development and metastasis compared to mice implanted with NLRP3-expressing HCC cells as well as was better in a sensitivity to NK-92 cells. Conclusively, the deficiency of NLRP3 in HCC might enhance NK immune-surveillance through the increased interaction of MICA/NKG2D on their surface.

D049

Novel Drug Combination for *Mycobacterium abscessus* Disease Therapy Identified in a *Danio rerio* Infection Model

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Mycobacterium abscessus is known to be the most drug-resistant *Mycobacterium* and accounts for ~80% of pulmonary infections caused by rapidly growing mycobacteria. This study reports a new *Danio rerio* (zebrafish)-*M. abscessus* infection model that can be used as an in vivo efficacy model for anti-*M. abscessus* drug potency assessment. The zebrafish-*M. abscessus* model enabled an assessment of the effectiveness of antibiotic treatment. As a combination approach for *M. abscessus* treatment, Rifabutin that is the most potent antibiotics for *M. abscessus* in animal model used as an anchor compound in combination with several anti-*M. abscessus* drugs or drug candidates against *M. abscessus*.

D050

Bacterial and Fungal Biofilm-related Gene Expression on Contact Lenses in Physiological Tear Solution

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Bacterial and fungal biofilm formation on contact lenses may increase the risk of eye diseases including keratitis, corneal ulcer and dryness. These eye diseases are closely related to tear stability, and tear circulation disorders reduce the antibacterial function of the eyes. In this study, we investigated the effects of tears on the biofilm related gene expressions on contact lens using *Staphylococcus aureus*, *S. epidermidis* and *Candida albicans*. Two types of soft lenses, etafilcon A and hilafilcon B, were used. The tear solution was prepared in phosphate buffer saline with lysozyme, globulin and albumin. The expression levels of biofilm formation-related genes were accessed using reverse transcription PCR and realtime PCR. In *Staphylococcus* spp., biofilm related genes including *ica*, *fnb*, *clf*, and *arc* were decreased after tear solution exposure. In *C. albicans*, the expression of biofilm related genes including *als3*, *ece1*, *hwp1* and *sap5*, which are hyphae-related genes mediating adhesion were investigated. The gene expressions were higher in etafilcon A than hilafilcon B. After exposure to tear solution, the gene expressions were decreased on both soft lens materials. These results suggest that tear fluid may protect eyes from bacterial and fungal infection and biofilm formation.

D051

Constructing Classification Model for Detection of Head and Neck Cancer by Oral Microbiome Profiling

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Head and neck cancer is the term that includes malignancies developed in several regions of head and neck except for brain and eyes. Recent evidences describe that oral microbiome is highly associated with pathogenesis and progression of head and neck cancer. Saliva microbiome harbors high diversity of microorganisms, however, little is known about which taxa mainly drives the pathogenesis of head and neck cancer. In this study, we analyzed oral microbiome profiles of saliva samples from patients with head and neck cancer and those from cancer-free participants using 16S rRNA amplicon sequencing. Overall, several genera, including *Capnocytophaga*, *Catonella*, *Oribacterium*, *Peptostreptococcus* and *Granulicatella*, were enriched in the saliva microbiome of patients with head and neck cancer, which were previously described as the genera correlated with high risks of tumors. We suggested the classification model using random forest to predict the pathogenesis of head and neck cancer and found the combinations of taxa that could be used as important biomarker for prediction of the aforementioned cancer. This model may be used as non-invasive method to diagnose malignant status of head and neck cancer.

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D052

Comparison of Assembly Results According to the Presence and Absence of Sequence 'K' When Performing WGS of IBV Using SISPA

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Infectious bronchitis virus (IBV), the coronavirus of chickens, has a significant economic impact on the poultry industry. It reduces egg productivity and causes poor growth of chickens. For IBV sequence analysis, it was common to analyze only the spike protein gene sequence, especially the S1 gene. However, recently, full genome analysis has been attempted for phylodynamic research. In this study, Whole Genome Assembly (WGA) of three IBV strains were performed. Sequence-Independent Single Primer Amplification (SISPA) was used to convert RNA into cDNA and amplify refined cDNA. The cDNA libraries were sequenced on an Illumina instrument and Illumina adapter sequences were removed with trimmomatic. After that sequence 'K' was removed by 'cutadapt' or 'fastp', de novo assembly was performed by SPAdes. To compare the relative quality of each sample's genome assemblies, genome assemblies were evaluated using QUality ASsessment Tool for Genome Assembly (QUAST). And then QUAST results of removing sequence 'K' and keeping it were compared. Comparing the results, all metrics of removing sequence 'K' had higher quality than the result of keeping it. Between 'cutadapt' and 'fastp', 'cutadapt' was about 2 times faster than 'fastp' and had the best results in almost all metrics. But in one strain, the results of running 'fastp' had higher k-mer coverage and more similar length of the longest contig with the reference genome than the result of running 'cutadapt'. Therefore, the sequence 'K' should be removed when performing IBV strains WGA using SISPA. When removing sequence 'K', performing both 'cutadapt' and 'fastp' and comparing the results provides an opportunity to achieve better results.

D053

Nationwide Surveillance of Infectious Bronchitis Virus on Live Bird Market in Korea, 2020

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Infectious bronchitis is an acute and highly contagious respiratory disease that results in severe economic losses to the global poultry industry. Its pathogen, Infectious bronchitis virus (IBV) is classified into different genotypes based on the S1 gene sequence. To investigate which types of IBV are present in Live bird market in Korea, Infectious bronchitis virus detection by RT-PCR, pathogen isolation and phylogenetic analysis were performed. As a result, IBVs were detected and isolated in 20 cases which accounts for 6.02%. Their genotypes were determined by phylogenetic analysis and comparison of S1 nucleotide sequence with other IBV reference strains. Among them, three strains were considered into-the vaccine strains since their partial S1 nucleotide sequences had 100% similarities with those of IBV vaccine strains. Others were classified into K1 (10 strains), QX (4 strains) and KM91 (2 strains). One sample showed a mixed pattern in S1 nucleotide sequencing and could not be analyzed. Our results showed that simultaneously multiple genotypes of IBV were present on the live bird market in Korea. As the bird market is a place where poultry from various farms are gathered, the existence of various genotypes of IB viruses is highly likely to result in the recombination of new genotypes of IB viruses. Therefore, it is necessary to take preventive measures such as disinfection management in the markets.

D054

Sero-epidemiological Surveillance of Newcastle Disease Virus in Wild Birds in Korea, 2020

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Newcastle disease (ND) is one of the most dangerous avian diseases in the world. ND is caused by virulent strains of avian avulavirus type 1 (AAV-1), formerly avian paramyxovirus, in the genus orthoavulavirus. Wild birds are considered as reservoir hosts for ND virus and may have the potential to act as a source of the outbreaks in poultry. For serological surveillance of ND, hemagglutination inhibition (HI) assay using LaSota antigen were performed using serums from 466 captured wild birds in 2020. The HI positive rate was 17%. Especially, 50% of tested Mandarin duck (*Aix galericulata*) was HI positive. However, the mean titer of positive Mandarin duck sample was not high ($2^{3.4}$). Because these can be false positive by infection of other type of avulavirus, an additional HI assay using strains of classified into AAV-2 to 4 and 6 to 9 was conducted on mandarin duck serum samples. In the HI assay, although thirteen specimens (32%) showed cross-reaction with AAV-6 and seven specimens (17%) showed cross-reaction with AAV-8. As well, there was no sample with a higher HI titer of AAV-2 to 4 and 6 to 9 than that of AAV-1. This result suggests that mandarin ducks were infected with several types of AAVs, but appear to be infected with a particularly high rate of ND virus. Therefore, more effort for surveillance and inspection is needed because wild birds, especially Mandarin duck can act as reservoirs for NDV.

D055

Dysbiosis of Oral Microbiota in a Mouse Model of Head and Neck Cancer Induced by 4-Nitroquinoline 1-Oxide

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Head and neck cancers (HNSCC) are mostly caused by tobacco and alcohol use. 4-Nitroquinolone oxide (4NQO) is used for establishment of a chemically induced HNSCC animal model for immunocompetent mouse. The 4NQO is a water-soluble carcinogen, resulting in tumors predominantly in the oral cavity. In our study, the mice were administered 4NQO in the drinking water for 16 weeks, and thereafter they were untreated up to week 26. At week 16, 4NQO-treated mice showed higher relative abundance of the family Streptococcaceae in the oral microbiota than 4NQO-untreated control mice. However, gut microbiome was affected by dietary type rather than 4NQO treatment. As expected, mice exposed to 4NQO for 16 weeks developed oral tumors at 26 weeks. Our results suggest that the carcinogen or carcinogen-induced tumor microenvironment leads to oral dysbiosis, which may be involved in tumor development.

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D056

Comparison of Intradermal (ID) and Intramuscular (IM) Vaccination of Foot-and-Mouth Disease Vaccine (FMDV) in Swine

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Foot-and-mouth disease (FMD) is a highly contagious disease that causes serious economic losses in terms of animal products as well as controlling disease prevention. In Korea, the outbreak of FMD in 2010 caused great damage to the livestock industry. Previous foot-and-mouth disease vaccine development consisted mainly of intramuscular (IM) vaccine. However, IM of FMD vaccine in pigs has a problem of necrosis or abnormal muscle in the inoculation site. Therefore, recent vaccine studies are developing vaccines through intradermal (ID) vaccination, which has a significantly low incidence of abnormal muscle in the inoculation site and has advantages in terms of cost-saving. Therefore, in this study, we compared ID and IM injection using FMD vaccine in pigs. In addition, the previously reported ID inoculation amount (0.5ml) was inoculated differently from 0.2ml to 0.5ml and compared. When compared ID and IM injection, ID showed similar PI value with lower inoculation amount than existing IM vaccine.

In conclusion, as previously reported, the ID vaccine, which can be expected to have economic effects with low incidence of side effect and low vaccination dose, has several advantages.

D057

Research on Adjuvant with High Immune Response from Early to Late Stage in Intradermal Foot-and-Mouth Disease Vaccine (ID FMDV)

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Foot-and-mouth disease (FMD) is a highly contagious disease that causes serious economic losses in livestock industry. It is single strand RNA virus and has seven serological type. FMD virus infects not only cloven-hoofed animals such as cattle and swine but also various species of animal. In a previous study, we researched to select a suitable adjuvant for the ID FMD vaccine. The ID FMD vaccine containing MVP-D (20%) showed a higher immune response in the early stage than in the late stage. We studied to determine changes in immune response by increasing the amount of MVP-D added to the vaccine. In addition, dimethyldioctadecyl ammonium bromide (DDA), which has an effect on early immunity, was added to ISA 207 to confirm the immune response from the early stage to the late stage. In addition, we plan to conduct an additional study to select an adjuvant with high immunity in the early, middle and late stages. As a result of sp ELISA, contrary to the expected results, even if a higher amount of MVP-D was added, there was no difference with the antibody titer of the existing 20% vaccine. Also, the ISA 207 group with DDA added showed similar or lower antibody titers than the other two adjuvants groups. Rather, the late immune response was more reduced than in the other groups. In conclusion, the adjuvant currently studied had the best effect when used alone, and additional studies on other adjuvant such as nanoparticle are needed.

D058

Selection of Effective Adjuvants for Research of Intradermal Foot-and-Mouth Disease Vaccine (ID FMDV) with High Immunity

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Foot-and-mouth disease (FMD) vaccine development was consisted mainly of intramuscular (IM) vaccine. However, IM of FMD vaccine in pigs has a problem of necrosis or abnormal muscle in the inoculation site. We studied to develop a intradermal (ID) domestic FMD vaccine. In the dermis, a lot of dendritic cells. So, ID inoculation can induce more stronger immune response. Existing vaccines are added with adjuvant that can produce a higher immune response. Therefore, It is very important to compare the immune response of adjuvants used in ID FMD vaccines. We compared to find an adjuvant with a better immune response. First, we compared adjuvants, ISA207 and MVP series, to find the adjuvant with the highest immune enhancing effect. We used guinea pigs in this experiments. And then, confirm the change in antibody titer through antigen sp ELISA. As a next step, in order to find adjuvant that high immune response in both early and late stages, we mixed that MVP-D with a high PI value in the early stage and ISA207 with a high PI value in the late period to checked whether the high PI value is maintained until the early and late stages. However, as a result of confirming the mixed adjuvant results up to 3 weeks, the PI value was rather lower than that of the group treated alone. In conclusion, as a result of comparing the adjuvant, the ISA207 value was confirmed best, and since the PI value of the MVP series increased at the beginning of inoculation.

D059

Guardians and Raiders of the Stomach: Microbes Associated with Gastric Disease States and Transplantation of Human Gastric Microbiota to Germ-free Mice

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Gastric cancer (GC) is one of the prevalent cancers in the world, for which microbial effects are well explained in cancer development: *Helicobacter pylori* infection triggers the carcinogenesis process. Recent reports, however, suggest the possibility that *H. pylori* is not the sole microbial factor causing GC. We analyzed microbiota structures of the stomach through 16S rRNA gene sequence analysis with respect to disease states that include chronic superficial gastritis, intestinal metaplasia (IM), and GC. Gastric microbiota structures were distinctive between disease states. We observed some microbial groups were abundant in superficial gastritis or GC respectively. Biopsied corpus or antrum tissues or gastric fluids of patients with chronic superficial gastritis, IM, or GC were inoculated into germ-free C57BL/6 mice to observe the effects of gastric microbiota related to gastric disease. Although the gastric microbiota from patients with CSG, IM or GC were selectively colonized in the mouse stomach, correlations between disease and taxa in transplanted mice which also showed a similar tendency in human gastric microbiota were identified. This study allows us to understand the gastric microbiota structure related to carcinogenesis and provide us useful model analyzing the causality of associations in human gastric microbiome studies.

D060

N-Acetylneuraminic Acid Promotes Extracellular Matrix Adherence by Pathogenic Bacteria

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Shigella flexneri, the causative agent of shigellosis, is a Gram-negative bacterial pathogen that initiates infection by invading cells of the colonic epithelium. The mechanism of adherence of the pathogen to the extracellular matrix remains poorly characterized.

In this study, we investigated the binding of *S. flexneri* strain 2457T to basolateral fibronectin, which is normally present on colonic epithelial cells. We also examined the role of exogenous N-acetylneuraminic acid for adherence of 2457T to fibronectin. The bacteria bound to fibronectin immobilized on microtiter plates in a concentration-dependent manner ($P < 0.01$). Soluble fibronectin inhibited by 73% ($P < 0.001$) the binding of 2457T to immobilized fibronectin. Various extracellular matrix components had no effect on adherence to fibronectin. However, pretreatment with mucin caused 33% inhibition ($P < 0.05$); whereas, pretreatment with N-acetylneuraminic acid resulted in a 156% increase in binding to immobilized fibronectin ($P < 0.001$). Addition of mucin-derived N-acetylneuraminic acid led to a 119% increase in adherence of 2457T to fibronectin ($P < 0.001$).

We provide strong evidence that *S. flexneri* binding to fibronectin is promoted by mucin-derived N-acetylneuraminic acid.

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D061

Genomic Characterization of Nine *Clostridioides difficile* Strains Isolated from Korean Patients with *Clostridioides difficile* Infection

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Clostridioides difficile infection (CDI) is an infectious nosocomial disease caused by *Clostridioides difficile*, an opportunistic pathogen that occurs in the intestine after extensive antibiotic regimens. Nine *C. difficile* strains (CBA7201–CBA7209) were isolated from nine patients diagnosed with CDI at the national university hospital in Korea, and the whole genomes of these strains were sequenced to identify their genomic characteristics. Comparative genomic analysis was performed using 51 reference strains and the nine isolated herein. Phylogenetic analysis based on 16S rRNA gene sequences confirmed that all 60 *C. difficile* strains belong to the genus *Clostridioides*, while core-genome tree indicated that they were divided into five groups, which was consistent with the results of MLST clade analysis. All strains were confirmed to have a clindamycin antibiotic resistance gene, but the other antibiotic resistance genes differ depending on the MLST clade. Interestingly, the six strains belonging to the sequence type 17 among the nine *C. difficile* strains isolated here exhibited unique genomic characteristics for these toxin gene loci and had similar antibiotic resistance genes. In this study, we identified the specific genomic characteristics of Korean *C. difficile* strains, which could serve as basic information for CDI prevention and treatment in Korea.

D062

Clinical Efficacy Evaluation of Recombinant Porcine Circovirus Type 2 (PCV2) Vaccine Composed of Virus-Like Particle (VLP) Expressing PCV2b and PCV2d Consensus Capsid Protein Sequence

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The pathogenic porcine circovirus type 2 (PCV2) is causative agent of porcine circovirus-associated disease (PCVAD) and is involved in a significant economic damage to the swine industry worldwide. The PCV2 can be divided into 8 genotypes (PCV2a - PCV2h). Since the early 2000s, PCV2a genotype was prevalent worldwide and the genotype shift to PCV2b occurred in 2003. PCV2d has been recently become a popular genotype. In Korea, PCV2d is most frequently isolated about 95% and is more prevalent genotype than PCV2b. Nevertheless, most commercial PCV2 vaccines are based on PCV2a or PCV2b genotypes remaining questions about vaccine effectiveness. In this study, we used a baculovirus expression system to express recombinant PCV2b and PCV2d consensus capsid proteins (PCV2b&d-ConCP), and the efficacy of recombinant virus-like particle (VLP) as a vaccine was analyzed. The results showed that PCV2b&d-ConCP VLP vaccination induced high levels of both PCV2b and PCV2d-specific immune responses including neutralization antibodies. In addition, it effectively reduced PCV2d viremia and prevented PCV2d infection in the lungs and lymph nodes in pigs challenged with virulent PCV2d.

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D063

Contribution of the Periplasmic Chaperone Spy to Flagella and SPI-2 Type III Secretion Systems of *Salmonella* Typhimurium

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Periplasmic chaperones maintain proteostasis in the extracytoplasmic space against environmental stresses. Spy is known as a stress-inducible molecular chaperone expressed and localized in the periplasm of *E. coli*. However, its client proteins and their physiological relevance in bacterial infections remain unexplored. We show that Spy is required for the fitness and pathogenesis of *Salmonella* Typhimurium. Mutant *S. Typhimurium* lacking *spy* showed attenuated virulence in a murine infection model. Comparative secretome analyses of WT and *spy* mutant cultured under *Salmonella* pathogenicity island (SPI)-1- and SPI-2-inducing conditions showed that *spy* mutation caused significant reductions in the secretion of proteins required for flagella biogenesis and SPI-2 type three secretion system. Accordingly, compared to WT, *spy* mutants were less motile, exhibited altered flagella phase variation, and showed impaired SPI-2 type three secretion system functions, including reduced formation of *Salmonella*-inducible filaments in infected epithelial cells and a notably lower proliferation rate in macrophages. The *spy* mutation made little difference to the mRNA levels of genes encoding proteins differentially secreted in *spy* mutant cultures, suggesting that the Spy chaperone may directly promote periplasmic proteostasis, which is essential for bacterial pathogenesis in animals.

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D064

Study of ssDNA Aptamers that Specifically Bind to Major Food Poisoning Bacteria

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Food is very important in human life. Recently, interest in food has been increasing due to the emergence of various functional foods. However, despite the development of food industry, food safety issues occur continuously. Therefore, this study examined ssDNA aptamer which has high affinity and specificity in the bacterial surface molecules and targets live bacteria with *Escherichia coli* O157:H7 (ATCC 700728), *Staphylococcus aureus* (ATCC 6538) and *Salmonella typhimurium* (ATCC 14028) the representative food poisoning bacteria in human. Through the Whole-cell Systemic Evolution of Ligands by Exponential enrichment (SELEX) process, 16,510, 2,139 and 27 aptamer candidates were obtained from *E. coli*, *S. aureus* and *S. typhimurium*, respectively. Among them, 39, 28 and 6 sequences expected to have high binding affinity were selected, respectively. Binding assay was performed by combining FAM with the selected sequence. And, the aptamer sequence observed to have high binding affinity at the 526-535 nm range was confirmed.

[This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT)]

E001

Amino Acid Availability-dependent Regulations of Peptidoglycan Endopeptidases in *Escherichia coli*

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Peptidoglycan (PG) is a mesh-like structure that is required for maintaining cell morphology and overcoming turgor pressure. PG endopeptidases (EPases) play a role as 'space-maker' for the insertion of new synthesized PG strands. Although physiological roles of EPases have been significantly revealed, the regulatory mechanisms of their expression are poorly unveiled. In this study, we analyzed novel nutrient-dependent regulations of the synthetically lethal EPases MepS and MepM. A *mepS mepM* double mutant was lethal in LB or amino acid-rich medium, but this lethality was almost completely abolished in minimal medium, indicating the unnecessary of MepS and MepM in minimal medium. Western blot experiments showed that protein levels of two EPases dramatically decreased in the minimal medium. Although both proteins are substrates of the periplasmic protease Prc, the decreased level of MepS was regulated by Prc in minimal medium, whereas the level of MepM was not affected by Prc. The protein level of MepS was increased by the presence of aromatic amino acids, while that of MepM increased in the presence of glutamate in the medium. In conclusion, these results suggest that both of MepS and MepM levels are regulated by amino acid availability, but molecular mechanisms of these regulations are distinct.

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E002

Functional Characterization of HigBA Toxin-antitoxin System in an Arctic Bacterium *Bosea* sp. PAMC 26642

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Toxin-antitoxin (TA) systems are widespread genetic modules in bacterial and archaeal genomes and consist of intracellular toxin protein and its cognate antitoxin. Toxin proteins interfere with vital cellular processes through various mechanisms and consequently cause growth arrest. However, under normal growth condition, antitoxins are tightly counteracting the activity of the toxin. Upon stresses, antitoxins are inactivated, which freeing and activating toxins. Therefore, it has been suggested that TA modules participate in the adaptation to stressful conditions. The BoHigB (AXW83_11955) toxin, one of HigB homologs in *Bosea* sp. PAMC 26642 living the Arctic lichen *Stereocaulon* sp., showed growth-inhibitory activity on *E. coli*, which was restored by the co-expression of BoHigA (AXW83_11960), suggesting that this BoHigBA module might be functional in *Bosea* sp. PAMC 26642. Microscopic observation and growth resumption assay revealed that the BoHigB had bactericidal effect on the growth of *E. coli*. The BoHigB toxin had a ribonuclease activity against the *crp*, *polA*, and *hisG* mRNAs being translated, resulting in the disappearance of polysomes. These findings imply that inhibition of protein synthesis by BoHigB might trigger growth arrest in *E. coli*.

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E003

Overexpressed NhaR Restores Cold-sensitivity of *Escherichia coli* *bipA*-deletion by Reinstating the Surface Polysaccharides Synthesis

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The BipA protein is ubiquitously conserved in various bacterial species and belongs to the translational GTPase family. The function of *Escherichia coli* BipA is not essential for cell growth under optimal growth conditions. However, cultivation of $\Delta bipA$ cells at 20°C leads to growth defect and several phenotypic changes in ribosome assembly, capsule production, lipopolysaccharides (LPS) synthesis, biofilm formation, and motility, suggesting its global regulatory roles at 20°C. In addition, BipA has been shown to regulate the virulence factors in a number of gram-negative bacteria, including *E. coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* serovar Typhi, *Vibrio cholerae*, and *Yersinia pestis*. Here, through genomic library screening, we found a suppressor clone containing *nhaR*, encoding a transcriptional regulator for genes involved in adaptation to high pH and salt conditions, which is not directly associated with ribosome biogenesis. Interestingly, overexpression of *nhaR* in $\Delta bipA$ cells restored the capsule synthesis, LPS maturation, and biofilm-forming ability at 20°C without resolving defects in ribosome assembly, indicating that NhaR may be involved in the regulation of exopolysaccharides and LPS synthesis as well as biofilm formation. In this study, we revealed novel roles of NhaR in surface polysaccharides biosynthesis at 20°C using $\Delta bipA$ strain.

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E004

Crystal Structure of Nuclease SbcD from *Staphylococcus aureus*

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The SbcCD complex is an essential component of the DNA double-strand break (DSB) repair system in bacteria. The bacterial SbcCD complex recognizes and cleaves the DNA ends in DSBs by ATP-dependent endo- and exonuclease activities as an early step of the DNA repair process. SbcD consists of nuclease, capping, and helix-loop-helix domains. Here, we present the crystal structure of an SbcD fragment from *Staphylococcus aureus*, which contained nuclease and capping domains, at a resolution of 2.9 Å. This structure shows a dimeric assembly similar to that of the corresponding domains of SbcD from *Escherichia coli*. The *S. aureus* SbcD fragment exhibited endonuclease activities on supercoiled DNA and exonuclease activity on linear and nicked DNA. This study contributes to the understanding of the molecular basis for how bacteria can resist sterilizing treatment, causing DNA damage.

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E005

Roles of the Inner Membrane Protein LapB in Regulation of the Proteolysis of LpxC and Adaptation to Cold Stress

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The lipopolysaccharide (LPS) assembly protein B (LapB) is an inner membrane protein that is involved in activation of the proteolysis of LpxC, a soluble enzyme that catalyzes the second reaction of LPS biosynthesis, by an essential inner membrane metalloprotease, FtsH. In this study, we determined the novel mechanism of the regulation of LapB. The essential inner membrane protein YejM inhibited LapB by direct interaction, which led to the accumulation of LpxC. The transmembrane domains of two proteins were indispensable for both their physical interaction and regulation of LpxC proteolysis. Additionally, we revealed that the *lapB* mutant was strongly sensitive to cold stress. Notably, this phenotype was not associated with increased accumulation of LpxC. The transmembrane domain of LapB was also required for its role in adaptation to cold stress. In summary, these results demonstrated that LapB plays an important role in both the regulation of LpxC proteolysis, which is controlled by its interaction with the transmembrane domain of YejM, and adaptation to cold stress, which is independent of LpxC.

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E006

Antibiofilm Activity of Phorbaketals from the Marine Sponge *Phorbasp.* against *Staphylococcus aureus*

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Biofilm formation by *Staphylococcus aureus* plays a critical role in the persistence of chronic infections due to its tolerance against antimicrobial agents. Here, we investigated the antibiofilm efficacy of six phorbaketals: phorbaketal A (**1**), phorbaketal A acetate (**2**), phorbaketal B (**3**), phorbaketal B acetate (**4**), phorbaketal C (**5**), and phorbaketal C acetate (**6**), isolated from the Korean marine sponge *Phorbasp.* Of these six compounds, **3** and **5** were found to be effective inhibitors of biofilm formation by two *S. aureus* strains, which included a methicillin-resistant *S. aureus*. In addition, **3** also inhibited the production of staphyloxanthin, which protects microbes from reactive oxygen species generated by neutrophils and macrophages. Transcriptional analyses showed that **3** and **5** inhibited the expression of the biofilm-related hemolysin gene *hla* and the nuclease gene *nuc1*.

E007

Microbiota is Associated with Anti-stress in the Intestine and Brain

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During psychological stress, the intestinal microflora changes, causing serious health problems worldwide. Integrity of the intestinal barrier and blood brain barrier can regulate the process of bacterial translocation and provide the nervous system with real-time information about the environment. We use a rodent model to explore the association between damaged intestinal and blood brain barriers and altered fecal microflora under psychological stress to improve understanding of the camphor axis. Here, clues converge to control the basic developmental processes of the intestine and brain, such as barrier function. This study presents a new direction for investigating the etiology of emotional disorders and the formula of clinical treatment.

E008

Melanogenesis Inhibitory Effect of Xanthatin Isolated from *Xanthium orientale* L.

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The purpose of this study was to investigate melanogenesis inhibitory activity of Xanthatin isolated from *Xanthium orientale* L.. Xanthatin was isolated from ethanol extract of *X. orientale* L. whole plant. Its chemical structure was measured by spectroscopic analysis, including, LC-ESI-MS, 1D-NMR, and UV spectroscopy. Xanthatin inhibited melanogenesis of B16F10 cell in a dose-dependent manner. Especially, Xanthatin was 21.5% decreased the melanin content at 5 μ M compared with control in B16F10 cell without cytotoxicity. Xanthatin presented inhibition of the body pigmentation in zebrafish embryo model. These results suggest that Xanthatin isolated from *X. orientale* L. is an effective whitening agent.

[Supported by grants from Nakdonggang National Institute of Biological Resources (NNIBR), funded by the Ministry for Environment (MOE) of the Republic of Korea (NNIBR202102101)]

E009

Cloning and Functional Characterization of Excitatory GABA-gated Cation Channel

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Gamma-aminobutyric acid (GABA) mediates fast inhibitory neurotransmission by activating anion-selective ligand-gated ion channels. Although electrophysiological studies indicate that GABA may activate cation-selective ligand-gated ion channels in some cell types, such a channel has never been characterized at the molecular level. Here we show that GABA mediates enteric muscle contraction in the nematode *Caenorhabditis elegans* via the EXP-1 receptor, a cation-selective ligand-gated ion channel. The EXP-1 protein resembles ionotropic GABA receptor subunits in almost all domains. In the pore-forming domain of EXP-1, however, the residues that confer anion selectivity are exchanged for those that specify cation selectivity. When expressed in *Xenopus laevis* oocytes, EXP-1 forms a GABA receptor that is permeable to cations and not anions. We conclude that some of the excitatory functions assigned to GABA are mediated by cation channels rather than by anion channels.

E010

Swd2/Cps35 Protect Set1 Protein Stability from Degradation

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H3K4 methylation is a well-conserved histone modification from yeast to human. Because H3K4 methylase Set1 and its complex, COMPASS (Complex of proteins associated with Set1), are conserved from yeast to humans, budding yeast has been studied as an acceptable model organism. Since COMPASS components affect Set1 protein stability and H3K4 methylation activity variously, it is important to study how Set1 is regulated by complex components. However, deletion mutant of Swd2 component of COMPASS is not viable, although overexpression of Sen1 fragment enables the construction of Swd2 deletion mutant. This study found that positioning epitope tag to the N-terminal of Swd2 did not decrease interaction between Swd2 and Set1, but reduced the stability of both proteins, Swd2 and Set1, and global H3K4 methylation. Also, we observed that overexpression of N-terminal tagged Swd2 caused increased Set1 protein level and bulk H3K4 methylation. Therefore, Set1 protein can maintain its protein level only when enough Swd2 exist to cover the protein amount of Set1. Also, by comparing RNA sequencing analysis of N-terminal tagged Swd2 and Swd2 deletion mutant with Sen1 fragment overexpression, we isolated genes regulated by Swd2. In conclusion, we suggest that the abundance of Swd2 is important to regulate the protein stability of Set1 and the regulation of gene expression.

E011

Role of IplA in the Regulation of Cell Migration in *Dictyostelium*

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Cell migration is an essential cellular process and is mediated by the asymmetric regulation of the cytoskeleton at the anterior and posterior of migrating cells. It has been shown that Ca^{2+} influx was stimulated by the chemoattractant and that the intracellular calcium ions were involved in the regulation of cell migration. Inositol-1,4,5-trisphosphate receptor-like protein A (IplA) is located at the endoplasmic reticulum membrane and plays a role in the control of calcium concentration in the cytosol with InsP_3 binding in response to the external signals. Disruption of *iplA* in *Dictyostelium* abolishes intracellular Ca^{2+} rise in response to chemoattractants. Here, we investigated the role of IplA in cell migration in *Dictyostelium*. *iplA* null cells were smaller and showed decreased cell adhesion compared to wild-type cells. In cell migration assay, *iplA* null cells displayed increased migration speed and decreased directionality towards the chemoattractants. Similar results were observed in electrotaxis. Cells lacking IplA showed no significant phenotype in the developmental process. To understand the regulation of calcium influx in response to the stimuli, we developed a *Dictyostelium* specific calcium-sensor protein using pGCaMP3. The sensor proteins were expressed in wild-type cells and *iplA* null cells, and the calcium influx in response to external stimuli, chemoattractants and electric fields, are under investigation.

E012

Pi3-kinases Play Different Roles Depending on the Developmental Stage in Regulating Cell Motility

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Cell migration is essential for diverse cellular processes including wound healing, immune response, development, and cancer metastasis. Pi3-kinase is a key regulator for actin cytoskeleton and phosphorylates phosphatidylinositol (4,5)-diphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3). High levels of PIP3 by Pi3-kinase are associated with increased levels of F-actin and pseudopod extension in neutrophils and *Dictyostelium*. We investigated the role of Pi3k on cell migration in electrotaxis and found that the roles of Pi3K in regulating cell motility differs depending on the developmental stage of the cells. Vegetative cells showed increased cell motility in the presence of LY294002, a Pi3K inhibitor, whereas developed cells displayed decreased motility. Similar results were found in the experiments using *pi3k* null cells. Further confirmation experiments are in progress.

E013

Characteristics of *Aspergillus oryzae* Strains Isolated from Meju and Analysis of Aflatoxin Reduction Effect during Fermentation of Meju

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Woosuk University

Filamentous fungi of 7 genera, 30 species, and 260 strains were isolated from fermented Meju commercially available in Korea. As a result of morphological analysis, ITS region sequencing and aflatoxin-producing ability test, a total of thirty-two strains of *Aspergillus oryzae* were identified. We analyzed 6 highly frequent mutation regions in the aflatoxin gene cluster (AGC) by PCR method and identified a total of six types of AGC deletions. In addition, the isolated *A. oryzae* strain did not show the high amylase activity seen in the commercial yellow-koji strain, and the activity of lipase and peptidase did not show a significant difference between the strains. Meanwhile, we found that the production of aflatoxin in Meju was reduced when *A. oryzae* and *A. flavus* strains were simultaneously inoculated and fermented. The isolated *A. oryzae* strain was inoculated into Meju at a rate of 12% of the total seed inoculum (5 g/kg), and *A. flavus* was inoculated to 88% and fermented, it was confirmed that the production of aflatoxin was completely inhibited. By selecting *A. oryzae* strains representing the characteristics of their AGC mutation patterns and properties of enzyme activity, we intend to analyze their potential to be used as seeds for soybean Meju fermentation.

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E014

CRISPR/Cas9-mediated Genome Editing of rapA and rasG in Dictyostelium

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Ras proteins are small GTPases to regulate various cellular processes. RapA among 19 Ras subfamily is a key regulator of cell adhesion and cell motility in Dictyostelium. In this study, we determine if rapA is essential using CRISPR/Cas9 system and investigate roles of RapA in cell migration using rapA-edited cell lines. RasG is another key Ras protein involved in the regulation of actin cytoskeleton and cell survival. There are several cell lines lacking rasG. However, the phenotypes of rasG null cells are slightly different for each cell line. Here, we are planning to generate an independent rasG null cell line using CRIPR/Cas9 system and determine the roles of RasG in cell migration. CRISPR/Cas9-mediated genome editing in Dictyostelium has been recently developed and used for editing multiple genes and testing the essentiality of a gene. We obtained the Dictyostelium-specific CRISPR/Cas9 system (all-in-one plasmid, pTM1285). A 20-nucleotide target sequence for RapA and RasG was designed using web-based tools and cloned into the single-guide RNA (sgRNA) site of the plasmid using Bpil restriction enzymes and Golden Gate Assembly kits. The CRISPR/Cas9 plasmids were transformed into Dictyostelium cells by electroporation followed by incubating the cells in the medium containing 10 ug/ml G418. The cells were collected after 1 days and then plated on SM agar plates with *K. aerogenes*. Further studies using cells expressing RapA- or RasG-edited proteins are in progress.

E015

Oxidative Stress Causes Vacuolar Fragmentation in the Human Fungal Pathogen *Cryptococcus neoformans*

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Vacuoles are dynamic cellular organelles, and their morphology is altered by various stimuli or stresses. Vacuoles play an important role in the physiology and virulence of many fungal pathogens. For example, a *Cryptococcus neoformans* mutant deficient in vacuolar functions showed significantly reduced expression of virulence factors such as capsule and melanin synthesis and was avirulent in a mouse model of cryptococcosis. In the current study, we found significantly increased vacuolar fragmentation in the *C. neoformans* mutants lacking *SOD1* or *SOD2*, which respectively encode Zn, Cu-superoxide dismutase and Mn-superoxide dismutase. The *sod2* mutant showed a greater level of vacuole fragmentation than the *sod1* mutant. We also observed that the vacuoles were highly fragmented when wild-type cells were grown in a medium containing high concentrations of iron, copper, or zinc. Moreover, elevated temperature and treatment with the antifungal drug fluconazole caused increased vacuolar fragmentation. These conditions also commonly cause an increase in the levels of intracellular reactive oxygen species in the fungus, suggesting that vacuoles are fragmented in response to oxidative stress. Furthermore, we observed that Sod2 is not only localized in mitochondria but also in the cytoplasm within phagocytosed *C. neoformans* cells, possibly due to copper or iron limitation.

[Supported by grants from National Research Foundation of Korea.]

E016

Structure-based Identification of Broad Inhibition Activities of Halisulfates against β -Lactamases

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AmpC BER is an extended-spectrum (ES) class C β -lactamase. Compared to its narrow spectrum progenitor, AmpC BER has a two-amino-acid insertion in the H10 helix region defining the boundary of the active site. This insertion widens the active site by restructuring the flexible H10 helix region, which is likely to be associated with enhanced catalytic activities towards bulky β -lactam antibiotics. Furthermore, two tightly-bound sulfates from the crystallization solution were observed in the active site. The ability of AmpC BER to accommodate sulfate and ring-structured chemical scaffolds led us to perform *in silico* molecular docking experiments with halisulfates, natural products isolated from marine sponge. We demonstrated that halisulfates 3 and 5 noticeably inhibit ES class C β -lactamase, particularly the inhibition efficiency of halisulfate 5 is comparable with avibactam; nitrocefin-hydrolyzing activity of AmpC BER is inhibited with a K_i value of 5.87 μ M in a competitive manner. In addition, halisulfate 5 showed moderate and weak inhibition activities against class A and class B/D enzymes, respectively. The broad inhibition spectrum of halisulfates indicates that their structural scaffold could be used to develop novel inhibitors that can escape bacterial resistance mechanisms mediated by β -lactamases.

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E017

Novel Catalytically-incompetent Pose of Carbapenems Observed on Acylated ACC-1

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The hydrolysis of β -lactam antibiotics by class C β -lactamases proceeds through the acylation and the rate-determining deacylation steps mediated by the nucleophilic serine and the deacylation water, respectively. The pose of poor substrates such as carbapenems in the acylated enzyme is responsible for the low efficient deacylation reaction. Here we present the crystal structures of the Y150F variant of the ACC-1 class C β -lactamase in the apo and acylated states. In the acylated enzyme complexed with two carbapenems, imipenem and meropenem, the lactam carbonyl oxygen is located in the oxyanion hole. However, the five-membered pyrrolidine ring displays a novel orientation that has not been reported so far. The ring is rotated such that its C3 carboxylate makes salt bridges with Lys67 and Lys315, which is accompanied by the side-chain rotamer change of Phe150. The C3 carboxylate is placed where the deacylation water occupies in the apo-enzyme, which, together with the displacement of the catalytic base residue at position 150, explains why carbapenems are poor substrates of ACC-1. [This study was supported by a grant NRF-2015M1A5A1037480 (the National Research Foundation of Korea), the project entitled 'Development of Biomedical materials based on marine proteins' (the Ministry of Oceans and Fisheries, Korea), a grant from the Collaborative Genome Program 20180430 (the Ministry of Oceans and Fisheries, Korea).]

E018

Identification and Functional Description of *Clostridioides difficile* Hydrogen Peroxide Sensor (PerR)

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Clostridioides difficile, before known as *Clostridium difficile*, is a ubiquitous anaerobic, Gram-positive, toxins-producing, and spore-forming pathogenic bacteria. Because this intestinal pathogen is one of the major causes of antibiotic-associated diarrhea around the world, it is important to understand the ways that permit *Clostridioides difficile* to survive in the host environment. Being an obligate anaerobe, oxygen (O₂) and ROS (Reactive Oxygen Species) in the human intestine represent a challenge for *C. difficile*. ROS, which include hydrogen peroxide, superoxide anion, hydroxyl radical, and organic hydroperoxides can damage DNA, proteins, and lipids, and bacteria developed oxidative stress response systems that help bacterial survival. Among these enzyme-mediated pathways to resist ROS, PerR is a metal-dependent peroxide sensor that regulates the genes coding for proteins involved in the oxidative stress response. We found *Bacillus subtilis* homolog in *Clostridioides difficile*. By understanding the function and the structure of *C. difficile* PerR, we will be able to contribute to the study for a viable therapeutic drug. For this reason, we would like to study the PerR functions and their role in the defense mechanisms against hydrogen peroxide. Here we show that *C. difficile* PerR is an effective sensor of hydrogen peroxide and may play an important role in the defense against ROS.

E019

Effects of Ornithine Lipids on Biofilm Architecture

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Biofilm formation is a very integral part of infection for opportunistic pathogens, particularly for chronic infections in the lung by *Pseudomonas aeruginosa*. Often, *P. aeruginosa* encounters a variety of environments in the cystic fibrosis lung and must adapt. For instance, in phosphate-limiting conditions, *P. aeruginosa* can replace standard phospholipids in the membrane with ornithine lipids (OLs). As OLs increase in the membrane, the overall net charge of the surface of the cell changes to a positive charge. We hypothesized that as the net charge of the membrane changes due to the accumulation of OLs, exopolysaccharide architecture in the biofilm may also change. Particularly, the placement of the positive Pel exopolysaccharide may localize to the outer edges of the biofilm rather than the stalk region where cell concentration is highest. To investigate this, we integrated the GFP gene into the neutral *attB* site of the *Pseudomonas aeruginosa* strain PA01 genome to examine the overall biomass of the biofilm, and then enhanced OL production by overexpressing the OL-synthesizing operon (*olsBA*) in a flow cell system. Finally, we stained the biofilm with Pel- and Psl- specific lectins to examine the biofilm architecture under a confocal microscope. These results could give a better understanding of biofilm formation under various conditions.

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E020

Implications of SigB and SigE in an Increase in Rifampicin Resistance in *Mycobacterium smegmatis* under Respiration-inhibitory Condition

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Rifampicin (RIF) resistance was found to increase in an *aa₃* cytochrome *c* oxidase mutant (Δaa_3), a condition in which respiration is suppressed by 50% compared to the wild type of *Mycobacterium smegmatis*. The results of zone inhibition assay and MIC test showed that the increase in RIF resistance in the Δaa_3 mutant was almost abolished in the $\Delta aa_3 \Delta sigB$ and $\Delta aa_3 \Delta sigE$ mutants, which revealed that these two alternative sigma factors, SigB and SigE, are related to RIF resistance. Through comparative RNA sequencing analysis, we discovered differently expressed genes (DEGs) in the $\Delta sigB$ and $\Delta sigE$ mutants relative to the wild-type strain. The expression of the identified SigB and SigE regulons was induced in the Δaa_3 mutant, and SigB regulon significantly overlapped with the SigE regulon. Promoter assay showed that the expression of *sigB* was decreased in the $\Delta sigE$ mutant, and the consensus promoter sequence of SigE was identified in the upstream region of *sigB*, which suggests a mechanistic basis for the overlap of the SigB regulon with SigE regulon. Taken together, the results imply that induction of SigB and SigE regulon in the Δaa_3 mutant might contribute to an increase in RIF resistance in *M. smegmatis* under respiratory-inhibitory condition.

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E021

Expression of Ribosomal Protein Genes under Respiration-inhibitory Conditions in *Mycobacterium smegmatis*

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Mycobacterium smegmatis is an obligate aerobic bacterium that requires oxygen for its growth. Deletion of the *aa₃* cytochrome *c* oxidase (Δaa_3) in *M. smegmatis* has been reported to lead to a reduction of respiration rate by ~50% relative to the wild-type (WT) strain. To identify differentially expressed genes (DEGs) in *M. smegmatis* under respiration-inhibitory conditions, we conducted RNA sequencing analysis of the WT and Δaa_3 mutant strains. In the Δaa_3 mutant, 529 among 6722 genes were differentially expressed (p -value <0.05 , |Fold change| ≥ 2) relative to the WT strain, of which 296 are induced and 233 are repressed. Clusters of orthologous groups of proteins (COGs) analysis demonstrated that the genes in the category of translation and ribosomal structure biogenesis were mostly down-regulated in the Δaa_3 mutant. Gene ontology (GO) enrichment analysis showed that the down-regulated DEGs were significantly enriched in the translation function. Among the 26 ribosomal protein genes identified in GO enrichment analysis, expression levels of the two genes were verified by qRT-PCR. The amount of ribosomes in the WT and Δaa_3 mutant strains were comparatively determined by ribosome profiling. The amount of polysome and monosome fractions in Δaa_3 mutant were decreased relative to that in the WT strain. These results suggest that the expression of ribosomal protein genes was repressed under respiration-inhibitory conditions in *M. smegmatis*.

[Supported by grants from NRF]

E022

Small Proteins Regulate *Salmonella* Survival Inside Macrophages by Controlling Degradation of a Magnesium Transporter

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All cells require Mg²⁺ to replicate and proliferate. The macrophage protein Slc11a1 is proposed to protect mice from invading microbes by causing Mg²⁺ starvation in host tissues. However, the Mg²⁺ transporter MgtB enables the facultative intracellular pathogen *Salmonella enterica* serovar Typhimurium to cause disease in mice harboring a functional Slc11a1 protein. Here, we report that, unexpectedly, the *Salmonella* small protein MgtR promotes MgtB degradation by the protease FtsH, which raises the question: How does *Salmonella* preserve MgtB to promote survival inside macrophages? We establish that the *Salmonella* small protein MgtU prevents MgtB proteolysis, even when MgtR is absent. Like MgtB, MgtU is necessary for survival in *Slc11a1*^{+/+} macrophages, resistance to oxidative stress, and growth under Mg²⁺ limitation conditions. The *Salmonella* Mg²⁺ transporter MgtA is not protected by MgtU despite sharing 50% amino acid identity with MgtB and being degraded in an MgtR- and FtsH-dependent manner. Surprisingly, the *mgtB*, *mgtR*, and *mgtU* genes are part of the same transcript, providing a singular example of transcript-specifying proteins that promote and hinder degradation of the same target. Our findings demonstrate that small proteins can confer pathogen survival inside macrophages by altering the abundance of related transporters, thereby furthering homeostasis.

E023

Reduced ATP-dependent Proteolysis of Functional Proteins during Nutrient Limitation Speeds the Return of Bacteria to a Growth State

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All living organisms require nutrients to grow, and nutritional starvation causes slow growth and dormant state. Dormant bacteria recover growth when they encounter plentiful nutrients. So far, little has been known of how bacteria regrow after slow growth and dormant state. Here, we show that protein homeostasis during dormancy speeds the return of bacteria to a growth state. Nutritional deprivation reduces ATP amounts in *Salmonella*, which leads to the stabilization of ATP-dependent protease substrates. Furthermore, preservation of functional proteins in nutrient starvation is essential for bacteria to rapidly escape from a slow-growth state. Our findings suggest that protein preservation during dormancy is a conserved microbial strategy that facilitates the return to a growth state once nutrients become available. This study likely unveils how dormant bacteria populations adapt to stressful environments and prepare regrowth by preserving functional proteins to maintain protein homeostasis.

E024

Impact of Carbohydrate Components in Both Dietary Fibers and the Gut Bacterial Glycans on Human Health

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The human gastrointestinal microbiota has an important role in human health. Daily dietary intake may influence to modulate the composition and the metabolic function of the gut microbiome colonized to the gastrointestinal tracts. In this study, we tested the effect of dietary fiber as prebiotics on the metabolization for short chain fatty acids, including acetate, propionate, and butyrate, by *in vitro* gut microbiome consortia. Two independent gut microbiome consortia showed the different fermentation abilities toward various polysaccharides derived from dietary fiber such as crops and mushrooms within a certain molar ratio. Interestingly, the metagenome analyses of these microbial consortia displayed the genetic diversity of carbohydrate-active enzymes and the taxonomic profile of the gut rumen microbial communities. In addition, we isolated two Gram-positive bacterial strains from the microbiome consortia and analyzed the effect of their cell-wall associated glycans on the signal pathways for the host immune systems. One strain showed the modulation of the cell signaling pathways dependent of TLR2 and TLR4 in transfected HEK293 cells. However, the other strains did not show any responses to the cells. This study revealed that the carbohydrate components in both the dietary fibers and the gut bacterial glycans could be influential to stimulate the human immune systems directly and indirectly via modulation of the gut microbiota.

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E025

Amino Acid Deficiency Leads Defective in Lateral Growth and Mis-regulation in Iron Homeostasis in *Escherichia coli*

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Amino acid biosynthesis pathway plays roles in regulating intracellular free amino acid pool for protein synthesis. Most fast-growing and free-living bacteria retain the pathways despite of their metabolic cost, while symbiotic bacteria which uptakes amino acids from host cell has evolved to remove these biosynthetic pathways. Amino acid metabolism has been extensively studied and proven to have roles in many other cellular physiological processes. But most previous studies focus on one or a group of amino acids, and potential and other systematic roles of the entire biosynthetic pathway for all 20 amino acids have not been pursued due to technological difficulty. We have generated an amino acid deficient mutant in *Escherichia coli* which is not capable of synthesizing all 20 amino acids. Here, we demonstrate the effect of deletion of these pathways on cell physiology including changes in its growth and morphology. By applying adaptive evolution, we further characterized the deficient mutant by analyzing whole genome sequence to identify suppress mutations for phenotypes. Our study revealed that amino acid biosynthesis has roles not only in feeding into free amino acid pool in the cell but also in regulating many other cellular processes for optimal growth.

E026

Anthranilate, a Modulator of Pathogenicity-related Phenotypes in *Pseudomonas aeruginosa* and Surrounding Bacteria

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Pseudomonas aeruginosa is an outstanding pathogen for their ability to colonize most environmental niches. Since these niches are generally nutrient-limited and quite harsh, it is necessary to form a stronger community by inducing more protective phenotypes and selecting more resistant individuals from within the community. Here, we report that anthranilate, a diffusible molecule produced by *P. aeruginosa*, acts as a key factor in the pathogenicity-related physiology of *P. aeruginosa* and other surrounding bacteria, influencing phenotypes such as biofilm formation, antibiotic resistance, and virulence. We found that the anthranilate levels in *P. aeruginosa* cultures rapidly increased in the stationary phase and then decreased again, forming an anthranilate peak. Biofilm formation, antibiotic susceptibility, and virulence of *P. aeruginosa* were significantly altered before and after this anthranilate peak. In addition, these phenotypes were all modified by the mutation of *antABC* and exogenous addition of anthranilate. Anthranilate also increased the antibiotic susceptibility of other species of bacteria. Before the anthranilate peak, the low intracellular anthranilate level was maintained through degradation from the *antABC* function, which induction of *antABC* was also limited to a small extent. The premature degradation of anthranilate, due to its high levels, and *antABC* expression early in the growth phase appears to be toxic to the cells. These results suggest that by generating an anthranilate peak as a signal, *P. aeruginosa* may induce some sort of physiological change in surrounding cells, and select the cells that are adapted to this high-level anthranilate, which are more antibiotic resistant individuals and better biofilm formers. This study is important in that it provides a new insight to how microbial signaling substances can induce changes in the pathogenicity-related phenotypes of surrounding cells and consequently lead to stronger populations.

F001

Cellular Abundance of a Transcription Factor, IscR, is Determined by the Cellular Levels of Fur Complexed with Ferrous Ions

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IscR is a well-known transcription factor controlling the expression of Fe-S cluster systems. It also regulates the expression of virulence factors in *Vibrio vulnificus*, and its presence is required for successful infection to a model animal. Thus, appropriate levels of IscR should be present in cells upon entry to host environments, where irons available to pathogens are critically limited. To achieve it, *V. vulnificus* maintains the *iscR* expression under iron-deprived conditions through iron-sensing regulatory systems. In this study, a regulatory mechanism for *iscR* expression was elucidated at the transcription level. Expression profiles of cellular IscR showed that its levels were maximal at the exponential phase and then decreased to undetectable level during the stationary phase. Stationary-phase repression of *iscR* expression was abolished if an iron-chelator was added. This repressive effect of iron ions on the *iscR* expression was mediated by binding of iron-Fur complex to the *iscR* promoter region. The similar levels of Fur were observed in both exponential- and stationary-phase cells. However, measurement of the cellular irons revealed that the concentrations of ferrous ions were significantly higher in stationary-phase cells than exponential-phase cells. Therefore, this study proposes that the degree of *iscR* expression is controlled by ferrous ion concentration-dependent alternation between repression and derepression via transition of iron-complex states of Fur.

F002

Microbiome of Scars in the Patients with Post-burn Pruritus

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Post-burn pruritus is the scar itching that occurs after burn during the healing process of burn wounds and the rehabilitation therapy. This pruritus is one of the common burn complications. The mechanism of post-burn pruritus appears to have pruritogenic and neuropathic aspects, but it has not been elucidated clearly. Little information has yet been published about the microbiome of scars in the patients with post-burn pruritus. We investigated the distribution of microbiome in seven burn patients with pruritus. Swab for 16S rRNS based next-generation sequencing of the skin microbiome were performed on burn scars with pruritus. *Staphylococcus aureus*, *Moraxella osloensis*, *Corynebacterium tuberculostearicum*, *Cutibacterium acnes*, *Pseudomonas stutzeri*, *Streptococcus periodonticum*, and *Streptococcus porcorum* were detected in burn scars of all subjects. *Staphylococcus aureus* was the most abundant in burn scars of six subjects except one subject with atopic dermatitis, who had *Corynebacterium tuberculostearicum* as the most common microbiome of burn scar. *Acidovorax radices* was detected in only one of seven subjects. Some changes in skin microbiota were shown in burn scars with pruritus. Further study should need to demonstrate the association between skin microbiome and post-burn pruritus.

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F003

Analysis of mRNA Decapping and Localization Factors in Translational Regulation of Autophagy Genes

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Macroautophagy (here after autophagy) is a well-conserved cellular process that facilitates the degradation and recycling of cytoplasmic components. The transcriptional regulation of autophagy-related (ATG) genes have been studied under nutrient deprivation condition. However, little is known about their translational regulation. In a recent report, translation of Atg1 and Atg13, two proteins essential for autophagy, are regulated by Dhh1, a DEAD-box RNA helicase. A positive role of Dhh1 in translational regulation has been reported in a subset of mRNAs including STE12 mRNA. Dhh1 functions as an mRNA decapping activator in the mRNA decay pathway. mRNA decapping enzymes (Dcp1/Dcp2), Xrn1 exoribonylease, and decapping activator (Edc3, Scd6, Pat1) interact to form an mRNA granule known as P-bodies. In this study, we will investigate whether mRNA decapping and localization factors affect the translational regulation of Atg1 and Atg13 genes. Deletion mutation of DHH1, EDC3 and LOC1 will be analyzed under autophagy-inducing conditions those genes regulate the Atg1, Atg13 protein at the translational level.

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F004

Functional Characterization of Pro- and Anti-apoptotic Factors in *Candida albicans*

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Candida albicans is an opportunistic pathogenic yeast and causes systemic infection called candidiasis. Apoptosis is a form of programmed cell death that is also found in *C. albicans*. *C. albicans* can undergo apoptosis upon various stresses, including oxidative stress, acetic acid and antifungal agents. As a key player of apoptosis, CaMca1 metacaspase was identified in *C. albicans* and CaNma111, a homolog of the mammalian pro-apoptotic serine protease Omi/HtrA2 and CaYbh3, a homolog of BH3-only protein, were shown to increase apoptotic phenotypes by overexpression analysis. In this study, these apoptosis regulators were further analyzed by deletion analysis. By examining the intracellular ROS accumulations, caspase-like activity, cell survival and TUNEL assay, we showed CaNma111 and CaYbh3 as pro-apoptotic regulators. The protein level of CaBir1, inhibitor of apoptosis protein (IAP), was increased in *Canma111/Canma111* cells compared with the wild type cells. These results suggest that CaNma111 stimulate apoptosis through down-regulating CaBir1. We also characterized the filamentation and virulence phenotypes of *Canma111/Canma111* and *Caybh3/Caybh3* mutant strains. The pro-apoptotic regulators CaNma111 and CaYbh3 appeared to be involved in *C. albicans* pathogenicity.

[This work was supported by a research grant from the National Research Foundation of Korea]

F005

Comparative Analysis of Genetic Characterization of β -Lactam-resistant *Escherichia coli* from Bulk Tank Milk in Korea

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This study was conducted to analyze the genetic characteristics of 41 β -lactam-resistant *Escherichia coli* isolates, which are one of the common causes of environmental mastitis, isolated from the bulk tank milk of 290 dairy farms in five factories operated by three dairy companies in Korea. Analysis of the phenotypic and genotypic characteristics of β -lactam-resistant *E. coli* isolates revealed differences between factories even within the same company. Isolates from factory A1 and C1 showed high resistance to cephalothin (76.9% and 100%, respectively), which is the first-generation cephalosporins. Although all the 41 β -lactam-resistant *E. coli* isolates were positive for *bla*_{OXA-1}, *bla*_{TEM-1} was highly prevalent in isolates from factories C2 (100%) and C3 (100%). Among 17 isolates resistant to both β -lactams and aminoglycosides, the most common multilocus sequence type was ST399 (76.5%). Furthermore, 2 (11.8%) and 12 (70.6%) isolates belonged to the phylogenetic groups B2 and D, respectively, which are invasive strains that cause intestinal infections, respectively. The predominant serogroup was O15 (70.6%), which is a globally distributed extraintestinal pathogen. Although *E. coli* isolates were isolated from bulk tank milk, and not the clinical mastitis samples, the presence of the phylogenetic groups B2 and D, and the serogroups O15 and O157, which harbor antimicrobial resistance genes and virulence factors, can pose a threat to public health.

F006

Activation of an Oxidative Signaling Regulator Overcomes Caffeine Toxicity in *Saccharomyces cerevisiae*

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Caffeine, a methylxanthine derivative, is known to affect various physiological conditions such as cell growth, proliferation, and energy metabolism. Caffeine also inhibits DNA damage repair and prevents cell cycle checkpoint activation, leading to mutagenesis, apoptosis, and potential carcinogenesis. Genome-wide screening of mutants in *Schizosaccharomyces pombe* revealed that Pap1 and its downstream target genes especially involved in caffeine efflux are required for caffeine tolerance. We found that Yap1, a budding yeast AP-1 homolog required for oxidative stress response, has function as to caffeine resistance. The *yap1* mutant is not sensitive to caffeine but overexpression of Yap1 renders cells resistant to high concentration of caffeine. Caffeine sensitivity shown in mutants that lacking the multidrug resistance transporters, Pdr5 or Snq2, are completely restored by Yap1 overexpression. Among Yap1-dependent target genes, *FLR1*, encoding fluconazole resistant protein, turns out to be responsible for caffeine resistance when overexpressed by Yap1 activation. We also identified a significant genetic crosstalk between ROS signaling and caffeine resistance.

F007

New Fungal Pathogen Identification Causing Root Rot Disease of *Gastrodia elata* in Korea

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Gastrodia elata (*G. elata*), as an obligate mycoheterotrophic orchid (Orchidaceae), depends on two fungal partner: a broad range of *Mycena* spp. for seed germination and *Armillaria mellea* for plant growth. *G. elata* is leafless and lives more than 80% during three-year life cycle underground as a tuber. Recently, more than 70% of *G. elata* has been loss in the actual yield in Korea due to root rot disease. Thus, there is a rising need for identification of new fungal pathogen causing root rot of *G. elata*. We have observed necrotic spots of *G. elata* from Anseong, which is one of the largest *G. elata* production areas in Korea. Symptoms appeared round and grayish brown spots, which eventually coalesced into larger black lesions on tubers. The six pure cultures were recovered from necrotic lesions of symptomatic tubers. For fungal identification and characterization, fungal genomic DNA was isolated from tuber of *G. elata*, and amplified from three fungal loci, the internal transcribed spacer regions 1 and 4 and 5.8S nrDNA, translation elongation factor alpha, and beta-tubulin. Three fungi were identified the new fungal species through multigene phylogenetic analysis and morphological observation, while three fungi were formally described as *Clonostachys rosea*. Therefore, these results may assist to make the proper method to control root rot disease in the fields of *G. elata* in Korea.

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F008

LAMMER Kinase is a Positive Regulator for Differentiation and Biofilm Formation of *Aspergillus fumigatus*

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The LAMMER kinases are found in all eukaryotes and have been reported to be involved in fungal differentiation. Morphological changes are known to play important roles during fungal infection, however, the function of LAMMER kinase in *Aspergillus fumigatus*, an opportunistic human pathogen, is unknown. In this study, we constructed LAMMER kinase deletion strain (Δ LkhA) and complementation strain to analyze LAMMER kinase (LkhA) function in *A. fumigatus* differentiation. We found that Δ LkhA showed a reduced growth rate. No significant difference in the morphology of asexual organ, conidiophore, was observed, but reduction in conidia production was observed in Δ LkhA. Expression of central regulatory genes for asexual development (*brlA*, *abaA*, and *vosA*) was also decreased by LkhA deletion. During sexual development, Δ LkhA produced abnormal ascospores with no equatorial crests and showed decrease in production of cleistothecia and viable ascospores. Expression of sexual genes (*vosA*, *veA*, *steA*, *cpcB* and *svfA*) was also decreased by LkhA deletion. Δ LkhA showed reduced biofilm formation ability and reduced expression of genes for biofilm formation (*ags1*, *aspF1*, *rodA*, *rodB*). Taken these together, it is postulated that LAMMER kinase is involved in vegetative growth, asexual development, sexual development, and biofilm formation in *A. fumigatus*.

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F009

Tempo and Mode of Evolvability in *Escherichia coli* Strains with Different Fitness Backgrounds

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Adaptation through the fitness landscape may be influenced by the gene pool or the expression network. However, genetic factors that determine the contribution of beneficial mutation in the fitness landscape during adaptive evolution are poorly understood. In this study, we conducted an experimental evolution to investigate the genetic factors that influence the adaptability using two *Escherichia coli* strains with different fitness backgrounds. Contrary to the presumption that the adaptive potential of a strain with higher background fitness will be comparable to that of the wild-type strain, the fitness of the two strains converged by 2,000 generations. When we introduced the mutations to two *E. coli* strains with different background fitness, the beneficial effects tended to be lower in the strain with higher initial fitness. Replication rate analysis showed that the replication index does not correlate with the growth rate. Besides, the degree of transcriptional change was proportional to fitness gain. Our study uncovers the relationship between fitness change and transcriptome dissimilarity, and provides insights into the understanding of the tempo and mode of evolvability

F010

Modification of Elastase B Propeptide and Inhibition of LasB in *Pseudomonas aeruginosa*

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Three major proteases of *Pseudomonas aeruginosa*, elastase B (LasB) protease IV (PIV), and elastase A (LasA) play a key role in the *P. aeruginosa* infection and pathogenesis. These are activated post-secretionally in a cascade manner in which the initial activation of LasB is controlled by quorum sensing (QS). Once activated, LasB activated PIV, which then sequentially activates LasA. Since the activities of these three proteases are regulated by their propeptides, in this study, we tried to inhibit LasB using its purified propeptide (LasB_{pp}). However, LasB was not inhibited by exogenous addition of LasB_{pp} unlike LasA and PIV that were inhibited by their propeptides. The reason was that once activated, LasB was able to degrade LasB_{pp}, leading to resistance of inhibition by LasB_{pp}. In order to overcome this problem, we tried to make a mutant LasB_{pp} that is resistant to cleavage by LasB through mutating the cleavage site. From the C-terminal deletion series, we obtained a mutant LasB_{pp} that are resistant to LasB (LasB_{pp}-R2). By overexpressing LasB_{pp}-R2, the LasB activity was inhibited by 30%. In addition, other plasmids were constructed by cutting more or less amino acids based on LasB_{pp}-R2. As a result, we got a mutant LasB_{pp} which has just one more amino acid cleaved from LasB_{pp}-R2, and this mutant inhibited LasB activity by 70%.

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F011

Identification of the Genes for Differential Phage Infectivity in *Pseudomonas aeruginosa* Strains

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Phages infect a limited number of bacterial strains of the same species. This strain-specific phage infectivity may be one of the major obstacles for phage engineering and therapeutic application. In this study, we observed that the *Pseudomonas aeruginosa* transposable temperate phage, MP29, displayed differential strain-specific infectivity, which is associated with the lysogen strains (PAO1, PAK, and PA14) that generate MP29: MP29 produced from the PAO1 lysogen could infect PAO1, PAK, and PA14; MP29 from the PAK lysogen could infect PAK and PA14, but not PAO1; MP29 from the PA14 lysogen could infect only PA14. This strain-specific infectivity correlated with the DNA synthesis of the infecting phages in each strain, suggesting that the determinant(s) may work after genome entry and before particle assembly during the phage lifecycle. The transposon mutagenesis using the PAK lysogen was followed by the screen of the 2,256 transposon insertion clones with altered phage infectivity on three strains. Fifteen non-redundant mutant clones were isolated and their transposon insertion sites were determined. Among those, two *ligD* mutant lysogens (12H8 and 14G12) allowed altered plaque formation by MP22 superinfection as well. Topics discussed will include our recent results that account for the roles of the genes in DNA metabolism during phage-bacteria interaction.

F012

Autolysis of *Pseudomonas aeruginosa* Quorum-sensing Mutant is Suppressed by *Staphylococcus aureus* Extracellular Proteins through Iron-dependent Metabolism

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Microorganisms usually coexist as a multifaceted polymicrobial community at mucosal sites of the human body as well as on abiotic surfaces in the natural habitats. The two opportunistic pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus* commonly coexist in the bacterial infections for immunocompromised individuals. In this study, we observed that *P. aeruginosa* mutant (*pqsA*) defective in 4-hydroxy-2-heptylquinoline-*N*-oxide (HQNO) synthesis displayed residual killing activity against an *S. aureus* respiratory mutant. A total of 1,734 transposant clones of the *lasRmvfR* double mutant were screened to isolate 3 mutants (*truB*, *cysB*, and *cysG*) that were devoid of the residual killing against *S. aureus*. Interestingly, these mutants did not exhibit the autolysis phenotype of the *lasRmvfR* mutant. The autolysis was suppressed by the growing *S. aureus* cells and the partially purified extracellular proteins from the *S. aureus* secretome. Most of the extracellular proteins lacked in the two *S. aureus* mutants (*comEC* and *saeS*) that had been identified from 2,885 random transposant clones. The autolysis was also suppressed by iron treatment as well. These results suggest that the interaction between *P. aeruginosa* and *S. aureus* might be governed by sophisticated mechanisms that necessitate the *P. aeruginosa* quorum-sensing circuitry as well as the polymicrobial metabolism involving the extracellular iron resources during the coexistence in human airway.

F013

Mutational Analysis of RNA Phage Lysis Protein to Define the Regions Required for Membrane Association

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Single-stranded RNA phages such as PP7 achieve host bacterial lysis by expressing a single subunit lysis protein (LP). However, the lysis mechanism by LP still remains elusive. In this study, we created an LP-mediated cell lysis system by using the LP gene from the *Pseudomonas aeruginosa* phage, PP7. The PP7 LP gene were cloned into an IPTG-inducible mini-Tn7-based vector, with the ribosome binding sequence optimized for translation in *P. aeruginosa*. Based on the sequence characteristics, the 5x-aa LP is divided into 4 regions: the positively charged region (1-26 aa), the hydrophobic region (27-37 aa), the LS motif (38-39 aa) and the C-terminal region (40-55 aa). A total of 14 point mutants were constructed and tested for their capability of membrane association and host lysis. Those from the hydrophobic region and the LS motif resulted in complete loss of the killing activity, suggesting their presumable role in membrane association for host lysis. The membrane association of the wild type LP was visually verified by N-terminal mNeonGreen tagging in *Escherichia coli*, whereas the mutants for the hydrophobic region lacked membrane association. Interestingly, membrane association was still observed in the mutants for the LS motif, despite the lack of their host lysis activity. These results demonstrate that LP is associated with bacterial cytoplasmic membrane and dismantle the roles of the 4 regions for membrane association and disruption to ensure the appropriate host lysis.

F014

Regulatory Mechanisms of Ribonucleotide Reductases in the DNA Replication Stress in *Cryptococcus neoformans*

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The balance in the intracellular concentration of deoxyribonucleotides (dNTPs) is critical for DNA replication and DNA repair processes. Our previous study reveals that *Cryptococcus neoformans*, which belong to the basidiomycete, Rad53 and Chk1 cooperatively and divergently regulated DNA damage response. However, the regulatory mechanism of *RNR* in the basidiomycete fungus remains elusive during DNA replication stress. In this study, we demonstrated that *RNR1* and *RNR2* are required for viability, but not *RNR21*. The expression levels of *RNR1* and *RNR2* were cooperatively or divergently controlled by Rad53 and Chk1 kinases under DNA damage and DNA replication stresses. However, the *RNR21* expression was in an Ssn6-Tup1 complex-dependent manner and *rnr21Δ* mutants showed WT-levels of resistance in response to DNA damage and DNA replication stresses. Notably, we found that Mbs1, which is cooperatively regulated by both Rad53 and Chk1 at the transcription level, played a major role in the regulation of *RNR1* whereas Mbs1 and Bdr1 cooperatively regulated *RNR2* expression under HU treatment. Supporting this, *bdr1Δ mbs1Δ* double mutant showed significant sensitivity in response to HU than each single mutant. Collectively, the DNA damage repair pathway mediated by Rad53 and Chk1 governs the regulation of *RNRs* during DNA damage and DNA replication stresses in *C. neoformans*.

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F015

Purification and Characterization of Type II Restriction Endonuclease from *Fervidobacterium islandicum* AW-1

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Prime purpose of restriction-modification system is to defend the bacterial cell against macrophages. Type II restriction endonucleases occur in bacterial kingdom and identification of this system is important for development of genetic tools. Whole genome sequence of *Fervidobacterium islandicum* AW-1 revealed the presence of three adjacent ORFs coding for the restriction endonuclease and the corresponding methyltransferase. Gene encoding a type II restriction endonuclease was cloned and the recombinant enzyme was expressed in *Escherichia coli*. The recombinant enzyme was purified to homogeneity with Ni-NTA affinity chromatography and molecular weight was 36 kDa. DNA containing two GATC sites from pUC19 was used as substrate. The effect of temperature, pH and metal ion were studied to determine optimal reaction condition. Optimal temperature for the restriction endonuclease activity was 65-70°C. Specific DNA cleavage was obtained at pH range 5.0-10.0 and 5-10 mM MgCl₂. Manganese and cobalt could replace magnesium as a cofactor for activity. Recombinant enzyme was stable at 70°C for more than two hours and at 75°C for 30 min. The recognition sequence of the restriction cleavage is /GATC. This enzyme named as *Fisl*, is a thermostable isoschizomer of the mesophilic prototype restriction endonuclease *DpnII*.

F016

A Genetic Network of Winged Helix Transcription Factors Regulating Sexual Reproduction in the Homothallic Fungus *Fusarium graminearum*

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Fusarium graminearum is the major causal agent of Fusarium head blight (FHB) in wheat and barley. This disease leads to yield losses and reduction in crop quality due to the accumulation of mycotoxins, such as deoxynivalenol and zearalenone. *F. graminearum* produces ascospores by sexual reproduction. Ascospores discharged from the fruiting bodies (perithecia) that overwintered on plant debris serve as primary inocula for next year epidemic. Therefore, sexual reproduction provides not only genetic diversity but also efficient disease propagation strategy in this fungus. Winged-helix transcription factors are found in core components of transcription systems and involved in diverse cellular responses and differentiation processes in both prokaryotic and eukaryotic organisms. *F. graminearum* genome contains 28 winged-helix transcription factors. Of these, ten genes showed a defect in the process of sexual reproduction. Objectives of this study were to identify the critical role of winged-helix genes involved in sexual reproduction and to construct a genetic network of them in *F. graminearum*. The deletion mutants were confirmed and in-depth phenotyping of these mutants was performed in the sexual reproduction. To functionally characterize these genes, we have been constructing complementation and overexpression/constitutive expression mutants. This study will figure out regulatory mechanisms of sexual development in fungi.

[Supported by the National Research Foundation of Korea.]

F017

***Malassezia-Staphylococcus* Interactions: Changes in Transcriptional Expression and Three-Dimensional Genome Organization**

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Malassezia and *Staphylococcus* are one of the most frequently isolated genera of human skin microbiome. To explore inter-kingdom interaction between two species, we examined the changes in gene expression of *M. restricta* by co-culturing with *Staphylococcus*. We found that genes encoding ribosomal proteins, rRNA and macromolecule biosynthesis are induced, but the genes encoding stress response and RNA-modification are repressed in co-cultured *M. restricta* strains, suggesting that co-cultured *Malassezia* cells may attempt to proliferate under less stressful condition. In addition, genes encoding major secreted enzymes such as lipase and aspartyl protease are repressed. We noticed that the expression of genes involved in cohesin subunit were decreased. Therefore, we performed Hi-C analysis to identify changes in three-dimensional organization of *M. restricta* genome, and found local associations were significantly decreased in co-cultured *M. restricta*. We also have investigated changes in the expression of bacterial genes by co-culturing with *M. restricta* and found that the expression of genes involved in ribosomal proteins, iron uptake and DNA metabolism are induced, but genes involved in riboflavin, nitrate reductase, amino acid transporter, and environmental stress are repressed, suggesting that bacterial cells are also in favorable conditions for growth. We concluded that both fungal and bacterial strains control their gene expression to live in harmony and coexist.

F018

Whole-genome Analysis of *Streptococcus salivarius* Strain 72, Isolated from the Stomach Biopsy Sample

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The commensal bacterium *Streptococcus salivarius* is one of the pioneer microbes colonizing the human gastrointestinal tract in early infancy. It was reported that *S. salivarius* has the antimicrobial activity against human pathogens by producing bacteriocins such as lanthipeptides and inhibits inflammatory cytokines activated by periodontal pathogens. Therefore, *S. salivarius* has emerged as a promising source of probiotics that can contribute to a healthy microbiota. From the stomach biopsy sample, we isolated *S. salivarius* strain 72 showing an antagonistic effect against *Fusobacterium nucleatum*, *Haemophilus parainfluenzae*, and *Helicobacter pylori*, which are human pathogens. The assembled genome of strain 72 had two circular replicons: the 2.17-Mb chromosome and the 193-kb plasmid. The genome sequence of the strain 72 as well as those of 20 *S. salivarius* strains retrieved from NCBI including well-characterized probiotic strains were used for evaluating genome contents. Analysis of the biosynthetic gene clusters (BGCs) revealed that strain 72 had two BGCs for lanthipeptide production on its plasmid. Antibiotic resistance and virulence genes were also analyzed, compared among the strains. In conclusion, we identified novel BGCs in strain 72 through comparative genome analysis, and further characterization of these potential BGCs that may confer antagonistic effects against human pathogens is to be pursued.

F019

The Conserved Roles of Conidial-specific Transcription Factor CsgA in *Aspergillus* spp.

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Aspergillus spp. mainly reproduce through asexual reproduction. The process of conidia formation is controlled by various transcription factors. Our previous transcriptomic analysis identified fifteen conidial-specific transcription factors. In this study, we characterized one of conidial-specific transcription factors CsgA which contains the GAL4-like zinc-finger domain. The roles of csgA were investigated in two *Aspergillus* species, *A. nidulans* (*Ani*) and *A. flavus* (*Afl*). The *AnicsgA* deletion mutants showed increase in conidiation, fungal growth, and conidial viability. The *AnicsgA* deletion mutant conidia were more resistant to thermal, oxidative, and UV stresses than wild-type conidia. Trehalose biosynthesis increased in *AnicsgA* deletion mutants. The *AnicsgA* deletion mutants showed delayed sexual development. Overexpression of *AnicsgA* showed decrease in conidiation but increased sexual development. The production of sterigmatocystin increased in the *AnicsgA* deletion mutant conidia than wild-type conidia. In *A. flavus*, deletion of *AflcsgA* showed decrease in fungal growth, but conidiation increased in dark condition. The *AflcsgA* deletion mutants exhibit defect in sexual development and aflatoxin production. Overall, CsgA plays crucial role in proper conidiation, fungal growth and regulates mycotoxin production in *A. nidulans* and *A. flavus*.

F020

Transcriptome-based Analysis of Spore-specific Transcription Factors in *Aspergillus nidulans*

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The filamentous model organism, *Aspergillus nidulans*, mainly propagates by forming the asexual spores, called conidia. The formation of conidia is regulated by a myriad of transcription factors. Previous our transcriptomic analysis identified the nineteen spore specific transcription factors and phenotypes of each deletion mutants were analyzed in *A. nidulans*. Among them, we characterized one of the spore-specific-C₂H₂ zinc finger A SscA. The $\Delta sscA$ mutants showed defect conidiation, sexual development and decreased conidial viability. The $\Delta sscA$ mutant conidia were more sensitive to various stresses than wild-type (WT) conidia. Also, the amount of trehalose in $\Delta sscA$ mutant was decreased compared to WT. Deletion of *sscA* causes induced germ tube formation with or without of glucose. Absence of *sscA* led to increase in the amount of sterigmatocystin in the conidia. In addition, transcriptomic data suggested SscA affected mRNA expression of a variety of genes in *A. nidulans* conidia. Interestingly, deletion of *sscA* resulted in alteration of genes associated with response to stimulus and stress in conidia, and these results are identical to results of various stress test. The mRNA levels of sterigmatocystin gene cluster were upregulated in the *sscA* mutant conidia. Overall, these results propose that SscA is a spore-specific transcription factor, that is essential for proper asexual and sexual development, conidial maturation, and secondary metabolites in *A. nidulans*.

F021

Role of Forkhead Gene *fkhB* in *Aspergillus nidulans*

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Aspergillus nidulans reproduces by producing asexual spores. Various transcription factors are involved in the formation of conidia. The Forkhead domain genes are generally known to be important for meiosis and cell cycle regulation. Six Forkhead genes have been found in *A. nidulans*. Transcriptomic analysis found a variety of the spore-specific genes in *A. nidulans*. Among them, we studied *fkhB* showing up-regulation in conidia. Deletion of *fkhB* affected both sexual and asexual development. The *fkhB* deletion mutant strain exhibited decreased colony size and produced reddish asexual spores unlike the wild-type strain. The number of conidia in the *fkhB* deletion mutant strain was significantly decreased compared to wild-type strains. These results propose that the *fkhB* gene is involved in asexual sporogenesis. The deletion of *fkhB* increased the sensitivity to heat stress, decreased the amount of conidial trehalose, and decreased conidial viability. In addition, the absence of *fkhB* resulted in a decrease in sterigmatocystin production. Taken together, these results show that *fkhB* is essential for proper bacterial growth, development, and sterigmatocystin production in *A. nidulans*.

F022

Identification of a Polyketide Pathway for the Biosynthesis of Atranorin by Genome Mining and Heterologous Expression

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Deposides and depsidones are widespread in lichens as cortical and medullary substances. Despite the taxonomic and ecological significance of lichen chemistry and its pharmaceutical potentials, there has been no genetic evidence linking biosynthetic genes to lichen substances. Here, we systematically analyzed lichen polyketide synthases (PKSs) to classify and identify biosynthetic gene cluster (BGC) involved in depside production. Among these, we identified highly synthetic BGCs found exclusively in lichens producing atranorin. 4-*O*-demethylbarbatic acid was obtained by heterologous expression of putative atranorin PKS gene (coined *atr1*). Its presence indicates an intermolecular cross-linking activity of Atr1 for depside formation. A cytochrome P450 monooxygenase gene (*atr2*) or a *O*-methyltransferase gene (*atr3*) was then introduced into the heterologous host (*atr1*-expressing strain) to oxidize or methoxylate 4-*O*-demethylbarbatic acid, respectively. Finally, atranorin was successfully synthesized in a strain expressing the three genes *atr1*, *atr2*, and *atr3*. In the course of atranorin production, proatranorins I -III were biosynthesized as precursors. Beside these compounds, 3-methylorsellinic acid and atraric acid were produced by hydrolysis of 4-*O*-demethylbarbatic acid and atranorin, respectively. Overall, we established a heterologous expression system for the production of atranorin, which is helpful for the genetics and chemical evolution of diverse lichen substances research.

F023

Understanding of Canine Probiotics (C1 and C5) Effect on the Modulation of Gut Microbiota

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Lactic acid bacteria are well-known microbes with probiotic potential in several animals as probiotics. However, gaps still remain in the exact roles played by Canine probiotics in modulation of gut microbiota and health. In this study, we isolated microbial strains from Canine feces and annotated (C1 and C5). And these two strains C1 and C5 were tested for probiotic characteristics, and their beneficial effects on experimental animal were evaluated in vivo using metagenome sequencing data. We collected feces samples from mouse (control and treatment group during the 4 week probiotic feeding trial). When the probiotics were applied to mouse, changes in microbial composition and relative abundance of bacterial strains were clearly observed in the experimental groups. Experimental groups before and after the application were separated from structure analysis results. In particular, it was confirmed that when the two strains were fed together steadily (4 week), the diversity of the microbial flora also increased. Mainly, proportion of Bacteroidaceae were significantly more than in control group after the probiotics treatment. Conclusively, these results could provide comprehensive understanding of the effects of probiotic strains and their industrial applications.

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F024

Characterization of RNA N6-Methyladenosine Methylation Factors during Perithecial Development in *Fusarium graminearum* and *Magnaporthe oryzae*

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N6-methyladenosine (m6A) RNA methylation is regulated by three factors, m6A writer (RNA methylation complex), m6A eraser (demethylation enzyme), m6A reader (RNA-binding protein). In mammals and plants, m6A RNA methylation factors are indispensable for embryonic development. Also, in budding yeast, the writer encoded by *IME4* plays an important role in developmental transition from vegetative growth to sexual development. To investigate the role of m6A RNA methylation factors in sexual reproduction and perithecial development, we characterized m6A factors in plant pathogenic fungi, *Fusarium graminearum* and *Magnaporthe oryzae*. Based on the sequences of the RNA methylation factors in yeast and human, we identified three putative m6A factors and generated knockout mutants lacking each m6A factor in *F. graminearum* and *M. oryzae*. However, the knockout mutants were able to form normal perithecia and ascospores, indicating that the m6A factors in these two filamentous fungi play non-essential roles during sexual development. To further investigate the role of the *IME4* homolog in *F. graminearum*, we generated *IME4*-overexpressing strains. An *IME4*-overexpressing strain exhibited defective phenotypes in both vegetative growth and sexual development: slow growth rate and low mycelial density and a delayed perithecia development, compared to the wild-type. This study suggests filamentous fungi may have lost m6A-dependent regulation for sexual development after divergence from budding yeast.

F025

Pathway Engineering in the Aromatic Compound Degradation of Fungal Strains Using Genome-editing Technology

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Some of the aromatic compounds are hard to degradable in ecosystem. Phenylacetate (PA) is one of such compounds and is an important side product in the synthesis of 6-aminopenicillanic acid (6-APA). We screened fungal strains of ascomycetes and basidiomycetes and isolated fungal strains that can degrade PA when PA was provided as a sole carbon source. Metabolic pathway analysis of PA degradation revealed that hydroxylation of the benzene ring in PA is a rate-limiting step. Subsequent transcriptome analysis has shown that two oxidative enzyme genes were highly expressed in the presence of PA, which were a cytochrome P450 monooxygenase and a dioxygenase gene. To confirm the involvement these genes, we applied genome-editing technology to generated fungal strains devoid of the two enzyme genes. A plasmid vector carrying Cas9 and gRNA genes were introduced to the protoplasts of fungal cells and the transformants were isolated under selective pressure. The sequence analysis of the target genes confirmed successful editing. Subsequent degradation activity assay using the edited strains showed that the cytochrome P450 monooxygenase-edited strain was unable to oxidize PA, indicating it is a key enzyme to hydroxylate PA.

[Supported by Next Generation Crop New breeding Technology Development Project (PJ015165022) of Rural Development Administration (RDA)]

F026

Aconitase-mediated mRNA Decay of Iron-uptake Genes in *Schizosaccharomyces pombe*Soo-Yeon Cho^{1,2}, Soo-Jin Jung^{2,3}, Kyoung-Dong Kim^{1*}, and Jung-Hye Roe^{2*}¹Department of Systems Biotechnology, Chung-Ang University, ²School of Biological Sciences, Seoul National University, ³Center for RNA Research, Institute for Basic Science

Aconitase, a highly conserved protein found in prokaryotes and eukaryotes, is required for converting citrate to isocitrate in the TCA cycle. It has been also known that the cytosolic aconitase in mammals 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. Fission yeast Aco2 is a fusion protein consisting of aconitase domain and mitochondrial ribosomal protein L21 (bL21) with 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 cytosol and the nucleus. To investigate the role of Aco2 in the nucleus, we conducted RNA-seq analysis with NLS deleted *aco2* strain (*aco2ΔNLS*) and revealed that the mutation caused an increase in mRNAs coding for iron uptake transporters, such as Str1, Str3, and Shu1. The mRNA decay assay showed that *aco2ΔNLS* has significantly longer mRNA half-lives for iron uptake transporters, suggesting that the Aco2 likely functions in the degradation of those mRNAs. The UV crosslinking RNA-IP (CLIP) analysis revealed that Aco2 directly binds to the mRNAs of iron uptake transporters. We further identified that Aco2 genetically interacts with exoribonucleases like Rrp6. Therefore, we propose that the role of Aco2 is involved in the intracellular iron homeostasis through post-transcriptional regulation of iron transporter genes.

F027

Optimization of Guide RNA for the Efficient Editing of Stress-responsive Genes in *Agaricus bisporus*

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Delivering CRISPR/Cas9 system into the cells of fungal microorganisms has been implemented through plasmid carrying genes for Cas9 and gRNA or ribonucleoprotein complex (RNP) of Cas9 protein and gRNA. Efficiency of genome-editing is dependent on various factors, including the intracellular levels of Cas9 protein and gRNA, and precise targeting of gRNAs on a target gene, which was assessed by *in vitro* assay targeting stress-responsive genes of *Agaricus bisporus*. For this, Cas9 protein was successfully purified from *E. coli* harboring a Cas9 expression vector. Four gRNAs were designed per gene and the gRNAs were prepared by *in vitro* transcription. The RNPs were incubated with PCR-amplified target genes at different concentrations and reaction conditions. The cleavage assay revealed that the targeting efficiency of gRNAs was dependent on the sequence and amount of gRNA, indicating the importance of gRNA design as well as intracellular expression of gRNA. All four target genes showed similar dependency on gRNA. We next investigated the gRNA efficiency by introduction of RNP complexes into the protoplasts of *A. bisporus*. The results showed direct correlation with the *in vitro* assay results. Our finding here will contribute to the generation of new strains and the understanding of physiology of *A. bisporus* under stress conditions.

[Supported by Next Generation Crop New breeding Technology Development Project (PJ015165022) of Rural Development Administration (RDA)]

F028

Genetic Interaction between Tubulin Folding Cofactor Alf1 and Recombination Protein Rad51 is Important for Genomic Instability and Cytotoxicity in *Saccharomyces cerevisiae*

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Homologous recombination (HR) serving for repair of DNA double-strand breaks (DSB) is critical for preserving genomic integrity and cancer prevention. Rad51 is key factor of HR that catalyzes a homology search and DNA strand exchange in *Saccharomyces cerevisiae*. Microtubules are heterodimer of α - and β -tubulin that play important roles for cell shape and motility and critical for cell division. Tubulin folding cofactor B is essential for the formation of α - and β -tubulin heterodimers. Alf1, the cofactor B homolog, is functionally and physically related with α -tubulin. In this study, we aim to investigate genetic interactions between DSB repair pathway and microtubules in *S. cerevisiae*. The *rad51 alf1* double mutant displays slow growth and synergistic sensitivity to caffeine and DSB generating drugs. Nuclear Rad52 foci representing DSB damage are accumulated more in *rad51 alf1* mutant than each single mutant. However, we observed that lack of Alf1 ameliorates highly-mutated phenotype of *rad51* mutant. Overexpression of *Alf1* rescued the impaired growth of *alf1* mutants. These results imply specific genetic interactions between homologous recombination (HR) pathway and the maintenance of cell morphology.

F029

Generation of A Mating Type-deleted Mushroom Strains Using CRISPR/Cas9 Based Genome Editing Technology

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Mating of mushrooms is determined by the tetrapolar system, unlike other kingdoms. This involves *A* and *B* mating type loci, and each gene in the mating type loci has been known to be independently involved in this process. However, our recent studies have shown that the gene expression in the *A* mating type locus is dependent on the mating pathway via the interaction between pheromone and pheromone receptor in the *B* mating type locus. To determine interaction between the *A* and *B* mating type loci, we have tried to delete genes in the *A* mating type locus in various combinations of mating strains. For this, guide RNAs (gRNAs) and Cas9 genes were introduced to a plasmid vector pBGgHg which is a binary vector normally used for *Agrobacterium tumefaciens*-mediated transformation. In this vector, expression of Cas9 and gRNAs was controlled by glyceraldehyde-3-phosphate dehydrogenase promoter (pGPD) and U6 promoter from *Pleurotus eryngii*, respectively. gRNAs were designed to target homeobox domain of *HD1* and *HD2* genes of *A3* and *A4* mating type loci. The constructed plasmids were introduced to the protoplasts of *P.eryngii* of different mating type combinations (*A3B3* and *A4B4*) using polyethyleneglycol-mediated transformation and the transformants were selected against hygromycin. Subsequent interaction study using the edited strains will verify the interaction between *A* and *B* mating type loci.

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F030

Hyperactive Mutants of *Escherichia coli* RNase E Confer Relaxed Cleavage Specificity

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RNase E plays a major role in RNA degradation and processing in *Escherichia coli*. It is a single-stranded specific endoribonuclease, cleavage sites of which are well characterized in many RNA substrates. Here, we show that upregulation of RNase E cleavage activity by a mutation that affects either RNA binding or enzyme multimerization accompanies relaxed cleavage specificity. Both mutations (Q36R and E429G) at positions distant from the catalytic site led to enhanced RNase E cleavage in RNA I, an antisense RNA of ColE1-type plasmid replication, at a major site as well as cryptic sites. Expression of a variant of RNA I with a major RNase E cleavage site deletion at the 5'-end led to increased copy number of ColE-type plasmid in *E. coli* cells expressing wild-type RNase E, indicating this variant RNA I does not efficiently function as an antisense RNA. These results suggest the existence of noncanonical action mode of RNase E and a previously unidentified property of the cleavage product of RNA I.

F031

Evolutionary Aspects of Divergent rRNAs in *Vibrio vulnificus* Species

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A previous study showed involvement of l-rRNAs, the most divergent rRNA of *Vibrio vulnificus* CMCP6, in preferential translation of a subset of mRNAs that include *hspA* and *tpiA* mRNAs in response to environmental changes. In this study, we show that heterologous expression of l-rRNAs in the *V vulnificus* MO6-24/O strain, which does not express highly divergent rRNAs, results in preferential translation of *hspA* mRNA. In addition, heterologous expression of l-rRNAs enabled the MO6-24/O strain to survive better in mouse. This study suggests disparate evolutionary pathways of divergent rRNA genes in *Vibrio vulnificus* species.

F032

Functional Study on *Escherichia coli* AmiC

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Amidase C (AmiC) is one of the peptidoglycan enzymes that perform the hydrolysis of C-N bonds in cell wall. However, a recent study identified AmiC as a positive regulator of RNase E activity *in vivo*. To characterize functional role of AmiC on mRNA stability, we analyzed the *amiC* expression level in growth phase and transcriptome profiles using the wild-type and *amiC*-deleted *Escherichia coli* strains. Our results suggest RNase E substrates whose abundance is controlled by AmiC levels.

F033

FgCon7 is an Essential Transcriptional Regulator of Development and Conidiation in *Fusarium graminearum*

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In plant pathogenic fungi, including *Fusarium graminearum*, vegetative growth, asexual sporulation, and fruiting body development should be precisely regulated temporally and spatially as these biological processes are keys for the survival and pathogenicity. Transcription factors (TFs) play essential roles in each developmental stage by regulating gene expression. Understanding the regulatory functions of TFs is crucial for elucidating molecular mechanisms underlying plant-pathogen interactions. Con7p was first identified as a central regulator of infection-related morphogenesis in *Magnaporthe oryzae* and its orthologs have also been known to be important for virulence in several plant pathogenic fungi. We identified a Con7p homolog that showed 59 % similarities in *F. graminearum*. A green fluorescence protein (GFP) -tagged FgCon7 was localized to nuclei of all developmental structures. Deletion of *FgCON7* displayed severe defects in not only conidia formation but also vegetative growth. We investigated the genetic relationship between *FgCON7* and *FgAbaA*, which plays a significant role in conidiation. Several morphological deficiencies, such as vegetative growth, in the deletion *Fgcon7* strain were partially recovered when *ABAA* was overexpressed. Relative transcriptional levels of conidiation-related genes, *ABAA* and *WETA*, notably decreased in *Fgcon7* deletion mutant. Collectively, our results suggest that Fgcon7 is a master morphological regulator in *F. graminearum*.

F034

A Complete Genome Sequence Analysis of *Paenibacillus polymyxa* JE201, Antagonistic to Plant Disease by *Corynespora cassiicola* and *Septoria* sp.

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A *Paenibacillus polymyxa* JE201 strain was isolated from the rhizosphere soil in the natural habitat of Chwinamul in Jeongeup, Korea. We screened antagonistic microorganism against plant pathogen by replacement cultural method, and JE201 showed strong antagonistic activities against plant pathogen such as *Corynespora cassiicola* and *Septoria* sp.. The phylogenetic analysis of the JE201 using 16S rRNA sequences showed the highest similarities with *Paenibacillus polymyxa*. The JE201 was length of the completely assembled genome sequences of 6,167,064 bp, with a G+C content of 45.57%. The genome encoded 5,357 putative ORFs, of which 5,169 ORFs were assigned with COGs categories onto 448 ORFs (8.57%) carbohydrate transport and metabolism, 412 ORFs (7.88%) translation, 290 ORFs (5.55%) amino acid transport and metabolism, 260 ORFs (4.97%) inorganic ion transport and metabolism, and 229 ORFs (4.38%) signal transduction mechanisms and the others. Furthermore, anti-SMASH analysis of genome showed 19 putative biosynthetic gene clusters responsible for various secondary metabolites.

F035

A Complete Genome Sequence of *Enterobacter oligotrophica* BC01, Antagonistic to Cucumber Target Leaf Spot Disease by *Corynespora cassiicola*

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A *Enterobacter oligotrophica* BC01 strain was isolated from the soil of a greenhouse in Bucheon, Korea. We screened antagonistic microorganism against plant pathogen by replacement cultural method, and BC01 showed antagonistic activities against *Corynespora cassiicola*. The phylogenetic analysis of the BC01 using 16S rRNA sequence showed the highest similarities with *Enterobacter oligotrophica*. BC01 was length of the completely assembled genome sequence of 4,818,618 bp, with a G+C content of 55.33%, 4,414 protein-coding genes, 25 rRNAs and 84 tRNAs. The genome encodes 4,414 putative ORFs, of which 4,366 ORFs were assigned with COGs categories onto 395 ORFs (8.87%) carbohydrate transport and metabolism, 377 ORFs (8.47%) amino acid transport and metabolism, 365 ORFs (8.19%) transcription, and 307 ORFs (6.89%) inorganic ion transport and metabolism and the others. Furthermore, anti-SMASH analysis of genome showed 5 putative biosynthetic gene clusters responsible for various secondary metabolites.

F036

Comparative Transcriptome Analysis Reveals the Phosphopantetheine Transferase-related Genes in the *Aspergillus nidulans*

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4'-Phosphopantetheinyl transferases (PPTases) catalyze the post-translational modification of fatty acid synthases, polyketide synthases, and nonribosomal peptide synthetases, which are central components of various metabolites. The filamentous fungus *Aspergillus nidulans* harbors a broad-range single the PPTase, NpgA, which is involved in phosphopantetheinylation in the biosynthesis of primary and secondary metabolites. To identify genes involved in PPTase in *A. nidulans*, we performed comparative transcriptomic analysis in loss-of-function mutation of PPTase gene, using Illumina HiSeq platform. *De novo* assembly of these sequences revealed 10,776 representative transcripts in two different strains of *A. nidulans*. A total of 9,968 unigenes were annotated and subjected to Gene Ontology, EuKaryotic Orthologous Groups, and Kyoto Encyclopedia of Genes and Genomes analyses. Analysis of 6 KEGG categories indicated that wild type strain specific transcripts were significantly enriched in 'metabolic pathways' and 'biosynthesis of secondary metabolites' pathways, which are important for secondary metabolites biosynthesis. These transcriptomes would be useful in elucidating the molecular mechanisms of PPTase for metabolites biosynthesis, and contribute to the characterization of PPTase-related genes in *A. nidulans*.

F037

Draft Genome Sequence of a '*Aurantibacter crassamentii*' KCTC 52207 belonging to the Family *Flavobacteriaceae*

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Here we report draft genome sequence of '*Aurantibacter crassamentii*' KCTC 52207, belonging to the family *Flavobacteriaceae*. Its draft genome sequence was determined using Illumina HiSeq X-ten platform. The assembled genome of '*A. crassamentii*' KCTC 52207 consisted of two contigs with a total length of 4,519,628 bp and the genomic DNA G+C content was 34.3 mol%. The draft genome encoded 3,739 protein-coding genes, 6 rRNA genes, 38 tRNA genes, 4 non-coding RNA genes and 8 pseudo genes. Its draft genome sequence contained carotenoid biosynthesis related genes and various biopolymer degradation-related genes. This genomic information is expected to be useful for application to biopolymers degradation.

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F038

Identification and Characterization of the *ndrC* Gene Which is Regulated by the *nsdD* Gene of *Aspergillus nidulans*

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Aspergillus nidulans is a filamentous model fungus that has both of asexual and sexual life cycles, which depend on environmental factors such as nutritional conditions and stresses. The *nsdD* gene is a well-known GATA type transcription factor that is responsible for the regulation of sexual and asexual development. In this study, we identified a gene, named *ndrC* (*nsdD* dependently regulated gene) by using RNA-seq experiment followed by DEG analysis. The protein encoded by the *ndrC* gene has no known domain and reported as hypothetical protein. The characterization of the gene was performed after making the gene deletion mutants, and its phenotypes under the various differentiation induction conditions were observed by comparing mutations with the host strain as well as wild-type strain as the control. The colony size of the mutant was similar to the host strains and the control strains, but more conidia were produced compared to the control strains, suggesting that the gene is negatively regulate asexual development or conidia production. Microscopic observations showed that there was no cleistothecium or hüll cell formed after incubation of sexual induction condition. Taken together, the *ndrC* gene is responsible for the sexual development and repression of asexual development.

F039

Two Tandem Termination Signals at the End of *gal* Operon Regulated by Transcription-translation Coupling

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The *gal* operon of *E. coli* has four structural genes, *galE*, *galT*, *galK*, and *galM*, each of which is about 1 kb in size. The last structural gene, *galM* has both Rho-dependent (RDT) and Rho-independent termination (RIT) signals. The 3' end of the full-length mRNA of *gal* operon, *galETKM*, is located at 4,313 (from the transcription initiation site of the *galP1* promoter, +1), which is 28 nucleotides from the stop codon of the *galM* gene. In our previous work, we identified that both RDT and RIT generate the 3' end of *galETKM* mRNA. However, in mutants, where one type of terminations is impaired, the other type remains functional. Here we show the effect of transcription-translation coupling on the 3' end of the full-length mRNA of *gal* operon, *galETKM* mRNA. Since in bacteria transcription is coupled with translation by the leading ribosome, the functions of both RDT and RIT depend on the transcription-translation coupling status. We found that termination is not an arbitrary event, rather a predetermined event that is dependent on transcription coupling status. Our results propose that coupling impairs the Rho-independent terminator since the active site of the coupled RNA polymerase is at the Rho-independent termination site, when the leading ribosome reaches the stop codon of *galM*, while the termination of the transcription-coupled with translation happens at the adjacent Rho-dependent termination site. Furthermore, we demonstrated that a mutant *gal* terminator hairpin containing eight consecutive uridines terminates translation-coupled transcription, perhaps explaining why some protein-coding genes have only strong intrinsic termination sites at their ends.

F040

Participation of Transcript Cleavage by RNase E Together with Rho-dependent Transcription Termination in sRNA-mediated Regulation of *gal* mRNA in *E. coli*

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In bacteria, non-coding small RNAs (sRNAs) bind to the target mRNA and regulate its translation and/or stability. In *E. coli*, the galactose (*gal*) operon comprises of 4 structural genes: *galE*, *galT*, *galK*, and *galM* producing 6 mRNAs of different sizes harboring a Spot 42 binding site (SBS) at *galT-galK* junction. Spot 42, small RNA of 109 nucleotide-long functions through base-pairing to target mRNA. The binding of the Spot 42 sRNA to the operon transcript leads to the generation of *galET* mRNA. However, the mechanisms of these regulations are unknown. Here we show that sRNA-mRNA base-pairing at the beginning of the *galK* gene leads to both transcription termination and transcript cleavage within *galK*, and generates *galET* mRNAs with two different 3'-OH ends. We found that transcription termination requires Rho, and transcript cleavage requires endonuclease RNaseE depending on the ribosome-free mRNA promoting that *galK* translation actually inhibits the generation of the two *galET* species. Our results demonstrate that the base-pairing segments between the sRNA and mRNA required for each of the two processing events are different, indicating different sequence requirements for the two events. Furthermore, we report here the presence of two targets in an mRNA, each of them causing a different outcome, seems to be a new mode of action for an mRNA. Considering the prevalence of potential sRNA targets at cistron junctions, the generation of new mRNA species by the mechanisms described here may be a general function of sRNA in gene regulation.

G001

Delivery of Protein Cargos for Biotechnological Engineering in Prokaryotes

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In bacterial biotechnology, instead of producing functional proteins from plasmids, it is often necessary to deliver functional proteins directly into live cells for genetic manipulation or physiological modification. Here, we developed a method to deliver protein cargos into living bacteria using cell-penetrating peptides (CPP). We evaluated the cell penetration efficiency of 107 CPPs with our optimized delivery method. We found that cell penetration efficiency was enhanced for the CPP-tagged proteins up to 12-fold in *E. coli* compared with untagged protein. To demonstrate the applicability of the CPP-conjugated strategy for protein delivery, we used meganuclease I-SceI for plasmid removal in *E. coli* harboring different plasmid copy numbers. CPP-conjugated I-SceI successfully eliminated low-to-high copy number plasmids. In addition, we developed a marker gene excision method based on a CPP-conjugated Cre recombinase/loxP system. Our method showed high efficiencies in markerless gene editing for metabolic engineering and ease of conducting experiments in *Methylomonas* sp. DH-1. In summary, we demonstrated the utility of CPPs in bacterial engineering. The use of CPPs would facilitate bacterial biotechnology such as genetic engineering, synthetic biology, metabolic engineering, and physiology studies. [This research was supported by the C1 Gas Refinery Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2016M3D3A1A01913244). This work was also supported by the NRF grant funded by the Korean government (MSIT) (No. NRF-2018R1A5A1025077).]

G002

Identification of a Cytosine Methyltransferase That Improves Transformation Efficiency in *Methylomonas* sp. DH-1

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Industrial biofuels and other value-added products can be produced from metabolically engineered microorganisms. *Methylomonas* sp. DH-1 is a candidate platform for bioconversion that uses methane as a carbon source. Although several genetic engineering techniques have been developed to work with *Methylomonas* sp. DH-1, the genetic manipulation of plasmids remains difficult because of the restriction-modification (RM) system present in the bacteria. Therefore, the RM system in *Methylomonas* sp. DH-1 must be identified to improve the genetic engineering prospects of this microorganism. We identified a DNA methylation site, TGGCCA, and its corresponding cytosine methyltransferase for the first time in *Methylomonas* sp. DH-1 through whole-genome bisulfite sequencing. The methyltransferase was confirmed to methylate the fourth nucleotide of TGGCCA. In general, methylated plasmids exhibited better transformation efficiency under the protection of the RM system than non-methylated plasmids did. As expected, when we transformed *Methylomonas* sp. DH-1 with plasmid DNA harboring the *psy* gene, the metabolic flux towards carotenoid increased. The methyltransferase-treated plasmid exhibited an increase in transformation efficiency of 2.5×10^3 CFU/ μ g (124%). The introduced gene increased the production of carotenoid by 26%. In addition, the methyltransferase-treated plasmid harboring anti-*psy* sRNA gene exhibited an increase in transformation efficiency by 70% as well. The production of carotenoid was decreased by 40% when the *psy* gene was translationally repressed by anti-*psy* sRNA. In this study, Plasmid DNA methylated by the discovered cytosine methyltransferase from *Methylomonas* sp. DH-1 had a higher transformation efficiency than non-treated plasmid DNA. The RM system identified in this study may facilitate the plasmid-based genetic manipulation of methanotrophs.

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G003

Characterization of Stress Responsive Genes in the Saxitoxin Producing Dinoflagellate *Alexandrium pacificum* Under Algicidal Agents and Metal Stresses; Transcriptomic Survey, Molecular Identification, and Expression Research

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Dinoflagellates are important contributors to marine primary production, and unarmored marine dinoflagellate *Alexandrium pacificum* is causative organisms of harmful algal blooms (HABs) and toxin production. Here, we performed RNA sequencing of *A. pacificum* under various stresses, including algicidal agents and heavy metal stresses. As a result, 18.1 Gb nucleotides sequencing data that assembled to 297,808 transcripts were obtained. Of them, 207 and 134 transcripts were assigned to the antioxidant enzyme system and heat shock protein (*HSP*) families, respectively. In addition, the chlorophyll synthesis pathway and other typical stress-responsive genes (e.g., cold shock proteins and mitogen-activated protein kinases) were widely expressed. Phylogenetic analysis these genes showed they might be acquired from different origins. Expressional levels of *CuZnSOD* and *HSP70* of *A. pacificum* significantly responded to algicides and metals. These suggest that stress-resistant genes response to different source for defense and/or adaptation to various environment conditions. These may allow the organism to survive in a new environment.

G004

Loss of Saxitoxin Biosynthesis Genes and Evolutionary Modification in the Dinoflagellates *Amphidinium carterae* and *Prorocentrum micans*; Transcriptome Survey and Toxin Measurements

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Saxitoxins (STXs) produced by marine dinoflagellate accumulate in shellfish by filter-feeding, causing paralytic shellfish poisoning (PSP). Many studies reported the relation between the STXs producing ability and related genes in toxic dinoflagellates. In the present study, we characterized the genes potentially related to STXs synthesis; polyketide synthase (*PKS*) and *sxt* genes using the mRNA sequencing of two non-STX producing dinoflagellates *Amphidinium carterae* and *Prorocentrum micans*. We identified 94 and 166 *PKS* contigs in *A. carterae* and *P. micans*, respectively and type III *PKS*, which was closely related to bacteria. In addition, homologs 20 STX biosynthesis genes were detected and the core STX-synthesizing genes (*sxtA* and *sxtB*) were only found in *P. micans*. The two core genes (*sxtA* and *sxtG*) showed a low sequence similarity (37.0–67.6%) comparing with PSP causative dinoflagellates, such as *Alexandrium pacificum*, even organization of functional domains were different. These modification of genes may explain disruption of the initial reactions in STXs production and ultimately the loss of the STXs producing ability in both dinoflagellates. Our results imply that *PKS* and *sxt* genes are commonly found in non-toxic dinoflagellates, however their evolutionary modification and loss may alter STXs metabolism. In addition, the genes may also have other molecular metabolic functions.

G005

Alarmin Protein-derived Peptide Regulates the Inflammatory Response by Inhibition of RAGE Signaling Pathway

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Alarmin proteins such as high mobility group box 1 (HMGB1), pentraxin-related protein, and C-reactive protein are released by nuclear after that bind to DNA. Alarmin proteins can also be secreted from cells and binds receptor for advanced glycation end-products (RAGE) that receptor protein concerned in inflammatory diseases. The interaction between alarmin proteins and RAGE causes upregulation of NF- κ B that leads to increased production and secretion of cytokines. So, alarmin proteins-derived peptide can affect inhibitory response in many inflammations related diseases through binding inhibition. In previous studies, RAGE pathway signaling activated a variety of the inflammatory diseases such as sepsis, inflammatory bowel disease, and rheumatoid arthritis. So, we challenged to regulate the RAGE signaling for new strategy of other diseases. Here, we found that alarmin-derived peptide inhibits inflammation and leading to regulate RAGE expression and NF- κ B activation *in vitro*. Moreover, we demonstrated alarmin proteins-derived peptide reduced cytokine serum levels *in vivo*. Our results suggested that alarmin proteins derived peptide is effective inhibitor against RAGE signaling pathway, offering a novel potential therapeutic approach to patients and RAGE signaling has been proposed as a target for inflammatory diseases. [This work was supported by the NRF grant funded by the Korea government (MSIP) (2019R111A2A01064237).]

G006

Multi-functional rMPT Protein as a Therapeutic Agent against *Mycobacterium*

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Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), avoids the host immune system through its virulence factors. MPT family protein is the virulence factors secreted by MTB which regulate host proteins for the survival and proliferation of MTB in the host. In a previous study, we identified that MPT63 interacts with TBK1 and p47phox in macrophages, increasing the levels of reactive oxygen species (ROS) and pro-inflammatory cytokines, and decreasing the expression of IFN- β . Additionally, MPT64 interacted with TBK1 and HK2, specifically targeting the MTB-infected macrophages and regulating the level of pro-inflammatory cytokines and IFN- β . Here, we showed that a MPT63/64-derived multi-functional recombinant protein (rMPT), which was able to interact with TBK1, p47phox, or HK2, reduced the burden of MTB by regulating IFN- β levels and increasing inflammation and the production of ROS, both *in vitro* and *in vivo*. Furthermore, rMPT downregulated the survival of MTB through interacting with TBK1, p47phox, and HK2 in MTB-infected mice. Taken together, we suggest rMPT can be considered as a potential therapeutic agent against MTB via activating the inflammatory responses. [This work was supported by the NRF grant funded by the Korea government (MSIP) (2019R111A2A01064237).]

G007

Metabolic Engineering of *Escherichia coli* to Produce Unbound, Free Heme Secreted to the Medium

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Heme, a prosthetic group of many important enzymes, has wide applications in healthcare and food supplement industries. Here we generated an engineered *E. coli* strain producing extracellular free heme. First, the endogenous C5 pathway and the downstream heme biosynthetic pathway were optimized. Next, the *ldhA*, *pta*, and *yfeX* genes were knocked out. As a result, 7.88 mg/L of total heme with 1.26 mg/L of extracellular heme was produced by flask cultivation. In addition, the *ccmABC* genes coding for the heme exporter involved in cytochrome *c* biogenesis was overexpressed in the engineered *E. coli* strain. Fed-batch fermentation of the resulting strain produced 115.5 mg/L heme from glucose and secreted 73.4 mg/L (63.5%) out of the total heme produced. Furthermore, l-glutamate supplementation further enhanced the production of heme to 239.2 mg/L and also improved secretion of heme (151.4 mg/L; 63.3%) to the medium. These metabolic engineering strategies we report here will help generating microbial strains producing free heme at high efficiency.

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G008

RecET-based Recombineering System for Markerless Knockout and Integration of Genes to *Pseudomonas putida* Chromosome

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Pseudomonas putida has been considered as a promising engineering host for the production of high-value natural products. To construct stable industrial microbes producing heterologous products, integrating biosynthetic gene clusters to the chromosome is critical. However, chromosome engineering of *P. putida* has relied on time-consuming homologous recombination and transposon-mediated random insertions. Here we developed a RecET recombineering system that enables integrating heterologous genes to the *P. putida* chromosome without any antibiotic marker left. First, the capacity of the RecET recombineering system was demonstrated through deletion of various chromosomal regions spanning 0.6-101.7 kb. Then, we integrated four biosynthetic gene clusters (up to 7.4 kb) to *P. putida* chromosome and prove the efficiency of gene integration. Meanwhile, Cre/lox system and efficient plasmid curing system enabled markerless engineering of *P. putida* and generation of marker- and plasmid-free strains. The RecET-based markerless recombineering system developed and examined here will facilitate engineering *P. putida* for academic studies and developing industrially-relevant producers.

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G009

***Escherichia coli* as Chassis Strain for the Production of Diverse Natural Compounds**

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Natural products have been gained much attention around the world because of indispensable and diverse applications for our daily life, such as pharmaceuticals, food additives, nutraceuticals, and cosmetic ingredients. Plants have been a major source of many valuable natural products. However, their successful extraction is rather limited since plants are sensitive with weather and environmental conditions. Under this circumstance, an alternative means of biosynthesizing diverse natural products using *Escherichia coli* has emerged because of the development of various metabolic engineering strategies and high cell density culture strategies. Here, we reviewed novel metabolic engineering tools for the natural compounds overproduction in *E. coli*. Future perspectives on the biosynthesis of complex natural products through the development of robust microbial-cell are also proposed.

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G010

Malonyl-CoA Biosensor Development as Metabolic Engineering Tool for Various Microorganisms

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Here we repurposed RppA, which is a type III polyketide synthase and is capable to produce red-colored flaviolin as a malonyl-CoA biosensor applicable in three industrially important bacteria. Strains with enhanced malonyl-CoA pools were distinguished by the colorimetric screening of cells showing increased red fluorescence. Total 1,858 synthetic small regulatory RNA library was constructed for screening, thus discovering 14 knockdown gene targets that generally enhanced malonyl-CoA level in *E. coli*. Knocking down these genes allowed the engineered strains to produce enhanced production of two polyketide (6-methylsalicylic acid and aloesone) and two phenylpropanoid (resveratrol and naringenin) compounds.

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G011

Rapid and Multiplex Repression of Target Genes Using Synthetic sRNAs to Overproduce Natural Compounds in *E. coli*

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In this study, an *E. coli* genome-scale sRNA library was constructed based on the previously constructed synthetic small regulatory RNA (sRNA) technology. It specifically downregulates target genes by blocking translation. However, if the strains were already engineered, having incompatible plasmid(s), synthetic sRNA cannot be used. To overcome this limitation, by incorporating compatible antibiotic markers and origins of replication, an expanded sRNA expression platform was also constructed. This platform as well allowed rapid and multiplex knockdown of target genes. The new libraries were employed to construct violacein (5.19 g/L) and indigo (135 mg/L) producing *E. coli* strains. The expanded sRNA expression vectors in this study will enable construction of microbial cell factories capable of industrial-level production of valuable natural compounds, regardless of plasmid compatibility.

[This work was carried out with the support of Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (Grants NRF2012M1A2A2026556 and NRF-2012M1A2A2026557), and the Intelligent Synthetic Biology Center through the Global Frontier Project (Grant 2011-0031963) of the Ministry of Science and ICT (MSIT) through the National Research Foundation (NRF) of Korea.]

G012

Improved Production of Astaxanthin by Metabolically Engineered *Escherichia coli*

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Astaxanthin is a reddish keto-carotenoid, found in various marine organisms and microbes. It has powerful antioxidant and anti-inflammatory characteristics, compared to other carotenoids. In this research, we metabolically engineered *Escherichia coli* to produce astaxanthin in high concentration and high productivity. First, the heterologous *crt* genes and the N-terminal truncated *BKT* gene were introduced to construct astaxanthin synthetic pathway. Then, to promote membrane localization and stable expression, eight different signal peptides were attached to the N-terminus or C-terminus of the truncated BKT protein. To further increase the titer, *in silico* flux variability scanning based on enforced objective flux (FVSEOF) was performed and total 432.82 mg/L of astaxanthin was produced by *E. coli*. By adapting the strategies we have taken, enhanced production of astaxanthin, and other carotenoids will be possible in *E. coli*.

[This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01550602)" Rural Development Administration, Republic of Korea, the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (Project No. NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea.]

G013

Cytoplasmic pH-Dependent Regulation of TORC1 Downstream Processes

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The target of rapamycin kinase complex I (TORC1) connects nutrient availability to cell growth in eukaryotes. Recently, it was reported that cytoplasmic pH (pH_c) acts as a cellular signal to regulate TORC1 activity in response to glucose availability, proposing a molecular mechanism linking pH_c, carbon source, and cell growth in *Saccharomyces cerevisiae*. However, it remains unknown which downstream processes of TORC1 are subject to the pH_c-dependent regulation. To answer the question, we constructed and analyzed the Hypo-Pma1 strain that has a lower pH_c than the wild type in the presence of glucose due to reduced expression of Pma1, the major proton pump regulating pH_c. The replicative lifespan of the Hypo-Pma1 mutant was longer than that of the wild type. And, the transcriptome analysis revealed that expression of some PHO regulon genes, which are activated by the Pho4 transcription factor, was significantly increased in the Hypo-Pma1 mutant, suggesting that the signaling pathway for phosphate metabolism may be regulated by pH_c. We found that Pho4 was localized in the nucleus in the Hypo-Pma1 mutant in the glucose and phosphate-rich medium, indicating Pho4 is active regardless of phosphate availability in the Hypo-Pma1 mutant. We will present and discuss our results of whether TORC1 is involved in the Pho4 activation and which downstream processes of TORC1 are regulated by pH_c.

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G014

Identification of Chromomycin A3-Overproducing Mutant Strains in *Streptomyces* sp. SJ1-7

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Myongji University

Chromomycin A3 (CMA) is an anthraquinone glycoside-mithramycin A analog with the antitumor activity which is produced by *Streptomyces* strains. A novel *Streptomyces* strain, *Streptomyces* sp. SJ1-7, which produces CMA, was previously identified. In this study, we identified several CMA-overproducing mutant strains through a high-throughput screen using UV-induced mutation. Because CMA has an antibacterial activity, the production rate of CMA was measured by calculating inhibition zone diameters of *S. aureus*. We selected several mutants with strong antibacterial activities. Despite the strong antibacterial activities, most of them showed the growth rate comparable to that of the wild-type stain, indicating that the high production of CMA in these mutant stains is not caused by the fast growth rate. Through TLC analysis, we revealed that these mutant strains more highly produced CMA at all growth stages. HPLC analysis showed that the CMA production levels of some mutant stains were more than twice higher than that of the wild-type stain. Taken together, these results demonstrate the identification of CMA-overproducing mutant strains through the high-throughput screen using UV-induced mutation.

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G015

Evaluation of Antioxidant and Anti-inflammatory Activity of Ethanolic Extracts of *Polygonum senticosum* in Lipopolysaccharide-induced RAW 264.7 Macrophages

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Nakdonggang National Institute of Biological Resources

Polygonum senticosum (Meisn.) Franch. & Sav. (*P. senticosum*) has been used for treating various diseases (cholestatic hepatitis, pharyngitis, etc.) as a traditional Korean medicine. This study aimed to examine the antioxidant activity and anti-inflammatory effects of ethanol extract of *P. senticosum* (EPS) on LPS-stimulated RAW 264.7 macrophages. Antioxidant activity of EPS was assessed by radical-scavenging effects on DPPH free radicals. Pro-inflammatory markers produced by LPS-induced RAW 264.7 macrophages were quantified to assess the anti-inflammatory activity of EPS. Our results showed that EPS significantly increased DPPH radical-scavenging activity. Additionally, EPS inhibited LPS-induced proinflammatory mediators, such as nitric oxide (NO) and prostaglandin E2 (PGE2), along with pro-inflammatory cytokines, including tumor necrosis factor (TNF)- α and interleukin (IL)-1 β , without significant cytotoxicity. EPS significantly downregulated the expression of inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), TNF- α , and IL-1 β in LPS-stimulated RAW 264.7 macrophages. Positive correlations were noted between DPPH radical-scavenging activity and anti-inflammatory capacity. This study highlights the potential role of EPS against inflammation and associated diseases.

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G016

Microbiota is Permeability Regulator in Brain

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Microbial guns and microbial guns and destruction of the intestinal-brain axis have been associated with various metabolic, immunological, physiological, neurodevelopmental and neuropsychiatric disorders. After a brief review of the relevant literature, we have intestinal serotonin that is produced by intestinal intestinal cell cells, and is picked up and stored by circulating platelets in the intestines, brain and other organs. Intestinal serotonin can also act as a lasting regulatory signal, such as hormones for the whole body, including the brain. This regulatory signal function is mediated by platelets and depends primarily on the actual state of health of the intestine. This hypothesis can partially explain why intestinal dysbiosis can be linked to neurodevelopmental and neuropsychiatric disorders, as well as to various human pathological conditions.

G017

Enhancement of the Lipid Accumulation of *Chlorella* sp. HS2 by EMS-induced Mutation in Conjunction with FACS Based High Throughput Screening

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Despite being widely heralded as a promising replacement for fossil fuels, microalgae derived biofuels and value added products suffer from high production costs which precludes their commercialization. In order to improve the cell size and lipid content of the microalgae *Chlorella* sp. HS2, ethyl methanesulfate (EMS) induced random mutagenesis was used in conjunction with fluorescent assorted cell sorting (FACS) for the selection of cell population with desirable phenotypes. The growth, biomass yield, biomass productivity, lipid content, and lipid productivity of the selected mutagenized strains (SMS, T_{E+F}) were determined and compared with parent wild strains (PWS, T_C) and FACS screened strains (FSS, T_F). The result showed that the specific growth rate, biomass yield, and biomass productivity of the SMS (T_{E+F}) grown for 6 days was significantly higher than those of the PWS (T_C). The SMS (T_{E+F}) strain also showed the highest lipid content and productivity than those of PWS (T_C) and FSS (T_F). The fatty acid methylester (FAME) profile showed the combination of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were also higher in the SMS (T_{E+F}), while the total amounts of poly unsaturated fatty acids (PUFA) were reduced. These results indicate that directed evolution using EMS mutagenesis with FACS is a powerful tool to achieve microalgal strain improvement for biofuel production.

G018

Desirable Bioremediation Process to Degrade PAHs and TPHs Using *Peniophora incarnata*

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Peniophora incarnata has been received attention as a rising biological resource to investigate degradation pathway of PAHs. Also, its ligninolytic enzyme activities were investigated to their gene expression and degrading ability against PAHs. 50 mg/L of phenanthrene was degraded up to 99.1% using *Peniophora incarnata* KUC10078 within nine days. And benzoic acid and dibenzothipene was detected as intermediates during the degradation. It demonstrated a specific degrading pathway of phenanthrene by *P. incarnata*. Additionally, two ligninolytic enzyme genes, laccas and MnP genes produced from *P. incarnata*, were expressed with the highest efficiency at the ninth day of the incubation. It is considered that ligninolytic enzymes from two genes were activated to degrade phenanthrene. Finally, hydrocarbons were degraded in the petroleum-contaminated soil. During the removal of TPH, the copy number variation analysis of genomic DNA was carried out with 3D digital PCR. It resulted in 227 copies/g of gene copy number variation showed with FAMTM dye. Thus, the study can suggest a novel technology to analyze bioremediation related with degradation mechanism from degrading pathway of xenobiotics.

G019

Neutralizing Human Antibodies against SARS-CoV-2 Isolated from a Human Synthetic Fab Phage Display Library

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SARS-CoV-2 has caused a pandemic outbreak resulting in a tremendous global threat due to its unprecedented rapid spread. The receptor-binding domain (RBD) of SARS-CoV-2 spike protein is a key player for the viral entry into cells through its interaction with angiotensin-converting enzyme 2 (ACE2) receptor protein, and the RBD has therefore been crucial as a drug target. Here, we used phage display to develop human monoclonal antibodies (mAbs) that neutralize SARS-CoV-2. A human synthetic Fab phage display library was panned against the RBD. Reformating the five Fabs into IgGs greatly increased their apparent affinities, without compromising their non-aggregating properties and thermal stability. Furthermore, two of the mAbs (D12 and C2) significantly showed neutralizing activities on pseudo-typed and authentic SARS-CoV-2. Given their desirable properties and neutralizing activities, we anticipate that these human anti-SARS-CoV-2 mAbs would be suitable reagents to be further developed as antibody therapeutics to treat COVID-19, as well as for diagnostics and research tools.

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G020

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

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The problem of resistance due to excessive abuse of antibiotics is the cause of the birth of multidrug-resistant bacteria, and the problem has not been solved until now. Therefore, as a solution to the problem of existing antibiotics, attention was paid to the search for antimicrobial peptides (AMPs) whose resistance problem was not revealed. In this study, AMPs derived from halophiles were obtained based on the gene information of halocin peptides. It was named HB384, and the gene encoding HB384 was cloned into pGST vector and the recombinant HB384 was expressed in *E. coli* BL21. The recombinant protein was purified by GST affinity chromatography, and the molecular weight of HB384 was 3.14 kDa. Disk diffusion assays were performed to evaluate antimicrobial activity. HB384 showed antimicrobial activity against Gram-positive bacteria *S. aureus*, *B. subtilis* and Gram-negative bacteria *S. typhimurium*, *E. coli*. Moreover, HB384 was stable at 99°C for 8 h, as the concentration increased, antimicrobial activity against pathogen. In less than 6 µg, HB384 almost reached the maximum antimicrobial activity against pathogen. When comparing the number of moles of antimicrobial activity substances per area of the clear zone, the antimicrobial activity of HB384 against *B. subtilis* was 3.2 times better than that of ampicillin. As a result, purified HB384 is expected to be applicable not only as a functional peptide material, but also as a substitute for existing antibiotics.

G021

Cloning and Expression of Thermostable α -Amylase and 1,4- α -Glucan 6- α -Glucosyltransferase Coding Gene for Isomaltodextrin Production

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Isomaltodextrin (IMD) is a water-soluble dietary fiber with various bioactive functions, including the intestinal microbiome and bowel movements, immunity, and blood sugar regulation. The manufacturing process of IMD liquefies food grade starch using thermophilic α -amylase. Thereafter, saccharification is performed using α -amylase and 1,4- α -glucan 6- α -glucosyltransferase. Recently, genome sequencing of *Fervidobacterium islandicum* AW-1, a hyper thermophilic strain, was investigated. In this process, NA23_07330 and NA23_09780, which are strongly presumed to be α -amylase and 1,4- α -glucan 6- α -glucosyltransferase, were discovered. The purpose of this study is to characterize of these two genes by cloning and expressing them. The gene α -amylase (FIAA) and 1,4- α -glucan 6- α -glucosyltransferase (FIGT) from *Fervidobacterium islandicum* AW-1 were cloned and expressed in *E. coli* BL21. Sequencing of FIAA revealed an open reading frame of 1,857 bps encoding a protein of 618 amino acid residues with a calculated molecular weight of 71.2 kDa. The isoelectric point (pI) of the enzyme was about 5.17. Sequencing of FIGT revealed an open reading frame of 2,442 bps encoding a protein of 813 amino acid residues with a calculated molecular weight of 92.5 kDa. The isoelectric point (pI) of the enzyme was about 4.94. This study will contribute to the production of IMD as a key enzyme in the manufacture of isomaltodextrin.

G022

Antioxidant and Anti-inflammatory Effects of *Abelmoschus manihot* L. Extracts Fermented with *Bacillus licheniformis* CP6 from Korea South Sea

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In this study, we investigated the antioxidant and anti-inflammatory effects of fermented *Abelmoschus manihot* L. extracts in lipopolysaccharide (LPS)-stimulated RAW 264.7 mouse macrophages. The fermentation was performed using *Bacillus* sp. in mixture of *Abelmoschus manihot* L. at 37°C for 1 days (Named CP6-1D). DPPH radical scavenging activity of CP6-1D was increased in a 73% to 76%. The CP6-1D suppressed reactive oxygen species (ROS), nitric oxide (NO) production and the expression of iNOS and COX-2. Also, CP6-1D showed inhibitory effect on the production of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α). Moreover, nuclear translocation of nuclear factor- κ B (NF- κ B) and phosphorylation of mitogen-activated protein kinases (MAP kinase) were strongly suppressed by CP6-1D in LPS-stimulated RAW 264.7 cells. The present results indicate that CP6-1D has an antioxidant and anti-inflammatory properties. Based on these results, it is expected to be able to provide basic information that can be used as a new natural functional material in various fields such as functional foods, cosmetics, and pharmaceutical materials.

G023

Development of Functionalized Gold Nanoparticle as a Highly Effective Antibacterial Agent against *Acinetobacter baumannii*

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Acinetobacter baumannii causes multidrug resistance and fatal infections in humans. In this study, we investigated whether a hexahistidine-tagged form of an antimicrobial peptide (AMP), Lys AB2 P3-His, can effectively inhibit *A. baumannii* infection in mice. When *A. baumannii*-infected mice were intraperitoneally injected with functionalized gold nanoparticle (AuNP-Apt) loaded with Lys AB2 P3-His, about 53% inhibition of *A. baumannii* colonization was observed in the mouse organs, leading to increased survival time and rate among the treated mice compared to the control mice treated with AuNP-Apt or Lys AB2 P3-His only. This study shows the potential of the AMP delivered by AuNP-Apt as an effective therapeutic tool against infection caused by multidrug resistant pathogens in humans. Further studies are needed to develop DNA aptamers that specifically bind to the AMP in order to minimize alterations of antibacterial activity of the AMP due to the addition of hexahistidine-tag.

G024

Production and Purification of Compound-K made by Fungal Edible Enzyme Originated from *Aspergillus niger* C2-2 Isolated from Nu-Ruk

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Minor ginsenosides such as Rg3, F2 and Compound-K (C-K) are more pharmacologically effective than major ginsenosides such as Rb1, Rb2, and Rc. However, C-K has a low production yield and restriction on usage. An enzyme that transforms ginsenosides was discovered from the strain *Aspergillus niger* C2-2. A crude enzyme extracted from solid fungal culture can be effectively transformed to ginsenoside Rb1 → C-K via Rd and F2. *Aspergillus niger* C2-2 was cultured in a wheat bran. After that, five times of 50 mM acetate buffer was added on and adjusted at pH 5.0 and rested for 10 days to extract crude enzyme. And then supernatant was collected by centrifugation and was concentrated by 20X using a membrane concentrator. To produce C-K, Rb1 50% was used as a substrate and reacted at 10,000 ppm and 50°C. Then, it was purified using Prep HPLC system. The crude enzyme could effectively bioconvert the ginsenosides Rb1, Rc and Rd into C-K. A scaled-up biotransformation reaction was performed in a 200 L fermenter at pH 5.0 and 50°C for 120 h with a concentration of 10 mg/ml of Rb1 50%. Finally, 498 g of C-K was produced from 1 kg of Rb1 concentrate 50% with 51.5±1.1% chromatographic purity. These results show that this crude enzyme could be efficiently used in the preparation of edible ginsenoside C-K in the cosmetics, functional food, and pharmaceutical industries.

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G025

Changes in Rumen Microbial Community and Rumen Fermentation Characteristics of Holstein Dairy Cows in Early Lactation during Heat Stress

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Dairy cows are considered the most sensitive in response to heat stress that globally challenges dairy industry. Thus, this study was conducted to compare the changes in rumen microbial community and rumen fermentation characteristics of Holstein dairy cows at early stage of lactation during heat stress. The temperature-humidity index (THI) was monitored at two-time points: heat stress period (HS) and recovery period (RC). Principal coordinates 1 and 2 showed 33.1% and 19.1% of the total variation, respectively. There was no significant difference in observed species, Chao1, Shannon and Simpson indices. Metataxonomic analysis revealed three predominant phyla: Bacteroidetes (61.95% in HS and 57.816% in RC), Firmicutes (26.38% and 22.00% in HS and RC, respectively) and Proteobacteria (6.87% and 14.25% in HS and RC, respectively). At the genus level, five genera predominated the HS and RC: *Prevotella*, *Succiniclasticum*, *Selenomonas*, *Duncaniella*, and *Ruminococcus*. The ruminal pH, ammonia-N (NH₃-N), acetate, and butyrate were not significantly different. However, propionate and total VFA were significantly higher in HS. Overall, heat stress changes the abundance of Bacteroidetes and Firmicutes, and *Prevotella* at the genus level, altering the propionate and total VFA production of Holstein dairy cows in early lactation.

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G026

Adaptive Evolutionary Trajectory of Bacterial Cells toward Non-utilizable Sugars

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Peripheral sugar utilization pathways play a pivotal role in cell survival across all microorganisms. Although sugar catabolic pathways have been evolved to be specific for various sugars, the evolution path of substrate preference remains elusive. Herein, we attempted to adapt *E. coli* to utilize D-Tagatose, a naturally occurring rare monosaccharide, as a carbon source. Adaptive laboratory evolution (ALE) integrated with omics analysis led us to track the adaptive evolution process of how bacterial cells can use unusual nutrients. Genetic mutation and growth rate were monitored with continuously subcultured bacterial cells using genomic and phenomic analyses. Resequencing with quantitative real-time PCR enabled us to identify the adaptive mutational sites in readjusting the glycolytic reactions and the phosphotransferase system on unusable sugars. These results provide insight into how microorganisms have evolved to gain preferences for various sugars. Moreover, the ALE-induced strain could serve as a high-throughput screening platform for engineering tailor-made sugar isomerase for the production of D-tagatose.

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G027

Isolation of Lactic Acid Bacteria from Equine Feces

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Horse is a herbivore, monogastric animal and hindgut fermenting animal. In the large intestine, cellulose can be digested by micro-organisms to the VFA and absorbed. The digestive disorders in the large intestine of horse may lead to colic, laminitis, diarrhoea, dehydration and often death. Therefore, to maintain a healthy condition in the horse, digestive activity is an important issue that must be constantly monitored.

The purpose of this study was to investigate the probiotic properties of lactic acid bacterial strains isolated from horse feces. The properties were tested on the basis of guideline for probiotic selection protocol such as tolerance for acid or bile salt, antibiotic resistance, and antimicrobial activity.

Total 25 lactic acid bacteria were isolated from horse feces, and their antibacterial activity was tested using an agar diffusion assay. Among them, 4 selected strains were identified by analysis of their 16S rRNA, as *Lactobacillus acidophilus* CH310, *Lactobacillus reuteri* CH332, *Enterococcus faecium* CH421, *Bifidobacterium longum* CH534.

Results show that resistance to low pH and bile salts. Also, the selected strains were resistant to bile acid up to 3% and their autoaggregation rates were as high as 60%. These results suggested that the three selected strains could serve as horse probiotics.

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G028

BhTS (Barcoded High-Throughput Screening) Platform Improving Productivity of Recombinant Proteins Using 1200 Secretory Signal Peptides

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The productivity of recombinant proteins in mammalian cells has been considered as a critical factor for the development of new biopharmaceuticals in terms of development efficiencies and economics. We have established BhTS platform with human secretory signal peptides library and high throughput screening (HTS) system to select optimal signal peptides, which make high productivity of recombinant proteins. We designed 1200 signal peptide tags with amino acid barcoding sequences which corresponds to 1200 signal peptides. Various barcoding sequences in the secreted target proteins are identified, and then ranked in order of productivity of the target protein using LC-MS. To validate hit signal peptides, the expression vectors coding each signal peptide and target protein are expressed individually, then the secretion levels of target protein are compared by western blot analysis. Using this platform technology, we performed BhTS to select optimal signal peptide for single chain variable fragment (scFv), which is not produced in mammalian system as a case study. The levels of secreted proteins were analyzed, following the individual expression of each hit signal peptide. The results showed that 5 signal peptides make high secretion levels of target scFv. In conclusion, we have developed BhTS platform with 1,200 secretory signal peptides and it could contribute to reduce the time, labor, and cost of the process of biopharmaceuticals development.

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G029

Evaluation of an Effective Detection and Quantification Method for Particular Microorganisms by Comparing NGS-based Metagenome Profiling Data

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Metagenome profiling research using next-generation sequencing (NGS), a technique widely used to analyze the diversity and composition of microorganisms living in the human body, especially the gastrointestinal tract, has been activated, and there is a growing interest in the quantitative and diagnostic technology for specific microorganisms. According to recent trends, quantitative real-time PCR (qRT-PCR) are still of considerable technique in detecting and quantifying bacteria associated with the human mouth, nasal cavity, and pharynx due to analytical cost and time burden of NGS technology. Here, based on NGS metagenome profiling data produced by utilizing 100 gut microbiota samples, we conducted a comparative analysis of identifying for five bacterial genera proportions (*Akkermansia*, *Bacteroides*, *Bifidobacterium*, *Phascolarctobacterium*, and *Roseburia*) within same metagenomic DNA samples through qRT-PCR assay in parallel. Genus-specific primer, targeting the particular gene of each genus for qRT-PCR assay, allowed a statistically consistent quantification pattern with the metagenome profiling data. Furthermore, results of bacterial identification through Sanger validation demonstrated the high genus-specificity of each primer set. Therefore, our study suggests that an approach to quantifying specific microorganisms by applying qRT-PCR method can compensate for the concerns (potential issues) of NGS while also providing efficient benefits to various microbial industries.

G030

Fine Chemicals Produced from Sulfonated Polyethylene by a Bio-Photo-Fenton Approach Using Glucose Oxidase Immobilized on Titanium Dioxide

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Polyethylene (PE) plastics are highly recalcitrant and resistant to photo-oxidative degradation due to its chemically inert backbone structure. We applied two novel reactions such as, Bio-Fenton reaction using glucose oxidase (GOx) enzyme alone and Bio-Photo-Fenton reaction using GOx immobilized on TiO₂ nanoparticles under UV radiation, for (bio)degradation of pre-activated PE with sulfonation (SPE). From both the reactions, GC-MS analyses identified fine chemicals, acetic acid and butanoic acid as major metabolites produced from SPE. In the presence of UV radiation, 21 fold and 17 fold higher amounts of acetic acid and butanoic acid were produced from SPE after 6 h of reaction using GOx immobilized on TiO₂ nanoparticles than GOx enzyme alone. Our results suggest that (bio)degradation and valorization of naturally weathered and oxidized PE using combined reactions of biochemistry, photochemistry and Fenton chemistry could be possible.

G031

Application of the Porcine Whole Blood Hydrolysate for the Microbial Culture Media

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Porcine blood is an agricultural byproduct in the meat processing industries. However, the blood could be a low-cost protein source for the higher value products. In this study, we identified the metabolic compounds and peptides in the hydrolysate of porcine blood for the application of microbial culture media. The whole blood hydrolyzed by protease cocktails was formulated to dried powder form to analyze the water soluble compounds. A total of 468 metabolites were identified through LC-MS analysis. Overall, 82 metabolites, including the twenty essential amino acids, were quantitatively analyzed. Other metabolites involved in glycolysis/ glyconeogenesis, TCA cycle, amino acid biosynthesis, and glutamate metabolism were also detected in the enzymatic blood hydrolysate. Based on these analysis, the blood lysate was used as a nitrogen source in the culture media preparation for two GRAS strains, *Lactobacillus plantarum* and *Bacillus* sp. B-100. These bacteria showed the normal growth patterns in the culture media supplemented with the blood lysate. In addition, *Bacillus* sp. B-100 also displayed successfully its anti-fungal activities toward pathogenic fungi strains, *Hytolphthora capsici*, *Colletotrichum gloeosporioides*, *Fusarium fujikuroi*, and *Cylindrocarpon destructans*. This analytical data could contribute the potential utilization of a blood byproduct as the supplementary compounds in the microbial culture medium.

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G032

Greenhouse Gas Mitigation via a Microalgae-methanotroph Co-cultivation Platform

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Finding sustainable alternatives to fossil fuels and coping with the increasing concentration of greenhouse gases in the atmosphere has emerged as a research interest in academia and industry worldwide. The development of renewable bioprocess using carbon dioxide present in biogas to cultivate algal species represents an alternative to generate microbial biomass and bioproducts. Meanwhile, co-cultivation of methanotroph in a combined algal species system offers an additional advantage of using methane derived from biogas as carbon feedstock to produce biomass and relevant bioproducts. In this study, a type II methane-oxidizing bacterium (MOB), *Methylocystis rosea* M20-1 (in 100 ml NMS medium) was coupled to a microalga *Chlorella sorokiniana* (in 100 ml BG-11 medium) via oxygenic photosynthesis in synthetic biogas ($\text{CH}_4:\text{CO}_2=80:20$; headspace volume 800 ml) based on the flexible platform consisting of two 500 ml screw-capped glass Erlenmeyer flasks connected by glass tube for gas exchange. *Chlorella* cultivation was carried out with an agitation speed of 100 rpm under 16 h light illumination of $110 \mu\text{mol}/\text{m}^2/\text{sec}$ at 28°C . *M. rosea* M20-1 grown under the same conditions showed a slower growth with less biomass in a batch co-cultivation system than a monoculture grown in 25 ml vented culture flasks kept in the airtight container supplied with fresh methane and oxygen mixture (1:1) every 24 h. However, compared to the monoculture of *Chlorella* grown in syn-gas and aerobic conditions, the co-culture achieved over two-fold increase in biomass. Although this system appears to be very efficient for the growth of microalgae, there are some problems with MOB culture. Therefore, it is necessary to explore the optimal conditions for MOB growth in the near future.

H001

Analysis of the Young-radish (*Raphanus sativus* L.) Microbiota for an Improvement in Kimchi Product Management

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Young-radish (*Raphanus sativus* L.) is a popular vegetable in many countries including South Korea. The fresh vegetable is commonly consumed uncooked, the microbiota of fresh vegetable cause food-borne illnesses. Therefore, analysis of the young-radish microbiota can improve understanding of outbreaks and causes of food poisoning and help to better manage fresh vegetables. Total of young-radish samples were collected in April and August in South Korea (farm of Gyeonggi province). The 16S rRNA gene-based sequencing was conducted using Illumine Miseq. Proteobacteria, Firmicutes, and Actinobacteria were dominant phyla, and Gammaproteobacteria, Bacilli, Alphaproteobacteria, Actinobacteria, and Betaproteobacteria were dominant class at both times. The 5 dominant genera were *Pseudomonas*, *Bacillus*, *Methylobacterium*, *Pantoea*, and *Sphingomonas*. Among them, *B. cereus*, *P. agglomerans* were detected and quantified by qRT-PCR. In addition, we observed the influence of Enterohemorrhagic *Escherichia coli* (EHEC) infection on shift in young-radish kimchi microbiota during storage at 4°C. The proportion of *Escherichia* decreased over time (0.04% to 0.00% in non-infection group, 11.00% to 0.00% in infection group), and the fermentation of the infection group was slower compared to the control group. This study can be used to better understand the young-radish microbiota and provides insights into the role of storage conditions in young-radish kimchi product management.

H002

Effects of Temperature on Bacterial Communities and Physicochemical Properties during Milk Storages

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Control of microbial milk spoilage is economically important to the dairy industry. To investigate the effects of temperature on milk storages, the total bacterial counts and community and physicochemical analysis, such as pH, titratable acidity and sugar content, were monitored during milk storages at 5, 10, 15, 20 and 25°C with replicate (n=5). As a result of physicochemical analysis, the pH ranged from 6.28 to 6.82, the titratable acidity were 0.14% to 0.29%, and the sugar content was 8.3~13.9. The bacterial communities were analyzed using Illumina miseq based on 16S rRNA gene. Bacterial community analysis revealed that *Proteobacteria* were dominant in early stage of storage, but they were displaced by *Firmicutes* and *Actinobacteria* at the end of storage at the phylum level. At the genus level, *Ralstonia* and *Bacillus* were dominant in initial milk, and *Baillus*, *Pseudomonas* and *Rhodococcus* became dominant as storage progressed. Interestingly, although the identical milk from a company was stored at the same conditions, each milk exhibited different patterns of milk spoilage. For example, among five milks stored at 10°C, three milks showed the spoilage in 12 days by dominant of *Baillus*, the spoilage was not revealed to others during 30 days. Additionally in this study the relationships between microbial communities, physicochemical parameters and milk spoilage will be more discussed.

H003

Impedance Characteristics and Polarization Behavior of a Microbial Fuel Cell in Response to Short-term Changes in Medium pH

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pH oppositely influences anode and cathode performance in microbial fuel cells. The differential electrochemical effects at each electrode and the resultant full-cell performance were analyzed in medium pH from 6.0 to 8.0. Potentials changed -60 mV/pH for the anode and -68 mV/pH for the cathode, coincident with thermodynamic estimations. Open circuit voltage reached a maximum (741 mV) at pH 7, and maximum power density was highest (712 mW/m²) at pH 6.5 as the cathode performance improved at lower pH. Maximum current density increased and apparent half-saturation potential (E_{KA}) decreased with increasing medium pH due to improved anode performance. An equivalent circuit model composed of two time constant processes accurately fit bioanode impedance data. One of these processes was consistently the rate-limiting step for acetate-oxidizing exoelectrogenesis, with its pH-varying charge transfer resistance R_2 ranging from 2- to 321-fold higher than the pH-independent charge transfer resistance R_1 . The associated capacitance C_2 was 2–3 orders of magnitude larger than C_1 . R_2 was lowest near E_{KA} and increased by several orders of magnitude at anode potentials above E_{KA} , while R_1 was nearly stable. However, fits deviated slightly at potentials above E_{KA} due to emerging impedance possibly associated with diffusion and excessive potential.

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H004

Comparison of Anode Bacterial Communities Performance in Microbial Fuel Cells with Different Electron Donors

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Microbial fuel cells (MFCs) harness the electrochemical activity of certain microbes for the production of electricity from reduced compounds. Characterizations of MFC anode biofilms have collectively shown very diverse microbial communities, raising ecological questions about competition and community succession within these anode-reducing communities. Based on 16S rDNA-targeted denaturing gradient gel electrophoresis, all anode communities contained sequences closely affiliated with *Geobacter sulfurreducens* and an uncultured bacterium clone in the Bacteroidetes class. Various other *Geobacter*-like sequences were also enriched in most of the anode biofilms. While the anode communities in replicate reactors for each substrate generally converged to a reproducible community, there were some variations in the relative distribution of these putative anode-reducing *Geobacter*-like strains. Firmicutes were found only in glucose-fed MFCs, presumably serving the roles of converting complex carbon into simple molecules and scavenging oxygen. The maximum current density in these systems was negatively correlated with internal resistance variations among replicate reactors and, likely, was only minimally affected by anode community differences in these two-chamber MFCs with high internal resistance.

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H005

Influence of External Resistance on Electrogenesis, Methanogenesis, and Anode Prokaryotic Communities in Microbial Fuel Cells

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The external resistance (R_{ext}) of microbial fuel cells (MFCs) regulates both the anode availability as an electron acceptor and the electron flux through the circuit. We evaluated the effects of R_{ext} on MFCs using acetate or glucose. The average current densities (I) ranged from 40.5 mA/m² to 284.5 mA/m² for acetate-fed MFCs (ARs), with a corresponding anode potential (E_{an}) range of -188 to -4 mV. For glucose-fed MFCs (GRs), I ranged from 40.0 mA/m² to 273.0 mA/m², with a corresponding E_{an} range of -189 to -7 mV. ARs produced higher Coulombic efficiencies and energy efficiencies than GRs over all tested R_{ext} levels. Biogas production accounted for 14 to 18% of electron flux in GRs but only 0 to 6% of that in ARs. However, total methane production in ARs increased as R_{ext} increased, suggesting that E_{an} might influence the competition for substrates between exoelectrogens and methanogens in ARs. An increase of R_{ext} to 9,800 Ω significantly changed the anode bacterial communities for both ARs and GRs, while operating at 970 Ω and 150 Ω had little effect. The anode-methanogenic communities were dominated by *Methanosaetaceae*, with significantly lower numbers of *Methanomicrobiales*. These results show that R_{ext} affects not only the E_{an} and current generation but also the anode biofilm community and methanogenesis.

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H006

Influence of Flowrates to a Reverse Electro-Dialysis (RED) Stack on Performance and Electrochemistry of a Microbial Reverse Electrodialysis Cell (MRC)

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An MRC is a bioelectrochemical system combining a microbial fuel cell (MFC) with a RED stack to generate electricity from salinity gradient and organic wastewater with simultaneous treatment. Operating an MRC at an optimum flowrate to RED is important because it is closely related with energy production rate and economic feasibility. However, influence of RED flowrates on MRC electrochemistry and power production have not been investigated. For this purpose, four different flowrates of high concentration and low concentration solutions were tested. Maximum power density was highest in 10 ml/min (3.71 W/m^2) and optimum current density was highest in 7.5 ml/min (5.36 A/m^2). By mere increasing the flowrate to MRC, maximum power and optimum current densities increased by 17.7% and 16.2%. EIS showed that impedances of anode, cathode and full-cell were decreased by 51%, 31% and 19%, respectively. Anode CV showed that peak current density was increased by 25.7%. COD removal and CE were not affected by RED flowrate. Power generation at 7.5 ml/min and 10 ml/min were not so different, but current production was better at 7.5 ml/min. Therefore, considering energy production, the RED flowrate of 7.5 ml/min is a reasonable choice for MRC operation.

[This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1A2C1013989).]

H007

Improved Structures of Stainless-steel Current Collector Increase Power Generation of Microbial Fuel Cells by Decreasing Cathodic Charge Transfer Impedance

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Microbial fuel cell (MFC) is an innovative environmental and energy system that converts organic wastewater into electrical energy. For practical implementation of MFC as a wastewater treatment process, a number of limitations need to be overcome. Improving cathodic performance is one of major challenges, and introduction of a current collector can be an easy and practical solution. In this study, three types of current collectors made of stainless steel (SS) were tested in a single-chamber cubic MFC. The three current collectors had different contact areas to the cathode (P 1.0 cm²; PC 4.3 cm²; PM 6.5 cm²) and increasing the contacting area enhanced the power and current generations and coulombic and energy recoveries by mainly decreasing cathodic charge transfer impedance. Application of the SS mesh to the cathode (PM) improved maximum power density, optimum current density and maximum current density by 8.8%, 3.6% and 6.7%, respectively, comparing with P of no SS mesh. The SS mesh decreased cathodic polarization resistance by up to 16%, and cathodic charge transfer impedance by up to 39%, possibly because the SS mesh enhanced electron transport and oxygen reduction reaction. However, application of the SS mesh had little effect on ohmic impedance.

[This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1A2C1013989).]

H008

Effects of Vertical and Horizontal Configurations of Different Numbers of Brush Anodes on Performance and Electrochemistry of Microbial Fuel Cells

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To maximize wastewater treatment and energy production by microbial fuel cells, it is important to design the optimal anode arrangement. In this study, four brushes were tested horizontally or vertically to the cathode as the number of the anodes increased from one to four. In the horizontal configuration, adding the anodes greatly reduce electrode resistance and enhanced cell performance, showing four anodes (H4) was the best. In the vertical configuration, two anodes (V2) showed greatest performance and greatest decrease in anode resistance. Cathode resistance was relatively constant, showing adding anodes had negligible effect on it. Because diffusion resistance increases with increasing distance between an anode and a cathode, the vertical anodes should have different diffusion resistance and performances. In this study, adding more anodes vertically decreased cell performance. However, in a cyclic voltammetry test, current production was substantially increased when the third and the fourth brush anodes were introduced in the both arrangements. It shows that the external electrical input relieved diffusion resistance and increased current generation and that installing anodes away from the cathode is a good strategy to increase current production in a system with external power supply such as microbial electrolysis cell. [This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1A2C1013989).]

H009

Effects of Wire-type and Mesh-type Anode Current Collectors on Performance and Electrochemistry of Microbial Fuel Cells

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Carbon-based material is commonly used for anodes in MFCs, but its low conductivity often limits anodic performance. Application of corrosion-resistive current collector to carbon-based anode can be a promising strategy for increasing the anodic performance. In this study, it was hypothesized increasing metal current collector improved anodic performance. Two different carbon-felt anodes with titanium wires (CF-W) or stainless steel mesh (CF-M) as a current collector were tested in a single chamber MFC. In the short-term tests such as polarization and impedance tests, CF-M with the larger current collector area (21.7 cm^2) had 33% higher maximum power (2311 mW/m^2), 81% lower anodic resistance (3Ω), and 92% lower anodic impedance (1.1Ω). However, in the long-term tests, CF-W with the smaller current collector area (0.6 cm^2) showed higher performance in power and current generation, COD removal, and CE (51%, 10%, 11%, and 5% higher, respectively) and produced 41% higher net current in cyclic voltammogram (20.0 mA vs. 14.2 mA). This result shows that larger current collector is advantageous in short-term performance and disadvantageous in long-term performance, because the larger current collector is good for current collection, but interferes with mass transfer and microbial growth. [This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1A2C1013989).]

H010

Establishing the Stable Cell Performance Time and Anode Maturation Time of a Single Chamber Microbial Fuel Cell

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In microbial fuel cells (MFCs), to obtain accurate and reproducible experimental results, it is important to determine the 'anode maturation time'. In this study, four single-chamber MFCs were tested to know when the cell can produce stable and maximum performance. According to the linear sweep voltammetry polarization tests, three MFCs could obtain stable maximum power densities after 9 weeks. Average maximum power densities from the 9th to the 17th week were highest in MFC-4, followed by MFC-2, MFC-3 and MFC-1. Polarization resistance showed that MFC-1 had much larger anode resistance than the other MFCs. Possibly due to the bad inoculation, MFC-1 showed the lowest performance with the highest anode resistance. Anodic cyclic voltammetry showed that current production increased by time and MFC-1 had much smaller current production than the other MFCs in the 17th week. Current production enhancement indicated anode biofilm became more mature by time, but overall cell performance did not increase accordingly. However, in the 17th week, anode resistance of MFC-1 was reduced by 47%, resulting in cell performance improvement. This study showed that the stable cell performance of a single chamber MFC with a brush anode was 9 weeks. Nevertheless, anode needed more than 17 weeks to attain the mature state.

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H011

Performance Enhancement of a Microbial Fuel Cell by Physico-chemical Treatments of Activated-carbon Catalyst of an Air Cathode

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Improvement of electrode technology is very important for the practical use of Microbial fuel cell (MFC). In this study, for developing practical cathode catalyst technology, physicochemical modified activated carbon (AC) catalysts and platinum were tested for performance and electrochemical characterization in an MFC under the same condition, potentially to replace expensive platinum catalysts. Comparing with a maximum power density of a platinum-coated cathode (976 mW/m^2), a Co-N-C/AC cathode made with activated carbon doped with cobalt and 1,10-phenanthroline at 800°C produced 1526 mW/m^2 in the MFC condition, which was 56% higher than the Pt-coated cathode. A 500AC cathode made with heat-treated activated carbon at 500°C produced 1394 mW/m^2 and non-treated activated carbon cathode (Plain AC) produced 1014 mW/m^2 . The tested activated-carbon electrodes showed electrochemical performance and power production superior to the Pt-coated cathode. Electrochemical performance of cathodes was increased as more physico-chemical treatments were added to activated carbon catalysts. Cathode impedance results showed that enhanced electrochemical performance was attributed to decrease of cathode charge transfer resistance, possibly due to the physical-chemical modification of activated carbon and the catalyst change.

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H012

Addition of Reduced Graphene Oxide to an Activated-carbon Cathode Increases Electrical Power Generation of a Microbial Fuel Cell by Enhancing Cathodic Performance

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Activated carbon (AC) is an inexpensive catalyst for oxygen reduction in an air cathode of microbial fuel cells (MFCs). In the AC-based cathode, carbon black (CB) is used as a conductive supporting material. In this study, it was hypothesized cathodic performance would increase if reduced graphene oxide (rGO) replaces CB in an optimum ratio. rGO replaced CB in the four different weight ratios of rGO to CB: 0:30 (rGO0); 5:25 (rGO5); 15:15 (rGO15); 30:0 (rGO30). Maximum power density was the best in rGO15 (2642 mW/m²) followed by rGO5 (2142 mW/m²). In the optimum external resistance operation, rGO5 and rGO15 showed similar power (~1060 mW/m²), higher than the others. Linear sweep voltammetry, cyclic voltammetry, and impedance spectroscopy also showed that the optimal rGO additions improved cathodic performance and reduced cathodic internal resistance. Due to the flatter and wider shape of rGO and 5 times higher electrical conductivity than CB, the rGO addition improved the cathodic performance, but the complete replacement of CB with rGO decreased the cathodic performance due to the increased thickness and the morphological crack. The optimum rGO addition is a simple and effective method for improving cathodic performance.

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H013

Characterization of Impedance and Polarization of Carbon-felt Bio-anodes and Activated-carbon Cathode in a Continuous Flow Microbial Fuel Cells

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Compared to the development of Microbial fuel cell (MFC) technology, however, understanding of its electrochemical characterization is still insufficient. The main reason is that its electrochemical analysis is very difficult due to the complex nature of the anode biofilm, which is a key to generating electricity. In this experiment, the influence of the measurement potential of impedance and the scanning rate for polarization curve on the MFC electrochemistry was investigated. The experiment was performed after stabilizing the system for accurate measurement. Unlike the previous batch tests showing the lowest anodic impedance at -400 mV vs. Ag/AgCl, the anodic impedance decreased and the current production increased as the anode potential increased up to +5.7 mV vs. Ag/AgCl in the continuous flow MFC. The polarization curves were produced at two scanning rates (1 and 0.1 mV/s) in a continuous mode, and those electrochemical data were comparatively analyzed. When it is difficult to maintain a steady state for a long time in an MFC, it will be possible to produce polarization curves in a short time with a faster scanning rate. When performance analysis is needed, the comparative analysis would be possible among the data at different conditions through extrapolation.

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H014

Reproducibility Evaluation of Linear Sweep Voltammetry Tests of Electrodes in Microbial Electrolysis Cells

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Electrode is a key component in a microbial electrolysis cell (MEC) and it needs significant improvement for practical implementation of MEC. For effective development of electrode technology, accurate and reproducible analytical methods are very important. Linear sweep voltammetry (LSV) is an essential analytical method for evaluating electrode performance. In this study, biological brush (BB), abiotic brush (AB), Pt wire (PtW), stainless steel wire (SSW) and mesh (SSM) were tested to explore the most suitable counter electrode in different medium conditions. Coefficient of variation (Cv) for I_{\max} of LSV was comparatively analyzed. In BB-anode LSV, SSW (0.45%) and SSM (0.48%) showed higher reproducibility as a counter electrode. Reproducibility of anode LSV test was good in stainless steel wire and mesh as counter electrode. It shows electrode used in the operation is an appropriate counter electrode in the acetate-added condition. However, in the absence of acetate, PtW (1.24%) and BB (1.71%) produced more reproducible results in SSM cathode and PtW (0.61%) and SSW (1.21%) did in the Ni-AC-SSM-cathode, showing PtW is an appropriate counter electrode. Reproducibility evaluation results in this study suggest that counter electrodes can be set according to the various conditions in anode and cathode LSV test.

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H015

Improvement of Air Cathode Performance in Microbial Fuel Cells by Using Catalysts Made by Binding Metal-organic Framework and Activated Carbon through Ultrasonication and Solution Precipitation

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Activated carbon (AC) is an inexpensive catalyst for the oxygen reduction reaction (ORR) in the air cathode of microbial fuel cells (MFCs). However, since the electrochemical catalytic activity of AC is poor, it is necessary to improve its performance. The metal-organic framework (MOF) is composed of a metal ion and an organic linker. It has high porosity and high electrochemical catalytic activity. Herein, ZIF-67 was combined with activated carbon through ultrasonication (U) and solution precipitation (H), which was used to make ZIF-67U and ZIF-67H cathodes, respectively. In maximum power density, ZIF-67U cathode produced 4203 mW/m², and ZIF-67H did 3881 mW/m², which is 60% and 48% higher than AC cathode (2625 mW/m²) and 160% and 140% higher than Pt cathode (1614 mW/m²), respectively. In atomic nitrogen contents of catalyst surface, pyridine-N was 28% in ZIF-67U and 38% in ZIF-67H, respectively; pyrrole-N was 56% in ZIF-67U and 25% in ZIF-67H, respectively; no nitrogen was detected in AC. These cobalt-nitrogen increased the active site of the ORR, improved the reaction rate, and decreased charge transfer impedance. AC and ZIF-67 were bonded using ultrasonication and tested in the MFC for the first time, producing the highest power ever among the MOFs in the 50-mM phosphate-buffer-saline condition so far. [This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1A2C1013989).]

H016

Comparative Evaluation of Performance and Electrochemistry of Microbial Fuel Cells with Different Anode Structures and Materials

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Various materials and anode structures have been applied to enhance microbial fuel cell (MFC) performance. However, their comparative evaluation of performance and electrochemistry has not yet been investigated in detail under a same condition. In this study, a carbon-cloth anode, an anode-cathode assembly, and a brush anode with two different orientations were tested under a same condition for comparative analyses on their performance and electrochemistry, in order to reveal their unique electrochemical characteristics. The brush anode cells exhibited better performance than the carbon cloth cells. The brush anodes showed 41-72% higher maximum power densities, 18-75% higher maximum current density and 24-32% higher optimum current densities than the carbon cloth anodes. The brush anodes showed 25-43 Ω lower anodic polarization resistance than the carbon cloth anodes. The brush anodes showed 1.6-21.2 Ω lower ohmic impedance, 7.7-10.6 Ω lower charge transfer impedance and 9.3-31.8 Ω lower anodic impedance than the carbon cloth anodes. Anodic ohmic impedance was greatest in the carbon-cloth-anode MFC (21.9 Ω), where loose contact between a carbon cloth and a current collector might cause the high ohmic resistance, and large solution resistance in the cell could diminish anode performance due to slow ion transport.

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H017

Comparison of Hydrogen Production and System Performance in a Microbial Electrolysis Cell Containing Cathodes Made of Non-platinum Catalysts and Binders

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Microbial electrolysis cell (MEC) is an innovative electrochemical technology that decomposes organic matter in anode and produces hydrogen in cathode. It is imperative to use a high-performance and a low-cost cathode material to make the application of MEC economically viable. In this study, five different cathodes made of low-cost materials were tested in MECs. The materials included activated carbon (AC) and nickel powder (Ni) as a cathode catalyst; polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (PVDF) as a catalyst binder; stainless steel mesh (SSM) as a cathode substrate or a cathode itself. Among the tested cathodes, Ni/AC/PTFE obtained the best performance, followed by Ni/AC/PVDF, AC/PVDF, flamed-oxidized SSM (SSM/F) and SSM. Ni/AC/PTFE exhibited the best performance in hydrogen production rate (HPR, 1.88 L/L d), hydrogen purity (97.5%), coulombic efficiency (124%), energy efficiency (216%), cathodic capacitance (0.9924 F), along with the lowest cathodic impedance (35 Ω). The worst performed SSM showed as follows: 0.57 L/L d of HPR, 71% of hydrogen purity, 36% of coulombic efficiency, 62% of energy efficiency, 0.0008 F of cathodic capacitance and 62 Ω of cathodic impedance. This study shows quantitatively the electrochemical and performance transitions of MEC according to the cathode component changes.

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H018

Effects of Brush-anode Configurations on Performance and Electrochemistry of Microbial Fuel Cells

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For practical implementation of MFC, increasing power generation is important because it is closely related with energy production rate and wastewater treatability. However, it is not known which relative arrangement of anode and cathode gives the best performance, and it is necessary to know electrochemical reference point of the brush anode for this. Various configurations, materials and anode structures were tested in a single-chambered cubic MFC. By merely changing a brush anode configuration, power and current densities were increased by 20% and 30%, respectively. The horizontally-positioned anode configuration (H1) with the closest anode-cathode distance produced the highest power and current. EIS showed that anode impedance and full-cell impedance were decreased by 60% and 49%, respectively. Coulombic efficiency (CE) and energy efficiency (EE) were not significantly affected by the anode-cathode distance, but the horizontal type cells showed relatively higher CE, EE and COD removal rate and shorter batch time. The center of a titanium current collector and the center of carbon fibers of a brush-anode were found to be statistically-significant reference points for MFC electrochemistry.

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H019

Anti-inflammatory Effects of *Lactobacillus fermentum* PL9988 and Its Characteristics as Probiotics

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Lactobacillus fermentum PL9988 was isolated from Korean who were over 80 years old in Korean longevity villages. The previous studies showed that *Lactobacillus fermentum* PL9988 was resistant to acid and bile acid, and showed ability to enhance immune-enhancing activity, adhesiveness to the intestinal cell line Caco-2, adhesiveness to intestines in *in vivo* experiment, adhesiveness to human, and inhibit intestinal pathogens. And *Lactobacillus fermentum* PL9988 was susceptible to various antimicrobials. In this study, *Lactobacillus fermentum* PL9988 was tested for its binding ability to HT-29 cells and for its anti-inflammatory effects with *in vitro* and *in vivo* experiments. When HT-29 cell line was treated with LPS in the presence of *Lactobacillus fermentum* PL9988, secretion of IL-10, which is an anti-inflammatory cytokine, was increased while TNF- α , which is an inflammatory cytokine, was decreased in a dose-dependent manner. Results from *in vivo* experiment with BALB/c mice showed the same results from *in vitro* experiment. Results from the previous study and this study showed beneficial characteristics of *Lactobacillus fermentum* PL9988 as a good probiotic which has immune-enhancing activity, improving the intestinal health as well as anti-inflammatory activity.

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H020

Evaluation of *Abeliophyllum distichum* Nakai Extract on Wound Healing Ability in ICR Mouse and Antibacterial Activity against Human Cutaneous Flora

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In this study, we evaluated wound healing rate, inflammatory cells effects of *Abeliophyllum distichum* Nakai (ADN) extract in mice. We also test stability of ADN extract by exposure to sunlight. Treatments were as follow: 1) CON (only saline solution), T1 (CON + 0.0125% ADN extract), T2 (CON + 0.05% ADN extract), T3 (CON + 0.5% ADN extract). 4mm punch (KEYES, Pakistan) was used in the central part of the dorsal area to separate it to the subcutaneous tissue, causing full-thickness skin wound. 1ml of sample by treatment section was sprayed on the wound with a pipette every day from the day of the wound creation was applied using brush. In the stability test, the pH was measured at the 1, 4, and 8 weeks by exposing the samples for each treatment section to sunlight. the higher concentration of the ADN extract. Result of this study indicated that the wound contraction rate of the mice to which ADN extract was applied was low effective, and the stability of the sample containing the high concentration of ADN extract was not verified. In addition, no significant results were obtained in the inflammatory reaction. Therefore, additional research on wound contraction, stability and inflammatory cell of the ADN extract is needed.

H021

The Occurrence of CTX-M–producing *E. coli* in the Broiler Parent Stock in Korea

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A number of antimicrobials are used for the treatment of bacterial infections, and the emergence of antimicrobial-resistant *Escherichia coli* (*E. coli*) in livestock and the transfer of resistant isolates to humans poses a serious risk to public health. In particular, broiler parent stock can transfer antimicrobial-resistant bacteria and drug-resistance genes to chicks. This study was conducted to investigate the prevalence and characteristics of 3rd-generation cephalosporin-resistant and ESBL-producing *E. coli* from the broiler parent stock in Korea. Among 51 cefotaxime-resistant *E. coli* isolates, 45 (88.2%) were identified as multidrug resistant and 21 showed phenotypic and genotypic CTX-M–producing characteristics. The CTX-M-14, CTX-M-15, CTX-M-1, and CTX-M-1 were detected in 10, 7, 3, and 1 isolates, respectively. ISEcp1 or IS261 ISEcp1 were identified upstream of all CTX-M–type genes, and orf477 and IS903 were detected downstream of 9 and 10 CTX-M–type genes, respectively. Thirteen (61.9%) of the 21 CTX-M–producing *E. coli* isolates harbored class 1 integrons with 4 different gene cassette arrangements. Among the plasmid replicons, CTX-M-1 was located on I1, F, and FIB; CTXM- 14 on F and FII; CTX-M-15 on FII, FIA, and FIB; and CTX-M-65 on FIB. The results indicate that surveillance and monitoring systems in broiler parent stock farms are necessary.

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H022

Molecular Characterization of Fluoroquinolone-resistant *Escherichia coli* from Broiler Breeder Farms

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Fluoroquinolones (FQs)-resistant *E. coli* from broiler breeders vertically transmits to commercial chicken. This study investigated the phenotypic and genotypic characteristics of FQ-resistant *E. coli* isolates from broiler breeder farms. A total of 106 FQ-resistant *E. coli* isolates had mutations in quinolone resistance determining regions; all (100%) had mutations in *gyrA*, 89 (84.0%) had mutations in *parE*, 8 (7.5%) showed the mutations with *parC* and *parE*, and none had *gyrB* mutations. The prevalent mutation type was double mutation in *gyrA*, and all FQ-resistant *E. coli* isolates that had mutations in *parC* or *parE* also had double mutations in *gyrA*. Especially, FQ-resistant *E. coli* isolates which possessed double mutations in *gyrA* and *parC* or single mutations in both *parC* and *parE* showed high levels of MIC. Of the 23 plasmid-mediated quinolone resistance (PMQR)-positive *E. coli* isolates, *qnrS* was detected in 10 (9.4%) isolates, followed by *qnrA* (6.6%), *qnrB* (3.8%), and *aac(6'')-Ib-cr* (1.9%). 16 (69.6%) of the 23 PMQR-positive *E. coli* isolates harbored class 1 integron with four different gene cassette arrangements and 9 plasmid replicon types were identified in 23 PMQR-positive *E. coli* isolates. The result supports that monitoring at the broiler breeder level are required to prevent the pyramidal transmission of FQ-resistant *E. coli*.

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H023

Genetic Characterization of Fluoroquinolone Resistance in *Salmonella enterica* Serovar Gallinarum Isolates from Chicken in Korea

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Salmonella Gallinarum (*S. Gallinarum*) causes fowl typhoid (FT). To control FT in commercial chickens, fluoroquinolones (FQs) are widely used in Korea. This study aimed to investigate the genetic characteristics of FQs-resistant *S. Gallinarum* isolates from chicken. A total of 35 ciprofloxacin (CIP)-resistant *S. Gallinarum* was tested, and 22 (62.9%) isolates were multidrug resistant. All isolates had a mutation in the *gyrA* gene, whereas three isolates had only double mutations. MICs of isolates with double mutations were relatively higher than those of other isolates with single mutation in *gyrA*. Among 35 CIP-resistant *S. Gallinarum*, plasmid-mediated quinolone resistance genes were detected in 6 (17.1%) isolates, and *qnrB* and *qnrS* were detected in 4 and 2 isolates, respectively. In 35 CIP-resistant *S. Gallinarum*, *ant(2'')-I* (54.3%) was the most prevalent, followed by TEM-1 (14.3%), *sul1* (11.4%), and *cmIA* (5.7%). 15 (42.9%) of the 35 CIP-resistant *S. Gallinarum* also carried class 1 integron, which showed 5 resistance gene cassette types: *aadA2* (7), *aadA+dfxA12* (5), and *aadA1+aadA2* (3). 23 isolates (65.7%) carried five different plasmid replicons: Frep (9 isolates), FIB (7 isolates), FIIA (6 isolates), B/O (4), and I1 (3 isolates). Therefore, monitoring of FQ resistance is necessary in poultry to prevent the transmission to humans through the food chain.

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H024

Comparative Pathology of Domestic Pigs with Highly Virulent African Swine Fever Virus Strain from Vietnam

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African swine fever (ASF), caused by the ASF virus (ASFV), is one of the most important and serious threats to the pig industry today. This study was focused on the comparison of the histopathological lesions in experimentally ASF-inoculated pigs depending on the death period. A total 10 pigs (8-week-old) were inoculated with highly virulent ASFV ("VNUA/HY/Vietnam", $10^{3.5}$ HAD₅₀/ml). *Post-mortem* examinations were conducted for four pigs with different death period. For histopathological analyses, all samples were fixed and routinely processed for paraffin embedding. Five micron sections were conducted and routinely stained with hematoxylin and eosin (H&E) for light microscopy examination. For immunohistochemical (IHC) detection of ASFV antigen in tissue sections, viral protein p72 of ASFV was performed. The pigs died at 7–8 day-post-inoculation (dpi) showed the sever necrosis lesions in spleen and the moderate congestion in inguinal lymphnode. The ASF-antigen labelled cells were more detected in various organs from death pigs at 7–8 dpi than 5–6 dpi. Our histopathological findings showed different results depending on the death period of infected pigs, which could support to the interpretation of the pathological findings in ASF diagnosis.

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H025

Antimicrobial Resistance Monitoring of Commensal *Enterococcus faecalis* in Broiler Breeders

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Enterococcus faecalis (*E. faecalis*) has rapidly acquired resistance to multiple antimicrobials, and the antimicrobial resistance of *E. faecalis* from broiler breeders vertically transmits to commercial chicken. The aim of this study was to investigate the antimicrobial resistance and genetic diversity of commensal *E. faecalis* from the broiler breeder farms. Among a total of 229 *E. faecalis* isolates from 9 broiler breeder farms, the resistance rate to tetracycline was the highest (78.2%), followed by doxycycline (58.1%) and erythromycin (43.7%), and the antimicrobial resistance prevalence were significantly different among the 9 broiler breeder farms ($P < 0.05$). The *tetM* (77.1%) and *ermB* (85.0%) were detected at the highest levels in 179 TE- and 100 E-resistant isolates, respectively. 24 high-level gentamicin-resistant isolates carried *aac(6'')Ie-aph(2'')-Ia*, and 9 high-level ciprofloxacin-resistant isolates showed point mutations in both *gyrA* and *parC*. All high-level gentamicin-resistant or high-level ciprofloxacin-resistant isolates showed one of the two different virulence gene patterns, *ace-asa1-efaA-gelE* complex or *ace-efaA-gelE* complex. These results indicate that epidemiological monitoring at the breeder level is required to prevent the pyramidal transmission of antimicrobial-resistant *E. faecalis*.

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H026

Monitoring and Characteristics of Major Mastitis Pathogens from Bulk Tank Milk in Korea

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In many countries, bulk tank milk (BTM) has been used for examining milk and analyzed as an important part of milk quality assurance programs. The objectives of this study were to investigate milk quality and the presence of major mastitis pathogens in BTM, and to compare the characteristics of BTM by dairy factory or company. A total of 1588 batches of BTM samples were collected from 396 dairy farms of seven dairy factories owned by four companies in Korea. The means of individual bacterial counts (IBC) and somatic cell count (SCC) were 3.7×10^4 cells/ml and 1.1×10^5 cells/ml, respectively, and no significant differences among dairy factories were observed. The most common pathogen was *Staphylococcus* spp. (60.1%), followed by *E. faecalis* (53.8%), *E. coli* (37.6%) and *Streptococcus* spp. (22.5%). *Enterococcus* spp. showed the highest resistance to tetracyclines (51.1% to 73.9%) and macrolides (46.5%). *S. aureus* and coagulase-negative staphylococci (CNS) showed the highest resistance to penicillin (28.4% and 40.2%, respectively), and three (3.2%) *S. aureus* and seven (3.3%) CNS were also methicillin-resistant. These data show the diverse prevalence and characteristics of major mastitis pathogens among factories, and support the development of strong monitoring and prevention programs of mastitis pathogens by commercial dairy operations.

H027

Blood Counts and Biochemical Parameters in Pigs Infected Experimentally with the African Swine Fever Virus Isolates from Vietnam

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African swine fever (ASF), caused by the ASF virus (ASFV), is a fatal disease causing serious economic losses in the pig industry. Despite extensive research on this disease, there is no report to evaluate the complete blood count (CBC) and serum biochemical parameters in ASFV-infected pigs. The study aimed to investigate the impact of ASF on blood parameters in experimentally viral inoculation pigs. Blood samples were collected daily at 0–8 days post-inoculation. Viral DNAs were extracted from the whole blood, and viremia was assessed by ASFV DNA detection in the blood sample. CBC and biochemistry parameter were analyzed by an automated haematology analyser (Mindray, China). The numbers of WBCs were dramatically increased until 5 days post viremia (dpv). During viremia, a tendency of a decreased in the number of RBCs, hemoglobin concentration, and platelet counts was identified. The ALT/AST ratio were showed > 1 after 3 dpv in infection group. A slightly increase level of the serum creatine and urea was recorded in ASFV-infected groups after viremia. The overall results could provide the information of ASFV ability to develop an inflammation process, which resulted in various changes in blood counts and biochemical parameters.

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H028

Genetic Characterization of High-level Aminoglycoside-resistant *Enterococcus faecalis* and *Enterococcus faecium* Isolated from Retail Chicken Meat

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Retail chicken meat can transfer drug resistance to humans through meat contaminated with resistant enterococci. High level aminoglycoside-resistance (HLAR) in enterococci emerged in human cases. Therefore, the prevalence and genetic traits of HLAR enterococci in retail chicken meat were investigated in this study. Among 345 enterococci strains, 29 (8.7%) showed HLAR. All HLAR in enterococci carried at least 1 of 2 aminoglycoside-modifying enzyme genes, *aac(6')Ie-aph(2'')-Ia* and *ant(6)-Ia*. Among the 13 isolates carrying *aac(6')Ie-aph(2'')-Ia*, 3 had pattern A, and the other 10 had pattern D (with or without IS256 at both ends). All HLAR enterococci also showed multidrug resistance. Among the 24 erythromycin-resistant isolates, 19 (79.2%) harbored *ermB*, and 1 (4.2%) harbored both *ermB* and *ermA*. A total of 21 enterococci were tetracycline-resistant and harbored one or more of tetracycline resistance genes. The Int-Tn gene was detected in 1 isolate (3.4%) carrying *tet(M)* and *ermB*. All 4 chloramphenicol-resistant isolates carried either phenicol resistance gene *cfr* alone (1 isolate), both *cfr* and *fexA* (1 isolate), or both *fexA* and *optrA* (2 isolates). Four efflux pump genes, *efr(A)*, *efr(B)*, *emeA*, and *Isa*, were detected in all HLAR in *E. faecalis* isolates. The results improve our understanding of the HLAR in enterococci from non-hospital sources to humans.

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H029

Comparative Study of Cell-selective Radiation Protecting Effect of Metabolite Constituents of *Kalopanax pictus*

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The stem-bark of *Kalopanax pictus* (KP, family Araliaceae) has been used for inflammation in China as a medicament. The anti-inflammatory efficacy of KP is well known, and recently the anti-cancer efficacy of saponin constituents as kalopanaxsaponin A and B have been investigated. When the extract of KP is ingested orally into the human body, it is metabolized to kalopanaxsaponin B, A and hederagenin by intestinal microflora. In this study, I conducted a comparative study on the anti-cancer effect of KP metabolite constituents. Lu-177 was treated in peritoneal macrophages and A549 cells to induce cell-death, and metabolites were further treated to compare viability. As a result, metabolites inhibited cell-death by radiation from Lu-177 in peritoneal macrophages, unlike in A549 cells. And, kalopanaxsaponin A showed the highest inhibitory ability than other metabolites.

H030

Study of Enteric Muscle Contraction through Excitatory GABA-gated Channel in Microbiome

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The enteric muscle contraction is the last step of the defecation behavior which occurs every 50 s in *Caenorhabditis elegans*. This enteric muscle contraction is regulated by intestinal and anal depressor muscles, which are innervated by GABA motor neurons. In here, we show that target chemicals are expressed in intestinal muscle and anal depressor muscle, and the gain-off function mutant, shows defects in enteric muscle contractions. In addition, the intracellular region of EXP-1, an excitatory GABA receptor, specifically binds to chemicals. This interaction between UNC-49 and EXP-1 appears to be independent of both target chemicals and excitatory GABA-gated channel in nematode. This results suggests that our target chemicals functions as a negative regulator of excitatory GABA receptor in GABA signaling in *C. elegans*. Gamma-aminobutyric acid (GABA) mediates fast inhibitory neurotransmission by activating anion-selective ligand-gated ion channels. Although electrophysiological studies indicate that GABA may activate cation-selective ligand-gated ion channels in some cell types, such a channel has never been characterized at the molecular level. We conclude that some of the excitatory functions assigned to GABA are mediated by cation channels rather than by anion channels.

H031

Analysis of the Microbiota of Farm-raised and Wild-caught Dotted Gizzard Shad (*Konosirus punctatus*) in South Korea

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Dotted gizzard shad (*Konosirus punctatus*) is a species of brackish water fish which widely consumed raw in Asian countries. Two different origins of products (farm-raised; FR, and wild-caught; WC) of 40 dotted gizzard shads in September and November were used. V3-V4 region of 16S ribosomal RNA genes from each bacterial DNA were amplified. Average 69229.88 sequencing reads were obtained. Total bacterial loads of FR (1.04×10^7) was lower than WC (1.61×10^8). Alpha diversity shows significant difference on Shannon (2.68; FR, 5.34; WC) and Chao 1 (274.37; FR, 428.00; WC) indices. Proteobacteria (71.30% and 85.10%), Bacteroidetes (27.80% and 7.10%) and Firmicutes (0.59% and 7.55%) were predominant phyla at both FR and WC. The top 3 predominant family were Moraxellaceae, Flavobacteriaceae and Pseudomonadaceae in FR, whereas Vibrionaceae, Pseudoalteromonadaceae and Shewanellaceae in WC. Potential pathogenic genera such as *Psychrobacter*, *Flavobacterium*, *Pseudomonas*, *Shewanella*, *Vibrio*, and *Photobacterium* were found. Within those genera *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* were quantified by qRT-PCR, but only *V. parahaemolyticus* was quantified in WC (80.00%, 4.48×10^2 mean CFU/g). Although further studies are needed, this study can be used to improve the food safety of seafoods.

H032

Changes in Expression of Nrf2 and Antioxidant Enzymes during Corticotropin-releasing Hormone-induced Stress in Dermal Papilla Cells

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There is a growing interest in the relationship between stress hormones and skin diseases, including hair loss. Corticotropin-releasing hormone (CRH) plays a crucial role in regulation of central and local stress responses. In addition, Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which maintains redox homeostasis, has been expressed in the human hair follicles. Therefore, the aim of the study is to investigate the role of oxidative stress in CRH-induced hair follicle damages in stress environments on cultured human dermal papilla cells (hDPCs). As a result, the expression levels of catalase, SOD, and GPx, known as antioxidant enzyme, were changed with CRH treatment, indicating that hormonal stresses caused oxidative damages and regulated antioxidant defense system. In addition, Nrf2 expression levels were increased in cultured hDPCs. And also, both Heme oxygenase 1 (HO1) and NAD(P)H dehydrogenase 1 (NQO1) expression levels were up-regulated by CRH treatment in cultured hDPCs. These studies indicated that CRH induced various oxidative damages. Therefore, the defense system against oxidative damage was activated in stress response on hDPCs.

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H033

Reverse Genetics of SARS-CoV-2

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The first outbreak of Coronavirus disease 2019 (COVID-19) was reported in Wuhan in December 2019 and approximately 175 million cases have been reported so far. To overcome the pandemic, it is important to develop experimental systems to study and evaluate effective countermeasures. SARS-CoV-2 genome consists of single-stranded positive-sense RNA, approximately 30 kb in length. It is one of the largest viral genomes which poses challenges to construct infectious cDNA clone using reverse genetic system. Here we report that the infectious cDNA clone of SARS-CoV-2 was generated by Gibson assembly reaction using homologous regions of the genome. Specifically, we generated 8 fragments of cDNA clones spanning the entire genome of SARS-CoV-2 and reassembled them using transformation-associated recombination cloning. The resulting reverse genetic system will serve as a tool to evaluate viral host interactions and more importantly in the development of vaccines.

H034

D614G Mutation in RBD Increases the Infectivity of SARS-CoV-2

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The entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the host cells is mediated by the interaction of viral spike protein (S) with Human Angiotensin-Converting Enzyme II (hACE2) and the domain of S that bind with hACE2 is called the receptor-binding domain (RBD). Currently, various mutations in SARS-CoV-2 result in newly emerging variants. Especially, mutations in RBD of B.1.351 (beta variant) and B.1.617 (delta variant) lineages are of prime concern as these variants have high infectivity, transmissibility, and are more resistant to neutralizing antibodies. In this study, to understand the mechanism of increased infectivity of B.1.351 and B.1.617 variants, we developed pseudoviruses bearing S of wild-type strain B.1.351, and B.1.617 and evaluated their infectivity. Our results demonstrate, that B.1.351 and B.1.617 variants have significantly higher infectivity compared with wild-type pseudovirus, which is mediated by D614G mutation in RBD.

H035

Antagonistic Potential of Kimchi against Human Corona Virus

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Kimchi, a fermented food, commonly consumed in South Korea is a rich source of lactic acid bacteria (LAB). LAB contains lectin that may bind the glycosylated spike in Coronavirus and can interfere in its infectivity. This study aimed to evaluate the effect of LAB, isolated from kimchi, on human coronavirus-229E (hCoV-229E). Lactic acid bacteria in the log phase or stationary phase of the life cycle were collected and heat-killed or were fixed by 4% paraformaldehyde or 2.5% glutaraldehyde. After fixation, the bacteria were mixed with GFP-tagged hCoV-229E and incubated at 4°C for 1 h. The mixture was used to infect Huh-7 cell line and incubated at 37°C for 24 h. Eventually, the samples were analyzed for the expression of GFP by flow cytometry. Our results demonstrate that in contrast to heat killing or paraformaldehyde fixation, log phase lactic acid bacteria fixed by glutaraldehyde significantly downregulates the frequency of GFP expressing cells, implying that this specific treatment of LAB inhibits hCoV-229E. Moreover, stationary phase bacteria did not show any significant effect. In conclusion, log phase lactic acid bacteria fixed by glutaraldehyde can be effective in lowering the infectivity of hCoV.

H036

Establishment of the Specialized Bank for Multidrug Resistant Pathogens in Korea National Institute of Health

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The dissemination of antimicrobial resistance is a global concern of public health. However, there are some limitations to gather or share the various pathogens having specific characteristics that researchers need. At least 8,000 of antimicrobial resistant bacteria identified to 13 different species has been collected from humans, animals, and environment sources every year by Korea National Institute of Health (KNIH). To contribute to research of combating antimicrobial resistance as providing those multidrug resistant bacteria collected in KNIH, the Specialized Bank for Multidrug Resistant Pathogens (MRP Bank) was designated in April 2020. To determine criteria for resources selection of MRP bank, a survey was performed to 164 experts who belong to laboratories of colleges, institutes, and companies. A total of 462 isolates were deposited to the MRP bank, and they were classified according to determined criteria based on information on sources and specimens of isolates and its phenotypic and genotypic characteristics on antimicrobial resistance. Additionally, various multidrug resistant isolates will be selected and deposited to the MRP bank every year. The deposited resources of MRP bank will be opened to and provide to researchers in multi-sectorial laboratories since 2022 to contribute to the research of antimicrobial resistant in the human and non-human fields.

[Supported by the Korea National Institute of Health (grant number 2020-NI-021-01)]

H037

Glycosylation of Bisphenol A (BPA) by the Dinoflagellate *Ostreopsis cf. ovata*

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In the course of the isolation of toxins from the massive culture of *O. cf. ovata*, three new BPA glycosides, not toxins, were isolated. Generally, the endocrine disruptor BPA is never reported to be produced by any organisms, and instead, bisphenol A (BPA, 4, 4'-isopropylidenediphenol) is used to manufacture polycarbonate plastic and epoxy resin lining of food and beverage cans. However, the isolated BPA glycosides was deduced as metabolites of *O. cf. ovata*, which were metabolized with BPA released from the culture vessel made of the polycarbonate material. Some literatures have reported that microorganisms like freshwater microalgae absorbed and transformed BPA dissolved in the culture medium to several BPA glycosides. In this respect, our results demonstrate that a dinoflagellate *O. cf. ovata* can also metabolize BPA to its glycosides. Further study will show whether most marine microalgae have a potential of metabolizing BPA glycosides. This may be a concerned issue because the accumulated BPA glucosides may be digested to BPA by β -glycosidase in animal intestines. In this poster, we will present the isolation and structure determination of three new BPA glycosides and the estimation of metabolizing of *O. cf. ovata* with BPA added in the medium.

H038

One Health Approach against AMR in Korea: Multi-sectoral AMR Research Strategies and Activities

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WHO has recognized the seriousness of AMR problems and thus responded to AMR bacteria. It is necessary to establish a monitoring system for the concept of One Health through inter-governmental cooperation involving all relevant government ministries. The One Health approach to AMR means the need for comprehensive management because antimicrobials are used in various fields of not only humans but also agriculture, livestock, fisheries, food, and the environment. The Korean government has been implementing the NAP on AMR, with the AMR consultative committee of 6 ministries that was established in 2016. Accordingly, the "Multi-sectoral joint Project to One Health approach against AMR" has been launched since 2019 and expanded to involve 7 ministries (KDCA, MSIT, MAFRA, ME, MOF, MFDS, RDA) in Korea. This project have since been carried out such as the investigation of AMR bacteria in Ecosystem, the research on the resistance mechanisms of AMR bacteria transmission and acquisition, development of diagnostic methods, discovery of alternatives to antibiotics, and development of stewardship programs for the proper use of antibiotics. We have been conducting additional research in the area of infrastructure construction such as establishment of networks for efficient operation of the project, establishments of AMR web portal and AMR isolates bank, conduct a symposium, publish of annual report, and the joint investigation on AMR in the domestic community through a One Health approach.

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H039

Characteristics of Colistin Heteroresistance in *Acinetobacter baumannii* Isolated from Human and Veterinary Medicine

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Colistin (CST) is being administered as last-line therapy for patients that have failed to respond to other available antibiotics that are active against *Acinetobacter baumannii* (ACB). The underlying mechanism of CST-resistance and -heteroresistance (HR) remains largely uncharacterized. The present study investigated the characteristics and mechanisms of CST-HR in ACB isolated from human and veterinary medicine in Korea. A total of the 78 CST-S ACB isolates, which were collected from multi-sectoral joint project to One Health approach against AMR from 2017 were tested. Isolates recovered from 31 patients, 7 guardians, 14 companion animals, and 26 environments. 40 human clinical isolates were assigned to CRAB. 4 CST-HR ACB isolates which were selected by PAPs. The HR rates in the isolates from human, companion animal and the environment were 5.2%, 3.8%, and 7.1%, respectively. The stability test was performed to investigate whether CST-R is maintained without antibiotic treatment. Only one CST-HR isolate showed a typical pattern that survived after exposure to high CST concentrations and was found to be CST-R, whereas no resistant colonies were identified in the other CST-HR isolates. Typical CST-HR isolate had a low MIC of an investigational aminoglycoside, belonged to ST 191. Time kill assays and detection of resistant genes were also conducted. Our data suggest that the presence of CST-R subpopulations seems to be common in CST-S ACB isolates in humans, animals, and the environment. CST-HR *A. baumannii* would be a significant threat to the treatment of *A. baumannii* infection because these isolates are not easily detected through routine susceptibility testing to CST and may lead to an increase of resistance in *A. baumannii* by suboptimal usage of CST.

[This research was supported by a fund (2019-NI-083-00) by Research of KDCA]

H040

Dissemination and Characteristics of High-level Erythromycin-resistant *Enterococcus faecalis* from Bulk Tank Milk of Dairy Companies in Korea

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Enterococci are environmental pathogens that can cause bovine mastitis and macrolides are widely used for the treatment of bovine mastitis caused by staphylococci and streptococci/enterococci. The aim of this study was performed to compare the phenotypic and genotypic characteristics of high-level erythromycin-resistant (HLER) *Enterococcus faecalis* (*E. faecalis*) collected from four dairy companies (A, B, C, and D) in Korea. Although isolates from company D showed the highest prevalence of *E. faecalis*, the prevalence of HLER *E. faecalis* in company A (73.1%) and C (57.0%) was significantly higher than company D (33.9%). A total of 149 HLER *E. faecalis* isolates showed high rates of resistance to tetracycline (93.3%), followed by doxycycline (70.0%). In the distribution of macrolides resistance genes, 147 (98.7%) isolates carried *ermB* gene alone and two isolates carried both *ermA* and *ermB* genes. For aminoglycosides resistance genes, the prevalence of both *aac(6')Ie-aph(2'')-Ia* and *ant(6')-Ia* genes (43.0%) was the highest. Virulence genes, such as *ace* (99.3%), *cad1* and *efaA* (each 98.7%) were also highly conserved in the 149 HLER *E. faecalis* isolates. Our results indicated HLER *E. faecalis* isolates from bulk tank milk showed significant differences in phenotypic and genotypic characteristics between dairy companies. And the prevalence of resistance genes and virulence factors was also high in HLER *E. faecalis* isolates.

H041

Modulation of Host Gene Expression Infected by Zika Virus

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Zika virus (ZIKV) including a single-strand positive sense RNA genome causes severe neurological diseases named microcephaly in newborns and Guillain-Barre syndrome in adults. ZIKV, which is a mosquito-born flavivirus, has been related to global pandemic infections since was isolated at Zika forest, Uganda in 1947. Although various researches have been going on, tracking host gene expression by ZIKV have not been understood completely. Therefore, we aimed 1) to identify the expression pattern of host genes modulated by ZIKV infection and 2) to understand ZIKV pathogenesis. ZIKV infected cells were collected from a placental cell line named JEG-3 at the different time points. The total RNA was isolated and RNA-seq was performed using Illumina HiSeq system. The analyses were conducted by CLC Genomics Workbench and various bioinformatics tools. In the results, we identified host genes regulated by ZIKV infection and tried to speculate host-virus interactions. In addition, our results could provide the critical knowledge to invent a ZIKV medicine.

H042

Rapid Genome Sequencing and Variant Typing of SARS-CoV-2 from Patient Samples

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A major concern in COVID-19 outbreaks is the rapid mutation of SARS-CoV-2 viruses. The current variant typing of SARS-CoV-2 solely depends on whole genome sequencing (WGS). Thus, rapid and accurate genome sequencing of the virus is essential. In this study, we aimed to develop a rapid WGS protocol using universal primers spanning the entire genome of SARS-CoV-2. We have designed 11 pairs of SARS-CoV-2 universal primers which cover 7 genome clades (Clade of S, L, V, G, GR, GH, and GV) and 2 variants (B.1.1.7 and B.1.135). The 9 viral strain's RNAs extracted from cell cultures were used as template of PCR. The 3 kb amplicons were synthesized from the 11 primer pairs. The MinION sequencing reads were assembled into a complete genome with accuracy of 99.94-99.98%. Finally, the developed protocol was applied to five clinical samples. The results showed that the complete genome sequencing and variant typing could be directly obtained from clinical samples. Consequently, this method would contribute to rapid COVID-19 diagnosis, particularly in public health surveillance.

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H043

Comparative Genomic Analysis of *Fusobacterium* Species Using Multiple Metagenome-assembled Genomes

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Fusobacterium genus is a rod-shaped Gram-negative anaerobe and is part of normal human microbiome. Among *Fusobacterium*, *F. nucleatum* has been extensively studied in terms of pathogenic roles in diverse diseases, and some virulence factors such as FadA and Fap2 were revealed to participate in inflammation and colorectal carcinogenesis. However, non-nucleatum *Fusobacterium* species remain relatively unexplored. Thus, we compared entire functional capacity of available *Fusobacterium* genomes, and analyzed pan-genome to understand the adaptation of *Fusobacterium* to human gut. We collected 231 isolate genomes and 609 metagenome-assembled genomes from public databases to dissect genetic factors associated with host gut environments. Then, we manually curated available metadata such as health status, body mass index, age, and geographic information from 41 projects. The genomes were filtered by criteria $\geq 80\%$ completeness and $\leq 5\%$ contamination, resulting in 527 refined genomes. Phylogenetic trees were constructed based on core and/or accessory genome. Furthermore, the trees were analyzed with gene annotation and host condition metadata to discover whether specific clades are associated with genes or metadata attributes. *Fusobacterium* genomes were clustered by functional differences such as virulence factors and redox-associated pathways. Our pan-genomic analysis would provide a comprehensive resource to understand the adaptation of *Fusobacterium* species to host.

H044

Differential Gene Expression Patterns in a Bacterial Wilt Resistant Tomato Plant Transplanted with Two Different Soil Microbial Fractions

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Bacterial wilt (BW) caused by *Ralstonia solanacearum* greatly affects the production of *Solanaceae* crops including tomato plants. In the previous study, BW-resistant tomato cultivar transplanted with upland soil microbial fraction (UpMF) showed strong resistance to BW, while the BW-resistance was completely abolished by forest soil microbial fraction (FoMF) transplant. The differentially expressed genes (DEGs) between rhizosphere transplanted with two different microbiotas were investigated by employing RNA-Seq. Interestingly, most of the genes involved in plant-microbe interactions were expressed equally irrespective of microbiota transplant. However, DEG analysis identified a total of 32 DEGs that showed robust and strong differential expression under two different MFs at all three time points. Most of these DEGs were associated with signaling pathways in tomato plants. The expression level of 15 genes of the 32 genes was increased in UpMF transplant compared to FoMF transplant, while that of the rest genes were decreased. To confirm the expression of DEGs using RNA isolated from the tomato plants transplanted with UpMF and FoMF, we are conducting quantitative reverse transcriptional PCR. This result suggests that microbiota-specific signaling occurs in tomato plants to modulate BW resistance in a BW-resistant tomato plant.

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H045

Whole Genome Sequencing of RVFV and Related Virus Using Universal Primers

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Rift Valley Fever Virus (RVFV) is one of the “Disease X” reported by WHO and causes an acute viral hemorrhagic fever. Rift Valley Fever (RVF) which is common zoonotic disease and is mainly transmitted through mosquitoes. In this study, we aimed to develop a whole genome sequencing (WGS) protocol of RVFV for diagnostic purpose. To amplify the genome, 4 pairs of primers stretched over the three genome segments (segment L of 6 kb, M of 4 kb, and S of 1.6 kb) were designed. The primers were applied to three RNAs of RVFV strains (ZH548, Kenya 56, and BIME 01) and produced amplicons of expected size (3 kb). The amplicons were purified and sequenced using MinION, MiSeq, and Sanger sequencer. The resultant sequencing reads were successfully assembled into complete genome regardless of the sequencing platform. The limit of detection of this amplicon-based sequencing was >190 copies/μl showing that the whole genome sequence of RVFV could be directly obtained from clinical samples. [This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI20C0558)]

H046

Whitening Function of *Lactobacillus* Fermented Extract Using *Coix lacryma-jobi* Bran

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Coix lacryma-jobi L. (Adlay) is a grass crop that has long been consumed both as an herbal medicine and as a nourishing food. Adlay has been widely used as a diuretic, stomachic, analgesic, and antispasmodic agent from ancient times. Recently, a number of pharmacologically and physiologically interesting substances have been isolated from the different parts of adlay, including antiinflammatory, antitumor, hypoglycemic, antimicrobial, and ovulatory-active agents.

The purpose of this study is to research the whitening effects of fermentation extract made from the Adlay bran. Two *Lactobacillus* were isolated from Korean traditional food. Both of the two strains are safe according to safety assessment including hemolytic activity, biogenic amine production, mucin degradation and antibiotic susceptibility. Then the fermented adlay bran was extracted with Prethanol (1:3, v/v) and concentrated under vacuum. The ethanol extract was suspended in water and partitioned successively with n-hxane (Hx), dichloromethane (CH₂Cl₂), ethylacetate (EtOAc), n-butanol (BuOH). The BuOH fraction exhibited the most potent inhibition rate of the melanin biosynthesis, the activity of tyrosinase in malignant melanoma, B16F10 cells. [This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Technology Commercialization Support Program Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA)(821030-03)]

H047

Isolation and Selection of Biosurfactant-producing Yeast

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Surfactants are amphiphilic compounds that have hydrophobic and hydrophilic moieties. Biosurfactants from microorganisms or plants have properties similar to chemical surfactants and can reduce the tension between the interface and surface. Biosurfactants have potential applications in the field of agriculture, detergents, food and cosmetics etc. In this study, screening of biosurfactant producing yeasts was performed using for drop collapse assay and a novel species yeast, *Rhizosphaera* sp., producing biosurfactant was selected. The growth of the isolate and surface tension (ST) of its culture supernatant were measured for 8 days. The results showed that optical density (O.D) was gradually increased over time and the ST decreased to the lowest value (34.5 mN/m) on day 5. It presents that the biosurfactant activity was not proportional to the growth of the isolate. Genome sequence analysis of the yeast, a novel species, was performed to accumulate fundamental data to discover gene clusters that produce biosurfactant. The genome size is approximately 27 Mb with GC contents 50.78%. The yeast-derived biosurfactant could be a potential alternative to the chemical surfactant, and further studies are needed to the commercial application.

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H048

Anti-CRISPR Identification Using Deep Protein Language Model

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Anti-CRISPR is a group of proteins that inhibit the CRISPR-Cas system of the prokaryotic immune system. Recently, anti-CRISPR has emerged as a natural inhibitor of the CRISPR-Cas system, enabling post-translational regulation of the CRISPR-Cas system in a variety of applications. Although experimental techniques have been developed for the discovery of anti-CRISPR, bioinformatic prediction may provide a cost-effective screening strategy. However, the lack of verified anti-CRISPR data and low sequence similarity make algorithm development difficult. Here, we describe an approach to predict anti-CRISPR proteins from amino acid sequences in which pre-trained deep sequence models are fine-tuned for classification task. We build an anti-CRISPR protein classifier by using Transformer-based protein-language model, a deep sequence model pre-trained for unlabeled amino acid sequences from Pfam, and fine-tuning the pre-trained model on the anti-CRISPR dataset. Performance is evaluated on an independent data set using a variety of metrics compared to existing predictors. Conventional predictors use additional feature calculations and pre-filtering steps, but the present approach only requires a protein sequence, making the present method convenient and suitable for genome-scale studies.

H049

Comparison of Microbial Community in Dry Aged Beef and Analysis of Microbiological Contamination for Aging Period

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This study was conducted to confirm the microbiological changes of dry aged beef by aging period. First, beef by aging period was primarily isolated through a solid medium, and then typical colonies that appeared were counted and identified by isolation/16S rRNA sequencing to compare microbial communities. Second, the spoilage and contamination of the edible parts were determined. In the fresh meat of the 0th week of aging, the total number of bacteria was 3.5 Log CFU/g, and bacteria were present at a low level, and the main bacteria were also very diverse. However, after the 2nd week, the total number of bacteria was very high at 7 Log CFU/g. *Pseudomonas* spp. has been identified as dominant bacteria in aged beef. By the 24th week of aging, in the edible part inside the aged beef, the putrefactive bacteria *Acinetobacter*, *Lactobacillus*, *Leuconostoc*, and *Enterococci* were below the detection limit. As a result of the analysis, which is the food-borne pathogen was not detected in all samples until the 24th week of aging. The total number of aerobic bacteria in the edible part by aging period and lactic acid bacteria were detected at a low level from the 0th to the 20th week of aging. In the case of yeast and mold, it was not detected in all samples. This study can be used to prepare safety indicators for aged meat in the future. [This work was supported by research grant from Korea Food Research Institute (KFRI, E0211400-01), funded by the Ministry of Science and ICT of Korea.]

H050

The Treatment Effects of Anti-diabetic Agents Related to Gut Microbiota in Obese Patients with Type 2 Diabetes

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A randomized controlled trial was performed to compare the efficacy of the gemigliptin and metformin combination (gemi+MTF) to the glimepiride and metformin combination (glime+MTF) as initial therapy on gut microbiota and glucose homeostasis in patients with type 2 diabetes (T2D). Drug-naïve Korean T2D patients with a glycated hemoglobin (HbA1c) level of $\geq 7.5\%$ were assigned either to gemi+MTF or to glime+MTF therapy for 24 weeks. Fecal bacterial DNA was sequenced of the 16S rRNA gene V3-V4 regions. Sixty-two patients finished the trial. The patients on gemi+MTF achieved HbA1c $\leq 7.0\%$ without hypoglycemia and weight gain more compared with glime+MTF ($P < 0.05$). The *Firmicutes/Bacteroidetes* ratio, the metabolic disease marker, decreased in the gemi+MTF group resulting in a borderline difference between the two groups ($P = 0.065$). *Bacteroides*, microbiota for good glucose control, was increased in the gemi+MTF group with borderline group difference. *Weissella* was high in those who did not achieve the glycemic target. It had increased in the glime+MTF group and showed group difference. The glime+MTF group significantly increased *Streptococcus*, the marker for weight gain after treatment, resulting in difference between the groups. Combined treatment with the gemi+MTF changed gut microbiota more favorably compared with the glime+MTF, leading to a greater glycemic target goal achievement without hypoglycemia and weight gain in drug-naïve T2D patients.