A001

***Sandarakinorhabdus ruber* sp. nov., and *Sandarakinorhabdus oryzae* sp. nov., Isolated from Rice Paddy Soil**

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A Gram-stain-negative, motile, rods-shaped, aerobic bacteria, designated strain MO-4^T and NM-18^T, were isolated from rice paddy soil in south Korea. The two strains grew at pH 5.0–11.0 (optimum, pH 8.0), at 15–45°C (optimum, 37°C) and at salinities of 0–1.5% (w/v) NaCl (optimum, 0.4% NaCl). Phylogenetic analyses based on 16S rRNA gene sequences comparisons showed that the two isolates belonged to the genus *Sandarakinorhabdus* with sequence similarity of 98.6 to 97.6 %. And the similarity between strain MO-4^T and NM-18^T showed 97.3%. Average nucleotide identity values between MO-4^T and NM-18^T was lower than 82.0%, and that of between the two strains and closet relatives, *S. limnophila* so42^T and *S. caynobacteriorum* TH057^T 84.31 to 85.73%. The major fatty acids were iso-$C_{18:1}$ *ω7c* and summed features 3. The G+C content of strains MO-4^T and NM-18^T, obtained from genome sequencing data, were 67.6 mol% and 66.6 mol%, respectively. On the basis of polyphasic analysis from this study, strains MO-4^T and NM-18^T represents a novel species of the genus *Sandarakinorhabdus*, for which the names *Sandarakinorhabdus ruber* sp. nov. (type strain NM-4^T = KACC 21378 = NBRC 113957), *Sandarakinorhabdus oryzae* sp. nov. (type strain NM-18^T = KACC 21379) are proposed.

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

Keywords: *Sandarakinorhabdus oryzae* sp. nov., *Sandarakinorhabdus ruber* sp. nov., Rice paddy soil

A002

***Agroterribacter humi* gen. nov. sp. nov., a Novel Bacterium of the Family *Chitinophagaceae* Isolated from Soil of a Farming Field**

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A Gram-stain-negative bacterium, designated strain YJ03^T, was isolated from a farming field soil at Shinan in Korea. Strain YJ03^T was found to be aerobic, non-motile bacterium which can grow at 10–30°C (optimum, 25°C), at pH 6.6–9.5 (optimum, pH 7.0–7.5) and at salinities of 0–1.0% (w/v) NaCl (optimum, 0% NaCl). Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain YJ03^T belongs to the family *Chitinophagaceae*, showing highest sequence similarity to *Pseudoflavitalea rhizosphaerae* T16R-265^T (93.9%) and *Terrimonas lutea* DY^T (93.7%), and formed an independent lineage separated from other described genera of the family *Chitinophagaceae*. The predominant isoprenoid quinone was identified as MK-7 and the major fatty acids were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and iso-$C_{16:0}$ 3-OH. The major polar lipids were identified as phosphatidylglycerol, unidentified aminolipids and unidentified lipids. The DNA G+C content of this novel isolate was determined to be 42.8 mol%. On the basis of the phylogenetic, phenotypic and chemotaxonomic analyses, strain YJ03^T is considered to represent a novel species of a novel genus of the family *Chitinophagaceae*, for which the name *Agroterribacter humi* gen. nov. sp. nov. is proposed. The type strain is YJ03^T (= KACC 19548^T = NBRC 113195^T).

Keywords: *Agroterribacter humi* sp. nov., Bacteroidetes, Rhizosphere, Taxonomy

A003

A Bacterium Representing Novel Species in the Genus *Dyadobacter*, Isolated from Green-House Soil

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A Gram-stain-negative, non-motile, aerobic and yellow pigmented bacterium, designated strain BO54^T, was isolated from green-house soil near Suncheon, Republic of Korea. Strain BO54^T grow at 10–35°C (optimally at 30°C) and 0–1% of NaCl (optimally at 0.5% NaCl). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain BO54^T formed a distinct lineage within the genus *Dyadobacter* and was closely related to *Dyadobacter koreensis* WPCB159^T (98.11% 16S rRNA sequence similarity). The predominant fatty acids are Summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), $C_{16:0}$ and Summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*). The only respiratory quinone is menaquinone 7 (MK-7). On the basis of phenotypic, chemotaxonomic data and phylogenetic inference, strain BO54^T should be classified into the genus *Dyadobacter*, as a member of a novel species, for which the name *Dyadobacter luteolus* sp. nov. is proposed.
[This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea.]

Keywords: Dyadobacter, Green-house soil

A004

Amylibacter sargassum* sp. nov., Isolated from *Sargassum fulvellum

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A Gram-reaction-negative, pale-yellow colored bacterium, designated SFDW26^T, was isolated from *Sargassum fulvellum*, collected from South Sea, Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate belonged to the genus *Amylibacter*, with the closest relatives being *Amylibacter marinus* 2-3^T (96.46% 16S rRNA gene sequence similarity), *Amylibacter kogurei* 4G11^T (95.94%) and *Amylibacter ulvae* 6Alg 255^T (95.88%). Cells grow on MA but not on R2A, TSA, LB and NA. Growth occurs with 2–6% (w/v) sea salts (optimum, 3%) and at 10–30°C (optimum, 25°C). Catalase- and oxidase-negative. The predominant fatty acids were $C_{16:0}$, 11-methyl $C_{18:1}$ *ω7c* and Summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*). The major respiratory quinone was ubiquinone 10 (UK-10). Strain SFDW26^T represents a novel species of the genus *Amylibacter*, for which the name *Amylibacter sargassum* sp. nov. is proposed.
[This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea.]

Keywords: *Sargassum fulvellum*, Amylibacter

A005

A Bacterium Representing Novel Species in the Genus *Robiginitalea,* Isolated from Tidal Flat

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A Gram-stain-negative, rod shaped, non-motile, aerobic and orange pigmented bacterium, designated strain SC105^T, was isolated from a tidal flat of the Suncheon bay in South Sea. *Robiginitalea biformata* HTCC2501^T was the nearest neighbor of strain SC105^T with 97.9% 16S rRNA gene sequence similarity. Growth occurs at 15–40°C (optimum, 25–30°C), at pH 6–9 (optimum, pH 7) and with 1–5% (w/v) sea salts (optimum, 3%). Catalase–positive and oxidase–negative. The major quinone was menaquinone 6 (MK-6). The DNA G+C content of the strain was 55.4 mol%. On the basis of phenotypic-, chemotaxonomic data and phylogenetic inference, strain SC105^T should be classified into the genus *Robiginitalea*, as a member of a novel species.
[This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea.]

Keywords: Tidal flat, Robiginitalea

A006

A Bacterium Representing Novel Species in the Genus *Emticicia,* Isolated from Soil

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A Gram-stain-negative, aerobic, non-motile and pink pigmented bacterium, designated strain BO119^T was isolated from soil from a cabbage field in Suncheon, Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain BO119^T formed a distinct lineage within the genus *Emticicia* and was most closely related to *Emticicia soli* KCTC 52344^T (98.02% 16S rRNA gene sequence similarity), *Emticicia ginsengisoli* KCTC 12588^T (97.82%) and *Emticicia fontis* KCTC 52248^T (97.75%). Strain BO119^T grow at 15–35°C (optimally at 25–30°C) and 0–1% of NaCl (optimally at 0% NaCl). Cells grew on R2A, TSA, NA but not on MA, LB agar. Catalase and oxidase-positive. The DNA G+C content of the strain was 37.7 mol%. The major respiratory quinone detected is menaquinone 7 (MK-7). Strain BO119^T were proposed as a new species of the genus *Emticicia*.
[This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea.]

Keywords: Soil, *Emticicia*, 16S rRNA

A007

The First Report on *Diversispora eburnea* and *Paraglomus laccatum* in Korea

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The fungi belonging to Phylum Glomeromycota generally exist as the asexual spores in the rhizosphere of plants. When they penetrate the hypha into the plant roots, they form arbuscular mycorrhiza with plants. Since Phylum Glomeromycota has been isolated to monophyletic clade from Zygomycota by C. Walker & A. Schüßler in 2001, more than 300 species of AMF have been recorded worldwide, however, only *c*. 100 species of AMF have been reported in Korea. In this study, we isolated fungal spores belong to phylum Glomeromycota from soils that cultured in pots. We identified the isolated fungal spores using sequences analysis of 18S partial rDNA region, and "Krüger fragment" which including internal transcribed spacer and 28S rDNA regions. As a result, we confirmed 2 unreported species belong to phylum Glomeromycota, *Diversispora eburnea* and *Paraglomus laccatum*. We described about morphological characteristics of novel fungal spores and results of phylogenic analysis.

Keywords: Glomeromycota, Mycorrhiza, Arbuscular mycorrhizal fungi, Fungal spore

A008

Aquatic Species Diversity of Genus *Graphium* from Korea

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Genus *Graphium* belonging to order Microascales has been known as wood pathogens in the world to cause sapstain in timbers leading to wood degradation. However, there are lack of information related to the genera in Korea. Therefore, current study was conducted to investigate the aquatic species diversity of genus *Graphium* from Korea. Eleven strains, CNUFC-YRW-7, CNUFC-HRW1-1, CNUFC-PYW4-15, CNUFC-WG-58, CNUFC-YR1-18, CNUFC-BCW-49, CNUFC-WG-41, CNUFC-BCW-44, CNUFC-JSN3-15, CNUFC-WG1-1 and CNUFC-YRW-12 were isolated from freshwater samples except the strain CNUFC-HRW1-1 from marine water samples in Korea. On the basis of morphological characteristics and phylogenetic analysis of the internal transcribed spacer (ITS), 28S rDNA and translation elongation factor-1 alpha (TEF-1α) gene sequences, the isolated strains CNUFC-YRW-7, CNUFC-HRW1-1, CNUFC-PYW4-15, CNUFC-WG-58, CNUFC-YR1-18, CNUFC-BCW-49, CNUFC-WG-41, CNUFC-BCW-44, CNUFC-JSN3-15 and CNUFC-WG1-1 were identified as *Graphium basitruncatum*, two strains of *G*. *carbonarium, G*. *euwallaceae*, *G*. *fabiforme*, *G*. *ilexiense*, *G*. *jumulu*, *G. kuroshium, G. penicillioides* and *G*. *pseudormiticum*, respectively. Out of eleven isolates, there are nine new records of *Graphium* species including two strains of *G*. *carbonarium* from different sources and one is new to science in Korea.

Keywords: Phylogeny, Morphology, Water environment, *Graphium* spp.

A009

Novel Zygomycetous Fungi from Different Niches in Korea

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During the survey of fungal diversity from different specific niches, two novel strains, EML-GFI-1 and EML-WW2-12 were isolated from insect larva and freshwater samples, using direct plating and dilution plate method, respectively. A BLASTn search of internal transcribed spacer (ITS) indicated that the EML-GFI-1 and EML-WW2-12 isolates were closest to *Backusella recurva* CBS 673.75 (GenBank accession no. JN206264) and *Gongronella butleri* JHR101-14 (GenBank accession no. JX076989) with identity values of 99.78% (448/449 bp) and 98.90% (542/548 bp), respectively. Based on their morphological characteristics and phylogenetic analysis of ITS and 28S rDNA regions, the EML-GFI-1 isolate was identified as *Backusella recurva*, and EML-WW2-12 isolate was identified as a new species of *Gongronella.* To the best of our knowledge, *Backusella recurva* has not been previously reported in Korea.

Keywords: Zygomycete, *Backusella recurva*, Gongronella, Unrecorded species, New species

A010

Novel Fungal Species from Aquatic Environments in Korea

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Fungi thriving in terrestrial environment play important roles in nutrient cycling and food webs and can shape microorganisms communities as parasites and mutualists. Only less than 10% of fungal species have been identified from 1.5 to over 5 million estimation of fungal species. To date, a relatively small percentage of species have been described from aquatic environments in comparison to terrestrial domain. Aquatic fungal communities are a diverse group of organisms and fulfill important functions in the food web dynamics of surface water ecosystems. They help in leaf litter breakdown in rivers and creeks, nutrient cycling and contributing food for detritus feeders. This study represents diversity of freshwater-derived fungi from Korea based on their molecular phylogenetic and morphological analyses. Knowledge on the geographic distribution of freshwater-derived ascomycetes and their asexual morphs in the Korean peninsula is limited. Freshwater samples were collected from Damyang, Yeosu, Hwangyong, Yocheon and Woncheoncheon streams, Korea. Our findings provide diversity of fungal community derived from freshwater niche in Korea.

Keywords: Ascomycete, Aquatic fungi, Ecology, Phylogeny, Morphology

A011

First Report of *Mucor ardhlaengiktus* Isolated from the Moth Niche in Korea

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During a survey of the order Mucorales, a fungal strain EML-MG-2 was isolated from a butterfly sample collected at Gangwon province, Korea. The strain grew rapidly at 25°C on synthetic mucor agar (SMA), and reaching 57–60 mm in diameter after 4 days of incubation. The colony was white with abundant mycelium. The sporangiophores were hyaline, smooth, and erect. Columellae were usually globose or subglobose, and measured 12.2–21.1 µm in diameter. Sporangia were globose, brownish, and measured 21.1–35.2 µm in diameter. Sporangiospores were ovoid or reniform, and measured 3.3–5.8 µm in diameter. A BLASTn search of the ITS region sequences via NCBI database indicated that the strain EML-MG-2 matched *Mucor ardhlaengiktus* (synonym: *M. ellipsoideus*) (GenBank accession no. NR_111683) with identity value of 100% (569/569 bp). Based on morphological characteristics and sequence analysis, the strain EML-MG-2 was confirmed as *M. ardhlaengiktus*. To our knowledge, this is the first report of *M. ardhlaengiktus* isolated from the moth as a specific niche.

Keywords: Mucorales, *Mucor ardhlaengiktus*, Morphology, Phylogeny, Moth

A012

Molecular and Biological Characteristics of a Novel Double-stranded RNA Mycovirus of *Trichoderma atroviride* NFCF377

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Mycoviruses are widespread in most fungal groups. We have characterized the genome of a novel double-stranded RNA (dsRNA) virus isolated from the fungus *Trichoderma atroviride* NFCF377, designated Trichoderma atroviride mycovirus 2 (TaMV2) using a high-throughput sequencing methodology. Genomic organization revealed the mycovirus contained two open reading frames (ORF) in different frame, which contained a larger segment (dsRNA1) of the TaMV2 genome comprised 5,062 bp encoding a RNA-dependent RNA polymerase (RdRp) and a smaller segment (dsRNA2) consisted of 4,522 bp with a single ORF encoding putative structural/gag proteins. Northern blotting analysis evaluated that the sequence was derived from the segments. Sequence and phylogenetic analysis revealed that TaMV2 was most closely related to known and proposed family *Fusagraviridae*. Following single-spored progeny of parental TaMV2-containing *T. atroviride*, two isogenic, virus-cured isolates were confirmed by electrophoretic analysis, RT-PCR and Northern blotting analysis. Comparison of virus-infected and -cured isogenic lines demonstrated that the virus-cured strains showed antifungal activity due to increased activities of antifungal enzymes. However, there were no significant differences in growth rate and morphology. This study will yield new insights on the virus taxonomy and the interaction between the mycoviruses and the pathogenic fungal hosts.

[Supported by grants from NRF.]

Keywords: Mycovirus, *Trichoderma*

A013

***Laccaria macrobasidia*, a New Species of *Laccaria* (Agaricales, Basidiomycota) from Korea**

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Species of *Laccaria* (Agaricales, Basidiomycota) are well-known ectomycorrhizal symbionts of a broad range of hosts. *Laccaria* species are characterized by brown, orange, or purple colored basidiomes and globose or oblong, echinulate, multinucleate basidiospores. While some *Laccaria* species are easily identified at the species level using only the morphological characteristics, others are hard to distinguish at the species level due to small differences in morphology. Ten *Laccaria* species have been reported from Korea. While studying the fungal diversity in Gayasan National Park, a new *Laccaria* species was discovered. Based on its morphological features and molecular analyses (ITS, 28S rDNA, rpb2, and tef1a), it was found that it has yet been discovered. This species is proposed here as *Laccaria macrobasidia*. The unique morphological characters of *L. macrobasidia* that distinguish it from its closely related species are orange-brown color basidiocarps with pectinate-striate inwords from the edge, thick and light-brown lamellae, brown and fibrillose stipe, globose or subglobose and echinulate basidiospore, and long basidia (52–80 μm). This new species is described and illustrated in the present paper.
[Supported by the project on the survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR201701104) and Korea Basidiomycota Resources Center of the National Research Foundation funded by the Korean government (NRF2015M3A9B8029237).]

Keywords: Agaricales, Hydnangiaceae, *Laccaria macrobasidia*, New species, Taxonomy

A014

Genome Sequencing and Description of *Weissella cryptocerci* sp. nov., Isolated from Gut of the Insect *Cryptocercus kyebangensis*

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A taxonomic study of a Gram-stain-positive, rod-shaped, non-motile, non-spore-forming, catalase-negative bacterium, isolated from the gut of an insect, *Cryptocercus kyebangensis* collected from the mountainous area of Seoraksan, Yangyanggun, Republic of Korea, was conducted. Its 16S rRNA gene sequence showed high similarity values to *Weissella ghanensis* LMG 24286^T (95.9%), *Weissella beninensis* 2L24P13^T (95.9%), *Weissella fabalis* M75^T (95.7%) and *Weissella fabaria* 257^T (95.7%). The genome size is 3,020,651 bp with 41.1 mol% G+C content and 2,707 genes. Genome sequence was deposited NCBI database (CP032630). Polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, two unidentified aminophospholipids, one unidentified phospholipid and four unidentified lipids. The cell-wall peptidoglycan was of A4a type with the interpeptide bridge of Gly-D-Glu. Based on these results, strain 26KH-42^T could be classified as a novel species of the genus *Weissella,* for which the name *Weissella cryptocerci* sp. nov. is proposed. The type strain is 26KH-42^T (= KACC 18423^T = NBRC 113066^T).
[This study was carried out with the support (PJ013549) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.]

Keywords: *Weissella cryptocerci* sp. nov., Genome sequencing, Novel species, *Cryptocercus kyebangensis*

A015

***Azospirillum ramasamyi* sp. nov., a Novel Diazotrophic Bacterium Isolated from Fermented Bovine Products**

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A Gram-stain-negative, rod-shaped, aerobic bacterium, designated M2T2B2^T, was isolated from fermented bovine products in Suwon, Republic of Korea. The strain displayed growth at 15–45°C (optimum, 28–30°C), pH 6.0–10.0 (pH 7.0) and 0.2% (w/v) NaCl. Colonies were light pink-coloured, round and convex. The polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, and three unidentified aminolipids. Ubiquinone 10 was the predominant ubiquinone. The genome size is 6,316,263 bp with 68.0 mol% G+C content and 5,589 genes. The strain could fix atmospheric nitrogen, which was evaluated by the acetylene reduction assay. Further, whole genome sequence analysis revealed the presence of a nif gene cluster. Strain M2T2B2^T showed the highest 16S rRNA, rpoD and nifH gene sequence similarity to members of the genus *Azospirillum,* and showed 97.6% 16S rRNA gene sequence similarity to *Azospirillum oryzae* COC8^T. The phenotypic, phylogenetic and genomic analyses support the proposal of strain M2T2B2T as being a novel species of the genus Azospirillum, for which the name *Azospirillum ramasamyi* sp. nov. is proposed. The type strain is M2T2B2^T (= KACC 14063^T = NBRC 106460^T).
[This study was carried out with the support (PJ013549) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.]

Keywords: *Azospirillum ramasamyi* sp. nov., Genome sequencing, Novel species, Fermented bovine products

A016

Genome Sequencing and Description of *Pulveribacter suum* gen. nov., sp. nov., Isolated from a Pig Farm Dust Collector

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An aerobic, Gram-stain-negative, polar-flagellated, rod-shaped bacterium, designated as SC2-7^T, was isolated from the dust collector at a pig farm located in Wanju-gun, Republic of Korea. Growth occurred at 10–37°C (optimum, 28–30°C), pH 6.0–10.0 (optimum, 7.0–8.0) and in the presence of 0.3% (w/v) NaCl on R2A medium. The phylogenetic tree based on the 16S rRNA gene sequences revealed that strain SC2-7^T was a member of the family *Comamonadaceae,* forming a robust cluster with the genera *Alicycliphilus, Oryzisolibacter* and *Melaminivora.* The 16S rRNA gene sequences of strain SC2-7^T showed the highest sequence similarities to *Alicycliphilus denitrificans* K601^T (97.2%). The polar lipids present were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, an unidentified aminolipid and an unidentified phospholipid. The predominant quinone was ubiquione-8. The genome size of strain SC2-7^T is 3,358,427 with 69.1 mol% G+C content and 3,082 genes (CP027792). On the basis of the phenotypic, phylogenetic and chemotaxonomic data presented here, strain SC2-7^T represents a novel species of a new genus, for which the name *Pulveribacter suum* gen. nov., sp. nov., is proposed. The type strain of *Pulveribacter suum* is SC2-7^T (= KACC 19309^T = NBRC 113102^T).
[This study was carried out with the support (PJ013549) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.]

Keywords: *Pulveribacter suum* gen. nov., sp. nov., Genome sequencing, Novel species, Pig farm

A017

Genome Sequencing and Description of *Protaetiibacter intestinalis* gen. nov. Isolated from Gut of *Protaetia brevitarsis seulensis*

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A Gram-stain-positive, strictly aerobic, rod-shaped, non-spore-forming, non-motile bacterium, designated strain 2DFWR-13^T, was isolated from gut of the larva of *Protaetia brevitarsis seulensis,* in the Republic of Korea. Strain 2DFWR-13T showed high sequence similarities to *Lysinimonas kribbensis* MSL-13^T (97.7%), *Homoserinibacter gongjuensis* 5GH26-15^T (97.2%), *Microbacterium deminutum* KV-483^T (97.1%) and *Herbiconiux ginsengi* CGMCC 4.3491^T (97.1%). The major menaquinones were MK-13 and MK-12. The peptidoglycan type was type B2 with the diagnostic amino acid D-DAB. The N-acyl type of the murein was glycolyl. The polar lipids consisted of diphosphatidylglycerol, an unidentified glycolipid and an unidentified lipid. The genome size is 3,097,107 bp with 71.5 mol% G+C content and 2,926 genes. Based on its phylogenetic distinctiveness and distinguishing phenotypic characteristics, we conclude that strain 2DFWR-13^T represents a novel genus and species of the family *Microbacteriaceae,* for which the name *Protaetiibacter intestinalis* gen. nov., sp. nov. is proposed. The type strain of *Protaetiibacter intestinalis* is 2DFWR-13^T (=KACC 1932^T=NBRC 113050^T).
[This study was carried out with the support (PJ013549) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.]

Keywords: *Protaetiibacter intestinalis* gen. nov., Genome sequencing, Novel species, *Protaetia brevitarsis seulensis*

A018

A New Species of *Graphis* and New Lichen Records from Ulleung Island, Republic of Korea

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The lichen genus *Graphis* is one of the most abundant genera which recorded more than 400 species in the world. It is characterized by lirellate ascomata, septate or muriform ascospores, and exicipular structure (proper exciple carbonized). This genus can easily find in the montane and tropical forest even in the island. The Ulleung Island is geographically closed and many endemic genera were discovered in plants and animals. We have surveyed lichen collections in all sites of the island from the seashores, mountains to around waterfall regions. We discovered a new species of *Graphis* and three unrecorded species were confirmed for the first time in Korea. Morphologically, the new species is similar to *G. scripta* with labia entire and apothecial disc thinly white pruinose, but differs in having proper exciple apically carbonized. In the present study, a new species and three new records were confirmed their phylogenetic positions based on molecular analyses (mitochondrial small subunit (mtSSU) and large subunit (LSU) of rDNA region).
[Supported by the research fund of Korea National Arboretum (project No. KNA1-1-22, 17-2)]

Keywords: Ascomycota, Graphis, New to science, Taxonomy

A019

Mushroom Flora of Ganghwa Island and a Candidate of New *Amanita* Species in Korea

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In order to inventory the flora of mushroom in Ganghwa island, Republic of Korea, the biodiversity and population of higher fungi were surveyed from June to September in 2019. In total 170 specimens were collected and classified into 10 orders, 34 families, 64 genera, and 101 species. The collected specimens were 65.9 % saprophytic, 32.9% symbiotic, and 1.2% parasitic. Among them, we collected an unusual Amanitoid mushroom from territory of *Quercus acutissima* Carruth. Based on morphological characteristics and molecular identification, this specimen (KA19-0899) was not matched with previously reported *Amanita* species. The main characters of collected specimen is brownish to red brown pileus with appendiculate margin, brownish lammellae, and stem base deeply rooting. Here, we taxonomically described this species as a candidate of new to science. In addition, we provided a morphological comparison with closely related species.
[This research was supported by the Korean National Arboretum (Project No. KNA 1-1-25, 19-2)]

Keywords: Amanita, Biodiversity, Korea National Arboretum, Mushroom flora, Taxonomy

A020

Mucilaginibacter ginkgonis* sp. nov., Isolated from Bark of *Ginkgo biloba

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Strain HMF7856^T, isolated from a ginkgo tree of the Yongin, Republic of Korea, was an –yellow pigmented, Gram-staining-negative, non-motile, strictly aerobic, rod-shaped bacterium. The isolate grew on R2A agar at 30°C, pH 6.0–8.0 and 0–1.0% NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain HMF7856^T belonged to the genus *Mucilaginibacter* and was most closely related to *Mucilaginibacter phyllosphaerae* PP-F2F-G21^T (96.4% sequence similarity). The major fatty acids (> 5%) were summed feature 3 ($C_{16:1}$ *ω*7*c*/iso-$C_{15:0}$ 2-OH), iso-$C_{16:1}$, $C_{16:0}$ and iso-$C_{17:0}$ 3-OH. The cellular quinone content was exclusively menaquinone MK-7. The composition of polar lipids was revealed phosphatidylethanolamine, two aminophospholipid, unidentified aminolipid, two unidentified phospholipids, unidentified glycolipid and two unidentified lipids. The genomic DNA G+C content was 43.9 mol%. Thus, based on the phylogenetic, phenotypic and chemotaxonomic data, strain HMF7856^T represents a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter ginkgonis* HMF7856^T sp. nov. is proposed. The type strain of the species is strain HMF7856^T (=KCTC-ing).

Keywords: 16S rRNA gene, Novel species, Bacteria

A021

***Pedobacter ginkgonis* sp. nov., Isolated from Stem Bark of Ginkgo (*Ginkgo biloba*)**

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A bacterium, designated as strain HMF7056^T, was isolated from Ginkgo (*Ginkgo biloba*). It was Gram-stain-negative, strictly aerobic, motile by gliding and light pink in color. Growth was observed at 4–30°C (optimum, 25°C), pH 7–8 (optimum pH 7) and with 0–0.5% NaCl (optimum 0%). Strain HMF7056^T showed highest 16S rRNA sequence similarities to *Pedobacter luteus* DSM 22385^T (93.9%). The predominant fatty acids (> 10%) were iso-$C_{15:0}$ and summed features 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major respiratory quinone was menaquinone-7. The polar lipids consisted of phosphatidylethanolamine, one unidentified aminolipid, three unidentified phospholipids, two unidentified aminophospholipids, two unidentified glycolipids, and two unidentified polar lipids. The genome of strain HMF7056^T comprised of approximately 5.2 Mb with a G+C content of 50.5%. Based on the results of phenotypic and phylogenetic characterizations, the strains HMF7056^T are considered to represent a novel species of the genus *Pedobacter*, for which the name *Pedobacter ginkgonis* sp. nov. is proposed. The type strain is HMF7056^T (=KCTC 72282^T =NBRC 113965^T).

A022

First Report of Seven Korean *Lactarius* Species Originated from China

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The genus *Lactarius* is characterized by the striking phenotype, secretion of latex and its discoloration. This genus is composed of cosmopolitan ectomycorrhizal fungi, which are important food resource and symbionts in various ecosystems. Recent molecular studies showed that most *Lactarius* species are endemic in one continent or in a narrow region. During a recent study, seven species of *Lactarius* that were previously unrecorded in Korea were identified based on morphological observations and sequence analysis of the internal transcribed spacer region. Six species belong to the subgenus *Russularia* (*Lactarius conglutinatus*, *L. neglectus*, *L. orientaliquietus*, *L. qinlingensis*, *L. subatlanticus* and *L. subhirtipes*) and *L. albidocinereus* belongs to subgenus *Plinthogalus*. Here, we provide detailed morphological characteristics and phylogenetic support for each species.
[This work was supported by the project on the survey and excavation of Korean indigenous species of the National Institute of Biological Resources (grant number NIBR 201701104) and Korea Basidiomycota Resources Center of the National Research Foundation (NRF) funded by the Korean government (grant number NRF-2015M3A9B8029237)].

Keywords: Lactarius, Conspecificity, New records, ITS, Morphological comparison, Chinese origin

A023

GenBank Sequence Validation of Korean *Penicillium* Species

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Penicillium is one of the most common genera found in various environments and many species in this genus play important ecological roles as decomposers and plant pathogens. Approximately 450 *Penicillium* species have been reported worldwide. In Korea, more than 142 *Penicillium* species have been introduced. Currently, most species are identified by morphology and/or ITS sequence in GenBank. However, it is difficult to distinguish *Penicillium* by its morphological characteristics, and ITS sequence is not suitable to identify *Penicillium* species. The accumulated misidentifications of *Penicillium* species by the use of ITS sequence in GenBank is misleading the phylogenetic classification. To build a Korean *Penicillium* inventory and to correct the species misidentifications, we re-analyzed 455 ITS and 266 *benA* sequences of Korean *Penicillium* available on GenBank. Out of the 455 ITS sequences, 239 were assigned as 107 *Penicillium* species, 72 were undetermined, 116 were misidentified, and 20 were new species. Out of the 266 *benA* sequences, 231 were assigned as 80 *Penicillium* species, 13 were misidentified, and 22 were new species. Our research shows that there are 110 *Penicillium* species known and potentially 24 new species in Korea.
[This research was supported by National Research Foundation (NRF) (No. 0409-20190173)] and the Ministry of Oceans and Fisheries (MOF) (No. 20170431)].

Keywords: GenBank, *Penicillium*, ITS, *benA*

A024

Taxonomic Re-evaluation of the Genus *Pholiota* in Korea

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A genus *Pholiota* that belongs to the family Strophariaceae is composed of small or medium-sized mushrooms. *Pholiota* species are known as saprotroph and are commonly found in temperate climate regions. Because many species are morphologically similar, it is difficult to identify *Pholiota* species on a species level solely based on their morphological characters. So far, out of 18 species that have been reported in Korea, most of them were identified using the morphological features. To evaluate the taxonomy of Korean *Pholiota*, we used 79 specimens deposited in fungal collections, including KA, NIBR, and SFC. The specimens were identified based on morphological characteristics and molecular analysis of their internal transcribed spacer sequence. We identified 10 species with 9 previously recorded species and one non-recorded species, which was identified as *Pholiota gummosa*. We'll describe this unrecorded species in more detail.
[This work was supported by the National Institute of Biological Resources [NIBR 201701104], the National Research Foundation (NRF 2015M3A9B8029237) and the Korea National Arboretum (KNA-18-C-09)]

Keywords: *Pholiota*, *Pholiota gummosa*, ITS, Reverse taxonomy, Agaricales

A025

Flavobacterium microcysteis* sp. nov., Isolated from a Culture of *Microcystis aeruginosa

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A Gram-stain-negative, yellow-pigmented, strictly aerobic bacterial strain, designated MaA-Y11^T, was isolated from a culture of *Microcystis aeruginosa* in South Korea. Growth of strain MaA-Y11^T was observed at 25–37°C (optimum, 30°C) and pH 6.0–9.0 (optimum, pH 7.5) and in the presence of 0–1.0% (w/v) NaCl (optimum, 0%). Strain MaA-Y11^T contained iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) as major cellular fatty acids and menaquinone-6 as the sole isoprenoid quinone. Phosphatidylethanolamine, three unidentified aminolipids and four unidentified lipids were detected as major polar lipids. The G+C content of the genomic DNA was 37.0 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that MaA-Y11^T formed a phyletic lineage with *Flavobacterium lindanitolerans* IP-10^T within the genus *Flavobacterium*. Strain MaA-Y11^T was most closely related to *F. lindanitolerans* IP-10^T with a 98.85% 16S rRNA sequence similarity and shared less than 93.87% sequence similarities with other type strains. Average nucleotide identity and *in silico* DNA-DNA hybridization values between strain MaA-Y11^T and the type strain of *F. lindanitolerans* were 87.0% and 32.3%, respectively. Here it was proposed that strain MaA-Y11^T represents a new species of the genus *Flavobacterium*, for which the name *Flavobacterium microcysteis* sp. nov. is proposed. The type strain is MaA-Y11^T (= KACC 21225^T = JCM 33501^T).

Keywords: Novel, Bacteria, *Flavobacterium microcysteis*, New taxa

A026

A Novel Bacterium Candidate belonging to the Family *Rhodospirillaceae*

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A novel marine bacterial strain, designated strain HT1-32, which is an aerobic, Gram-stain-negative, catalase- and oxidase-positive, motile and rod shaped, was isolated from coastal seawater, Tongyeong of Korea. Growth occurred between 15–35°C (optimum at 30°C) and 0–5.5% (w/v) NaCl (optimum at 2.5–3.0%). On API Zym kit test, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase were positive. Comparative phylogenetic analysis based on the 16S rRNA gene sequence showed that the strain HT1-32 was a member of the family *Rhodospirillaceae* and most closely related to *Aliidongia dinghuensis* 7M-Z19^T with 90.0% sequence identity. The draft genome sequence of strain HT1-32 contains 4,423,878 bases with G+C content of 57.7%, 4,116 CDSs, 48 tRNAs, 5 rRNAs and 1 tmRNA. On the basis of phylogenetic analyses with low 16S rRNA gene sequence similarity, strain HT1-32 is considered to represent a novel species into the family *Rhodospirillaceae.*
[Supported by NRF-2017R1D1A3B04033871]

Keywords: Rhodospirillaceae, Phylogenetic analysis, Draft genome sequence

A027

Phenotypic and Genomic Analysis of a Novel Bacterium of the Genus *Kriegella,* Isolated from Coastal Seawater of Korea

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An aerobic, Gram-stain-negative, and flexirubin-type pigment producing bacterium, designated strain EG-1, was isolated from coastal seawater, Guryongpo of Korea. Phylogenetic analyses based on 16S rRNA gene sequence indicated that strain EG-1 was affiliated to the family *Flavobacteriaceae* and showed highest similarity to *Kriegella aquimaris* KMM 3665^T (94.3%). Assimilation of mannitol, hydrolysis of agar and enzyme activities of cystine arylamidase, trypsin and *α*-fucosidase were able to distinguished between the strain EG-1 and *Kriegella aquimaris* KMM 3665^T. The draft genome of the strain EG-1 comprised 4,449,445 bp, with the G+C content of 33.5%. The average nucleotide identity (ANI) and the values of percentage of conserved proteins (POCP) between the strain EG-1 and *Kriegella aquimaris* KMM 3665^T were 71.4% and 65.5%, respectively. Based on the phenotypic characteristics, phylogenetic and genome analysis, strain EG-1 should be classified as a novel species in the genus *Kriegella*.
[Supported by NRF-2017R1D1A3B04033871]

Keywords: Kriegella, Phenotypic characteristics, Phylogenetic and genome analysis

A028

Taxonomy and Antimicrobial Potential of *Micromonospora* sp. Isolated from Riverside Soil

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Following the survey on the taxonomic diversity and antimicrobial potential of the genus *Micromonospora*, an isolate designated *Micromonospora* sp. R_77 exhibited an outstanding antimicrobial activity, and was thus subjected to further characterization. Based on the 16S rRNA gene sequence analysis, strain R_77 was mostly related to the type strains of *M. avicenniae* and *M. echinospora* with the sequence similarities of 99.00 and 98.93%, respectively. Strain R_77 could grow well on ISP7 medium at 30°C and possessed optimal culture condition in pH 6 and 1% salt concentration. The strain utilized monosaccharides and disaccharides as the sole carbon sources and aliphatic and aromatic amino acids as a sole nitrogen sources. The strain showed polymer degrading activity such as starch, lipid and carboxymethylcellulose. Furthermore, the strain was active against Gram positive bacteria and yeasts, whereas little activity was observed against Gram-negative bacteria or filamentous fungi. The PCR based screening of biosynthetic genes also yielded positive results for polyketides synthetase type Ⅰ, Ⅱ and non-ribosomal peptide synthetase. The ongoing analysis includes search for optimal culture conditions for antimicrobial activity, detection of antimicrobial compounds using high performance liquid chromatography-mass spectrometry (HPLC-MS), and also extraction and purification of the compounds as well as chemotaxonomic characterization.

Keywords: Micromonospora, Taxonomy, Antimicrobial activity

A029

Evolutionary Analysis and Protein Family Classification of Chitin-related Genes

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Cryptococcus neoformans is a fungal pathogen causing cryptococcal meningoencephalitis. In fungi, the chitin deacetylases (CDAs) are important for defense mechanism, morphogenesis and development. The *C. neoformans* genomes (H99, JEC21, and B-3501A) have three or four CDAs. Cryptococcal CDAs show deacetylation activity against the surface of insoluble chitins *in vitro*. Interestingly, one (CDA2/MP98) of them describes as immunogenic and eliciting T cell responses. Previous studies show fungi CDAs can be separated into two groups, cell-wall and infection autolysis types. However, protein family classification of whole chitin-related genes (CRGs) is not established. Here, we attempted to resolve the CRG protein classification using the phylogenetic analysis and the protparam analysis. We obtained 156 sequences (48 fungi, 31 bacteria, and 1 amoeba) from NCBI. A phylogenetic tree appeared that CRGs can be divided into two branches; the CDA I group are clustered in CDAs and other chitin-related proteins from amoeba, bacteria and fungi. The CDA II group are composed of other fungal CDAs and cryptococcal CDAs. The difference is the presence of a carbohydrate binding domain (CBD) and glycosylphosphatidylinositol (GPI). Whereas the CDA I group has the CBD domain, CDA II group has the GPI anchor as a motif for cell surface localization. This study illustrates the most comprehensive comparative analysis of fungal CDAs and provides a new insight of the protein family classification of CRGs.

Keywords: *Cryptococcus neoformans*, Chtiin deacetylase, Protein family classification

A030

Isolation of Wild Yeasts from Soils, Waters and Flowers of Nakdong River of Korea and Characteristics of Unrecorded Yeasts

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The goal of this study were to isolate wild yeasts from soils, waters and flowers of Nakdong river, Korea and characterize their unrecorded wild yeasts. Fifty eight strains of sixteen species of wild yeasts including *Rhodosporidium fluviale* NKS 1-1 were isolated from 55 soils samples. Twenty five of sugar tolerant wild yeasts and ten halotolerant wild yeasts were also isolated. *Sporisorium graminicola* NK 9-2 from soils and *Candida davisiana* NNK 8-2 from water of Nakdong river represented a newly recorded yeast strains in Korea. Microbiological characteristics of theses unrecorded yeasts were investigated. Unrecorded yeasts, *Sporisorium graminicola* NK 9-2 and *Candida davisiana* NNK 8-2 are oval shape and ellipsoidal, respectively.

Keywords: Nakdong river, Wild yeasts, Soils, Waters, Flowers

A031

Description of *Flavobacterium hydrocarbonoxydans* sp. nov., Isolated from Polluted Soil

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This study presents polyphasic analysis to describe the taxonomic position of a Gram-negative bacterial strain designated GA093^T, isolated from polluted soil. Phylogenetic analysis based on 16S rRNA gene sequence similarities indicated that strain GA093^T is a member of the genus *Flavobacterium*, and showed the high sequence similarity with *Flavobacterium psychroterrae* CCM8827^T (98.16%), *Flavobacterium panaciterrae* DCY69^T (98.06%) and *Flavobacterium aquidurense* DSM18293^T (98.05%). Strain GA093^T was facultative anaerobic, and could grow at temperature range from 4 to 33°C (optimum 30°C), pH range pH 6–11 (optimum pH 7), and in the presence of 0–2% (w/v, optimum 0%) NaCl. The API 50CH test indicated that strain GA093^T was capable of producing acid from various carbon sources compared to other related species of *Flavobacterium*. The strain contained MK-6 as the only isoprenoid quinone, and iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:0}$ 3-OH and a summed feature comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$ as the major cellular fatty acids. The constituents of major polar lipids were phosphatidylethanolamine, phosphatidylinositol, unidentified aminolipids, unidentified glycolipids, and an unidentified aminophospholipid. On the basis of polyphasic analysis, strain GA093^T was found to have distinguished properties as a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium hydrocarbonoxydans* sp. nov. is proposed.

Keywords: Flavobacterium, Polyphasic taxonomy, New species

A032

Taxonomic Analysis and Plant Growth Promoting Properties of *Paenibacillus chungnamensis* sp. nov., Isolated from Soil

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In order to establish the taxonomic location of MMS18-CY102 isolated from soil, a polyphasic study was conducted that combines phylogenetic analysis, chemotaxonomic analysis, and phenotypic analysis. Phylogenetic analysis revealed that MMS18-CY102 is a species belonging to the genus *Paenibacillus*. The strain formed a new evolutionary lineage in the genus *Paenibacillus*, with the highest similarity to *P. curdlanolyticus* (98.2%), *P. cellulosilyticus* (97.65%) and *P. kobensis* (97.02%), respectively. The isolate strain grew at 20–37°C (optimal growth at 30°C), pH 6–9 (optimal growth at pH7), and 0–0.8% NaCl (optimal growth at 0%) in TSA medium. The isolate possessed MK-7 as the major quinone, anteiso-$C_{15:0}$ as the major fatty acids, ribose and glucose in the cell wall, and diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidyl ethanolamine (PE) as the diagnostic polar lipids. Regarding the plant growth promoting properties, MMS18-CY102 was able to carry out nitrogen fixation, phosphate solubilization, siderophore production and indole-3-acetic acid (IAA).

On the basis of polyphasic study, the strain MMS18-CY102 is proposed to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus chungnamensis* sp. nov. is proposed. The type strain is MMS18-CY102.

[This work was supported by the National Institute of Biological Resources (NIBR) of the Ministry of Environment (MOE), Republic of Korea.]

Keywords: *Paenibacillus*, New species, Polyphasic taxonomy

A033

Novel Subspecies Candidate *Streptococcus anginosus* subsp. nov. Isolated from a Clinical Specimen in Korea

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National Culture Collection for Pathogens (NCCP) collects strains that are non-identified among clinical isolates and determines whether they are simple non-identified strains or novel species pathogens.
Initial identification of the non-identified strains were performed using MALDI-TOF MS and 16S rRNA gene sequencing. The 16S rRNA gene sequence similarity was calculated by comparing with sequences on the EzTaxon server. The whole-genome average nucleotide identity (ANI), a robust method for measuring genetic similarity, was calculated by OrthoANI. A phylogenetic tree was constructed by the neighbor-joining and maximum-likelihood methods with MEGA 6.0. Biological activity test was performed in comparison to reference strains (*Streptococcus anginosus* subsp. *anginosus* DSM 20563T).
The isolate (KS 6) was identified as *S. anginosus* by MALDI-TOF MS. Genetic similarity analysis between KS 6 and DSM 20563T was analyzed through 16S rRNA and ANI with 98.32% and 94.23% similarity respectively. In addition, in the quinone analysis results, the KS 6 had the MK-8, while the DSM 20563T had the MK-6 and MK-7. Also, according to the polar lipids analysis, DSM 20563T had PIMs 1, but not KS 6. Based on these results, KS 6 was considered as a novel subspecies.
We will distribute the characteristics and informations of the novel species pathogens discovered to health and medical treatment researchers.

Keywords: Novel subspecies

A034

Genetic Diversity and Toxin Genotypes for *Clostridium perfringens* 25 Strains Isolated from Human in South Korea

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The species of *Clostridium perfringens* were both a ubiquitous environmental bacterium and a major cause of human intestinal tracts disease, and toxins produced by this bacterium play a key role in pathogenesis.
This study focuses on the genetic diversity and toxin genotypes of *C. perfringens* isolated (N=25) from Korean patients with positive control strains (N=6). Multilocus sequence typing (MLST) analysis was performed on eight housekeeping genes (gyrB, sigK, sod, groEL, pgk, nadA, cola and plc). Classification of *C. perfringens* into the five toxin types A–E is based on the presence of the major toxins genes cpa(α), cpb(β), etx(ε) and iap(ι) by PCR. Type A strains have only α gene, type B α, β and ε genes, type C α and β genes, type D α and ε genes, and type E α and ι genes.
MLST analyses of total 31 *C. perfringens* strains clusterd kind of 23 different alleles; among the one strain was 11 sequence type (ATCC 3624), 22 different alleles were new and gene fragments varied in length from 950 to 650 bps. Also among the 31 strains, 25 strains were type A, 4 strains were type B, 2 strains type E. 3 strains were positive for the enterotoxin cpe gene.
Access to such databases will help international collaboration and support the global surveillance of *C. perfringens* pathogens. Genetic profiles dataset will use to standard for registration in NCCP

Keywords: Genotype, *Clostridium perfringens*

A035

Genomic Classification of *Campylobacter jejuni* Isolated from Korea

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The genomic classification of *Campylobacter Jejuni* isolated from Korea could provide detailed information on the genetic features of these foodborne pathogens in Korea. A total of 44 *Campylobacter Jejuni* was sequenced based on the Illumina NextSeq platform (2 × 150 bp read length) and phylogenetic analysis was conducted. All strains in this study formed the high quality of draft genomes with more than 100x coverage. Filtered sequence reads (> Q20) were assembled using CLC Genomics Workbench 12.0 and scaffolds were annotated by RAST (Rapid Annotation using Subsystem Technology) pipeline. Average Nucleotide Identity (ANI) was used to classify clusters based on similarity of genomes. The major cluster was selected for SNP analysis to identify variants between strains in same cluster isolated from Korea. Analysis of cluster with annotated gene profiles will provide more detailed phylogenetic insight to develop detection methods or control methods for this foodborne pathogen.

Keywords: *Campylobacter Jejuni*, Genomic classification, Average Nucleotide Identity (ANI)

A036

***Oricola sediminis* sp. nov., a Marine Bacterium Isolated from Tidal Flat Sediment**

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A Gram-negative, aerobic, rod-shaped (1.8–4.4 μm × 0.5–1.2 μm) and motile marine bacterium, designated as MEBiC13590^T was isolated from tidal flat sediment of the Incheon province, West Korea. The 16S rRNA gene sequence analysis revealed that strain MEBiC13590^T showed high similarity with the *Oricola cellulosilytica* CC-AMH-0^T (96.7%). Growth was observed at 22.2–50.9°C (optimum 45°C), at pH 5–9 (optimum pH 7) and with 1-6 % (optimum 3%) NaCl. The predominant cellular fatty acids were $C_{16:0}$ (7.6%), $C_{18:0}$ (12.2%), 10-methyl $C_{18:1}\omega7c$ (5.7%), $C_{19:0}$ cyclo$\omega6c$ and summed feature 8 (comprised of $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; 38%). The DNA G+C contents is 66.1 mol%. The major respiratory quinone is Q-10. Several phenotypic characteristics such as esculin, gelatin and Enzyme activities of Lipase (C14), α-chymotrypsin, Acid phosphatase, β-galactosidase and β-glucosidase differentiate strain MEBiC13590^T from *Oricola cellulosilytica* CC-AMH-0^T. On the basis of this polyphasic taxonomic data, strain MEBiC13590^T should be classified as a novel species in the genus *Oricola* and it is proposed as *Oricola sediminis* sp. nov. The type strain is MEBiC13590^T (=KCCM 43313^T).
[Supported grants from KIOST & MBRB.]

A037

Isolation of Wild Yeasts from Riverside Flowers and Soils of Aramchan Bridge and Daechung Birdge in Geumgang Midstream of Korea and Characteristics of Unrecorded Wild Yeasts

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The goal of this study was to elucidate wild yeast diversity of Geumgang midstream near Sejong city, Korea. Fifty four strains of twenty eight species of wild yeasts were isolated from 40 flowers and soils samples around the Aramchan bridge and Hapgang park of Sejong city, Korea. *Moesziomyces aphidis* (11 strains) and *Aureobasidium pullulans* (8 strains) were dorminantly isolated from samples. Among them, 28 wild yeasts were halotolerant and 4 strains were also psychrophilic. Twenty five strains of eighteen species of wild yeasts were isolated from 25 flowers and soils samples under the Daechung bridge of Sintanjin in Daejeon city, Korea. *Cryptococcus* spp. including *Cryptococcus flavus* and *Cryptococcus aureus* were dorminantly isolated from samples. Among them, *Sakaguchia dacryoidea* NCD 9-1 was thermotolerant and halotolent strain. *Sporobolomyces longiusculus* HD 31-1 and *Sakaguchia dacryoidea* NCD 9-1 represented also newly recorded yeast strains in Korea. Microbiological characteristics of theses unrecorded yeasts were investigated.

Keywords: Geumgang midstream, Wild yeasts, Riverside flowers, Soils, Unrecorded wild yeasts

A038

Isolation and Characterization of Nasal *Staphylococcus*

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Changwon National University

The species belonging to genus *Staphylococcus* are ordinary residents on human skin. They are commensal and sometimes pathogenic. For instance, *S. aureus* is a frequent cause of clinically relevant infections. In this study, we isolated 31 strains of *Staphylococcus* from the human nasal cavity. The16S rRNA gene sequence followed by database search showed that the predominant species was *Staphylococcus epidermidis* (19 strains), *Staphylococcus aureus* (9 strains) and 3 other strains. Identification of the species was also confirmed by amplicons produced by RAPD PCR using a primer $(GTG)_5$. Besides, when the antibacterial activity against *Micrococcus luteus* was tested with all isolated strains, only one strain *S. epidermidis* Sep20 was most active. In addition, the API ZYM test showed that the isolated strains possess activities of alkaline phosphatase, esterase, esterase lipase, and naphtol-AS-BI-phosphohydrolase. The isolated strains were catalase-positive except *S. aureus* (Sar 7), *S. cohni* (Sco 1), *S. warneri* (Swa 1), and *S. capitis* (Sca 18). We tested growth on the agar medium containing two different concentrations of skim milk for extracellular protease activity, and we found that most *S. epidermidis* produced extracellular protease with variable activities.
[This research was granted by IRB and supported by the Basic Science Research Program through the National Research Foundation of Korea (No. 2016R1D1A1B01007775)].

Keywords: *Staphylococcus*, Skin, Nasal cavity

A039

The Genus *Relicina* and New Record of Parmeliaceae with Bulbate Cilia from Vietnam

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The genera *Relicina* (Hale & Kurok.) Hale and *Bulbothrix* Hale were segregated from *Parmelia* on the basis of their marginal bulbate cilia. These two genera differ in the secondary metabolites of their upper cortex, that *Relicina* contain usnic acid and *Bulbothrix* have atranorin. During the field excursion in the southern part of Vietnam, some specimens of Parmeliaceae with bulbate cilia were collected. In current study, morphological examination, chemistry and phylogenetic analysis on these samples were conducted in the laboratory. As a result, they belong to two genera, *Relicina* and *Bulbothrix*, of which the genus *Relicina* was first reported from Vietnam. In these two genera, one species *Relicina albicans* D. Liu & J.S. Hur is newly described, three species *Relicina gemmulosa* (Kurok.) Streimann, *Relicina abstrusa* (Vain.) Hale and *Bulbothrix asiatica* Y. Y. Zhang & Li S. Wang were newly reported from Vietnam. The illustration and key to the two genera *Relicina* and *Bulbothrix* in Vietnam were given in this study.

Keywords: Lichen, Bulbothrix, New species, New records

A040

Description of Novel Strain *Corallincola* sp. 176GS2-150 from Marine Sponge

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A Gram-stain-negative, aerobic, straight or curved rods and formed cream-coloured colonies. designated strain 176GS2-150, was isolated from the marine sponge *Hymeniacidon sinapium* collected from the Yellow Sea coast of the Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain 176GS2-150 belonged to the family Corallincola_f and showed the highest 16S rRNA gene sequence similarity with *Corallincola spongicola* 176GS2-150T (96.58%). Strain 176GS2-150 grew at 15–30°C, with optimum of 25°C. The pH range for growth was between 6.5–8.0, with optimum of pH 7.0. The range of NaCl concentration for growth was between 1.0–9.0% (w/v), with an optimum of 2.0%. The DNA G+C content of strain 176GS2-150 was 49 mol%. The major fatty acids were Summed feature 3 ($C_{16:1}$ *ω6c*/$C_{16:1}$ *ω7c*), iso-C17:0. The major respiratory quinone was Ubiquinone-8. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol. On the basis of the polyphasic analyses, strain 176GS2-150 is considered to represent a novel species of the genus Corallincola.

Keywords: Marine sponge, 16S rRNA, Corallincola

A041

Description of Novel Strain *Histidinibacterium* sp. 176SS1-4 from Korean Saltfields

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A Gram-stain-negative, aerobic, non-spore-forming, motile, ovoid or short rod shaped bacterium, designated strain 176SS1-4, was collected from Korean saltfields. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain 176SS1-4 belonged to the family *Rhodobacteraceae* and showed the highest 16S rRNA gene sequence similarity with *Histidinibacterium lentulum* B17^T (95.04%). Strain 176SS1-4 grew at 10–37°C, with optimum of 25°C. The pH range for growth was between 6.5–8.5, with optimum of pH 7.0. The range of NaCl concentration for growth was between 1.0–6.0% (w/v), with an optimum of 2.0%. The DNA G+C content of strain 176SS1-4 was 66.23 mol%. The major fatty acids were iso-$C_{16:0}$ (12.9%), iso- $C_{19:0}$ cyclo *ω8c* (10.48%), Summed feature 8 ($C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*) (63.97%). The major respiratory quinone was Q-10. The major polar lipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol. On the basis of the polyphasic analyses, strain 176SS1-4 is considered to represent a novel species of the genus *Histidinibacterium.*

Keywords: Korean saltfields, *Histidinibacterium*, 16S rRNA, *Histidinibacterium lentulum*

A042

***Pontibacter oryzae* sp. nov., a Carotenoid-producing Species Isolated from a Rice Paddy Field**

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A taxonomic study using a polyphasic approach was performed on a Gram-stain negative, red-pink, aerobic, non-motile, asporogenous, rod-shaped bacterium, designated strain KIRAN^T, isolated from soil collected from a rice paddy field. The 16S rRNA gene sequence analysis showed that strain KIRAN^T is phylogenetically related to *Pontibacter actiniarum* KMM 6156^T, *Pontibacter korlensis* X14-1^T, *Pontibacter odishensis* JC130^T, *Pontibacter litorisediminis* YKTF-7^T and *Pontibacter aurantiacus* NP1^T (97.6, 97.5, 97.3, 97.3 and 96.7% sequence similarity, respectively). The major fatty acids of strain KIRAN^T were identified as iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH and summed feature 4. The predominant menaquinone was identified as MK-7. The polar lipid profile was found to be consist of phosphatidylethanolamine, four unidentified phospholipids, an unidentified glycolipid, an unidentified aminolipid and four unidentified lipids. The genome of strain KIRAN^T has a G+C content of 48.3 mol%. The *in silico* DNA-DNA hybridization and average nucleotide identity values between strain KIRAN^T and the closely related strains *P. actiniarum* KMM 6156^T and *P. korlensis* X14-1^T, were 21.2%/ 21.8% and 76.4%/75.1%, respectively. On the basis of the data from phenotypic tests and genotypic differences between strain KIRAN^T and its close phylogenetic relatives, strain KIRAN^T is concluded to represent a new species belonging to the genus *Pontibacter*, for which the name *Pontibacter oryzae* sp. nov. is proposed. The type strain is KIRAN^T (=KACC 19815^T =JCM 32880^T).

Keywords: Paddy field, Carotenoid, Phylogenetic analysis, Cell morphology, New taxa

B001

Degradation of Industrial Solvent, 1,4-Dioxane by Mixed Cultures of Bacteria

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We explored the feasibility of using mixed cultures for 1,4-dioxane degradation, with the ultimate aim of application for wastewater treatment. The present study reports on mixed cultures of bacteria which were developed to grow aerobically with 1,4-dioxane as the sole carbon substrate. Experiments were conducted to study the effects of 1,4-dioxane concentration, pH, temperature, and inocula on the degradation of mixed cultures in media containing 1,4-dioxane (250–1,000 mg/L) as target substrate. Changes in the optical density at 600 nm associated with cell growth and the degradation of 1,4-dioxane were monitored and compared. Complete depletion of 1,4-dioxane was achieved in this experiment within 168 h. Degradation of 1,4-dioxane was verified by HPLC analysis of the residual 1,4-dioxane concentration in the test cultures. Four predominant 1,4-dioxane-grown isolates were screened, and 16S rRNA sequencing was performed to identify the isolates, which were assigned to *Pedobacter nanyangensis*, *Rhodococcus ruber*, *Shinella granuli*, and *Shinella zoogloides*, respectively.

Keywords: 1,4-Dioxane, Bacterial degradation, Mixed culture, Industrial solvent

B002

A Case Study of Fungal Communities on *Zelkova serrata*, a Kind of Legally Protected Tree, Republic of Korea

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In Korea, legally protected trees refer to trees that are more than 100 years old and it is also important academically and culturally. The *Zelkova serrata* (tree age: 500 years old), located in Suwon city, was designated as a legally protected tree in 1982. The summer of 2018, this tree fell down due to heavy rain and wind. Although it was a healthy tree when viewed with the naked eye, a broken tree caused by wind weakened the durability of the wood. Therefore, we investigated the fungal community composition and richness in relation to differences in decomposing sapwood and heartwood of the tree. Based on next generation sequencing (NGS), 4 phyla, 22 orders, and 97 genera were confirmed in the tree. Ascomycota was most detected in sapwood. However, abundant Basidiomycota causing white-rot was contained more diverse species in heartwood during wood decomposition, but little in sapwood. The Pleosporales was dominant in sapwood and Coniochaetales was dominant in heartwood. The composition rate by genus showed that the genera *Pyrenochaeta*, *Exophiala*, *Coniochaeta*, and *Periconia* belonging to Ascomycota were the most. In conclusion, dead wood seems to be weakened fungal communities and increased potential tree decomposer. Our results provide a case study of fungal community structure on legally protected tree for the first time in Korea.
[Supported by grants from Korea National Arboretum (project No. KNA1-1-25, 19-2)].

Keywords: Deadwood, Diversity, Macrofungi, Species richness

B003

Antifungal Activity against Mycotoxigenic Fungi and Mycotoxin Removal by *Streptomyces* spp.

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This study aims to evaluate the capabilities of inhibition against mycotoxigenic fungi and mycotoxins removal by *Streptomyces sporoverrucosus* JS383, *S. lavendulae* JS669 and *S. shenzhenesis* YR226. Mycotoxin, a group of secondary toxic metabolites produced by filamentous fungi, is extremely toxic, carcinogenic and mutagenic. JS383, JS669 and YR226 removed aflatoxin B_1, ochratoxin A and fumonisin B_1 (100 μg/L each) by 81.5 to 97.1% in nutrient broth (72 h, 30 or 37°C). They showed a good thermostability in mycotoxin removal up to 60°C. The target organisms of antifungal activity were 10 mycotoxigenic fungi (3 aflatoxigenic *Aspergillus flavus*, 3 ochratoxigenic *Aspergillus* spp. and 4 fumonisinigenic *Fusarium* spp.). They produced inhibition zones of 5.7 to 25.3 mm dia. against all target organisms in a disc diffusion test. JS383, JS669 and YR226 suppressed sporulation of all target fungi up to 94.7, 99.2 and 99.9%, respectively, and also inhibited spore germination of target organisms (72.3–100.0%). They produced siderophore up to 113.2, 110.2 and 96.8 μM, respectively, and showed maximum chitinase production of 1.4, 1.6 and 2.1 U/ml, respectively. YR226 also produced biosurfactant that had antifungal activity. These results suggest these strains can be used for biological method for mycotoxin removal and toxigenic fungi inhibition in food and feed industry.

Keywords: Mycotoxigenic fungi, Antifungal activity, Antifungal substances

B004

Biodegradation of Diesel and Aromatic Hydrocarbons by Psychrophilic Bacteria Isolated from Arctic Soil

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In recent years, widespread consumption of petroleum products in cold climate area has led to soil pollution at cold site. Petroleum hydrocarbon contamination has received significant attention because the compound is fatal to human. Bioremediation has been considered to be more effective and less expensive option to remove petroleum products, causing less damage on cold climate environment. Diesel-degrading bacterium was isolated from the Arctic soil using enrichment culture technique. The isolates were identified by 16S rRNA gene sequence analysis. Growth profiles were assessed by measuring the absorbance at wavelength 600 nm on different temperature (4, 10, 18, and 28°C). Diesel and BTEX- degrading ability was analyzed by GC-FID and GC-MS. 9 diesel-degrading bacteria species isolated from Arctic soil were identified as *Pseudomonas* spp. (ML15R14, ML15P11), *Pseudarthrobacter* spp. (AL16L05, B33P09, ML15P08), *Arthrobacter* spp. (ML15L13, B33L26), *Streptomyces* sp. (ML15L03), *Massilia* sp. (ML15P13) by 16S rRNA gene sequence analysis. These bacteria species utilized diesel as a sole carbon source and energy for their growth. Three species were characterized by maximal activity at 4°C, 5 species at 10°C and 1 species at 18°C. Nine bacteria species utilized 83.1–50.5% of total diesel (200 mg/L) in 15 days at their optimum growth temperature. Among nine bacteria, strain ML15R14 could degrade BTEX mixture (initial concentration of each BTEX compounds, 30 mg/L) in 12 days at 10°C.

Keywords: Biodegradation, Aromatic hydrocarbon, Bacteria, Low temperature

B005

Inhibition of Sporulation and Aflatoxin B_1 Production of Aflatoxigenic *Aspergillus flavus* by Bacteria on Peanut

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Aspergillus spp. contaminate stored food and feed and produce a carcinogenic aflatoxin B_1 (AFB_1). The purpose of this study is to examine the inhibition of sporulation and AFB_1 production of aflatoxigenic *Aspergillus flavus* strains (KACC 44986, 45068, 45146) by *Bacillus subtilis* YR47 and *Streptomyces lavendulae* JS669 in peanut. Pre-inoculation of washed YR47 cells on peanuts could inhibit sporulation of *A. flavus* KACC 44986, 45068 and 45146 that applied lately, up to 92.93, 92.46 and 82.61%, respectively (100 rpm, 7 days, 30°C). While whole culture of YR47 reduced over 97% of sporulation of all three target fungi under the same conditions. Washed JS669 cells showed 86.72, 85.76 and 70.24% of sporulation inhibition against *A. flavus* KACC 44986, 45068 and 45146, respectively on peanuts, and JS669 culture displayed sporulation inhibition from 80.04 to 89.90%. When YR47 and JS669 were pre-inoculated on peanuts, AFB_1 produced by *Aspergillus flavus* KACC 44986 was not detected. These results indicated that *Bacillus subtilis* YR47 and *Streptomyces lavendulae* JS669 could prevent sporulation and AFB_1 production by *Aspergillus flavus*, and may be utilized as a biocontrol agent to reduce economic damage and health threat to human and animals in agriculture and food industry.

Keywords: Mycotoxigenic fungi, Antifungal activity, Antifungal substances

B006

Antifungal Activity and Synergistic Effects between *Streptomyces* Strains against Mycotoxigenic *Aspergillus* spp.

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The aim of this study is to evaluate antifungal activity of *Streptomyces* strains DS1379, DS1548, DS1923 and DS1938 against *Aspergillus alutacues*, *A. awamori* and *A. fresenii* that produce ochratoxin A and synergistic effects between 4 *Streptomyces* strains. All *Streptomyces* strains produced inhibition zone against 3 ochratoxigenic fungi. They also inhibited sporulation (~99.95%), spore germination (~98.53%) and mycelial growth (~95.02%) of target fungi. Consortium of DS1379 and DS1548 completely inhibited sporulation of *A. alutaceus* and *A. awamori,* and DS1379 and DS1923 combination indicated synergistic effects against all target *Aspergillus* strains. Spore germination of *A. alutaceus* was completely inhibited by synergistic effects of DS1379 and DS1938 combination. In mycelial growth inhibition, combination of DS1923 and DS1938 showed high synergistic effects as much as 99.89% against *A. alutaceus* and *A. awamori*, and DS1379 and DS1938 combination inhibited *A. alutaceus* and *A. awamori* up to 98.43%. Chitinase was produced by DS1548 and DS1923, and siderophore was detected in DS1379, DS1923 and DS1938 as antifungal substances. Only DS1938 could produce cellulase. Based on these results, 4 *Streptomyces* strains may be utilized as an environment-friendly biocontrol agent against ochratoxigenic fungi.

Keywords: Mycotoxigenic fungi, Antifungal activity, Antifungal substances

B007

Isolation of Phototrophic Purple Bacteria *Porphyrobacter* sp. and Evaluating Their Potential as PGPR Inoculant

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Phototrophic purple bacteria (PPB) have been isolated from rice paddy fields by combining the culturomic methods and molecular marker (*puf*LM gene) detection. These PPB were categorized as alpha proteobacteria (479 isolates), Firmicutes (5 isolates), and Actinobacteria (16 isolates) by 16S rRNA gene sequence analysis. Sixty-four percent of the total PPB were *Porphyrobacter* sp. belong to α-proteobacteria. Twenty-three of the total PPB isolates were able to produce indole-3-acetic acid (IAA), and production ranged from 170 to 1,970 μM OD_{540}^{-1}. These isolates were initially screened by employing seed germination and seedling vigor assays to evaluate their potential as inoculants. All of the isolates showed the same germination quality in cucumber as the non-treated control. The *Porphyrobacter* sp. COR-2 highest seedling vigor index (the number of lateral root), followed by PF-30 and the MO-37 isolates. Accordingly, the *Porphyrobacter* sp. COR-2 was selected for further experiments. We assessed the PGPR effects of the rice seedling with approximately 4.0×10^6 CFU of the COR-2 inoculant and *Rhodobacter capsulata* KACC 15298^T type strain applied to each plug tray. The promoted plant growth (fresh weight and root length) of COR-2 that was significantly greater than type strain and non-inoculated control, suggesting it has the potential PGPR inoculant.
[Supported by grants (Project No. 918016-4) from Agricultural Microbiome R&D Program.]

Keywords: Phototrophic purple bacteria, PGPR, *Porphyrobacter* sp.

B008

Effects of Cover Crops and Rice Cultivation on Community-level Physiological Profiles of (CLPP) of Soil Bacteria in Paddy Fields during the Non-growing Season

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National Institute of Agricultural Sciences

Soil bacteria perform ecological functions such as organic matter decomposition and nutrient cycling in paddy fields. The purpose of this study was to investigate the effects of cover crops and rice cultivation on the utilization of carbon resources by soil bacteria during the non-growing season. The Biolog EcoPlate was used for analysing community-level carbon substrate utilization profiles of soil bacteria. Effects of cover crops including *Brassica napus* and *Lolium multiflorum* soil bacteria were tested during the non-growing season. Cultivated paddy fields were also compared with adjacent interface of mountain. Cover crops altered bacterial utilization of carbon sources, as reflected by the increased utilization of amines under *Lolium multiflorum* culture. But PCA and MRPP showed that CLPP results were not separated among different groups. The utilization of carbon sources including amino acids and amines were greater in paddy fields than in interface soils. PCA and MRPP ($P = 0.02$) showed that CLPP of soil bacteria was different between interface of mountain and paddy fields. The results show that cover crops have a minimal impacts on the soil bacterial functioning in relation to carbon substrate utilization. It shows that rice cultivation greatly alters physiological profile of the soil bacterial community.

Keywords: Biolog Ecoplate, Carbon, Cover crop, Paddy fields, Spatial characteristics

B009

The Effects of Genetically Modified Herbicide Tolerant Soybean on Soil Microbial Community

Hyosun Lee[1], Dae Young Kim[2], So Yi Chae[2], Ji Yeon Han[2], Gi Bong Jang[2], and Dong-Uk Kim[2*]
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With the advance of gene technology, genetically modified (GM) crops have increased in recent years. GM crops offer us various benefits. But there are potential risks of GM crops on the environment. In this study, the impacts of transgenic plants on soil microbial community structures were assessed by using both cultivation and molecular methods. We using the total viable count and OTU-based community profiling with Illumina MiSeq platform for measure the changes of microbial density over time between GM and non-GM plants. The results showed that the microbial dynamics of GM subplots were quite similar compared to non-GM subplots. Only the density of Rhizobium associated with legume plants increased in soybean soils. This study showed that the bacterial communities of the experimental field soils were not significantly affected by cultivation of GM soybean. There were not meaningful differences between GM and non-GM lines based on culture-dependent and molecular approaches.
[This work was supported by the National Research Foundation of Korea Grant (NRF-2018R1D1A1B07047456)]

Keywords: GMO, Soybean, Metagenome, Soil microorganism

B010

Methylobacterium algicola* sp. nov., Isolated from Freshwater Alga, *Synura petersenii

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A Gram-stain-negative, strictly aerobic bacterium, designated strain SyP6R^T, was isolated from a freshwater green alga *Synura petersenii* in Nakdong river of South Korea. Cells were motile rods showing catalase- and oxidase-positive activities. Growth of strain SyP6R^T was observed at 20–35°C (optimum, 30°C) and pH 5.0–7.0 (optimum, pH 7), and in the presence of 0–0.25% (w/v) NaCl (optimum, 0.25%). The G+C content of the genomic DNA was 69.7 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain SyP6R^T formed a formed a phyletic lineage with *Methylobacterium platani* PMB02^T within the genus *Methylobacterium*. Strain SyP6R^T was most closely related to *Methylobacterium platani* PMB02^T, *Methylobacterium tarhaniae* N4211^T and *Methylobacterium aquaticum* DSM 16371^T with 98.6, 98.5 and 98.4% 16S rRNA sequence similarities, respectively. On the basis of phenotypic, chemotaxonomic and molecular analysis, strain SyP6R^T clearly represents a novel species of the genus *Methylobacterium*, for which the name *Methylobacterium algicola* sp. nov. is proposed. The type strain is SyP6R^T (= KACC 19923^T).

Keywords: *Methylobacterium algicola*, Alphaproteobacteria, Taxonomy, New taxa

B011

Ecological Revision on the Lichenicolous Fungi of Korea, with a New Species *Hydropisphaera phaeophysciicola* and Three New Records

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Lichenicolous fungi were unfascinated to mycologists and lichen taxonomists in the past. Studying the fungi is one of the most arduous tasks in collaboration of all morphological, anatomical, and molecular analyses, as well as detection of the fungi in the field. Difficulty in getting a nice hand-section due to their tiny size and little biomass of the fungi discourage microscopic and molecular works, respectively. This study comprehended all lichenicolous fungi records in Korea, and a statistical analysis in R revealed a significant positive relationships between the LF genera *Lichenochora*, *Lichenostigma*, *Rinodina* and *Taeniolella*, and the hosts *Heterodermia*, *Phaeophyscia* and *Pyxine* in the order Caliciales on barks in mid-elevated inlands, and between the LF genera *Endococcus*, *Lichenostigma*, and *Muellerella*, and the hosts *Aspicilia*, *Ochrolechia* and *Pertusaria* in the order Pertusariales on rocks in low-elevated areas of islands. *Hydropisphaera phaeophysciicola* Lee & Hur ad int. is described as a new lichenicolous fungus supported by morphological and molecular analyses. Three lichenicolous fungi, *Muellerella lichenicola*, *Stigmidium microspilum*, and *Vouauxiomyces santessonii* are introduced new to Korea. A surrogate key is provided to assist in the identification of all 31 taxa of *Hydropisphaera* in the world.

Keywords: Lichenicolous fungi, Taxonomy, Phylogeny, Biodiversity, Korea

B012

The Real-Time PCR for Monitoring of Toxigenic *Microcystis* spp. and the Potential Presence of Microcystin in Water

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Among harmful algal toxins produced by cyanobacteria, microcystins are mainly produced by *Microcystis* spp., and these can also be produced in part by *Anabaena*, *Oscillatoria*, and *Aphanizomenon*. In Korea, the algal blooming levels are determined by the number of counted algae. Although not all *Microcystis* spp. produce Microcystin, counting method for algae can't distinguish between toxin-producing *Microcystis* and non-toxin producing ones, therefore the amount of harmful *Microcystis* can be overestimated. In this study, we designed primers and probes for detecting *Microcystis* (16S rRNA) and toxin-producing *Microcystis* (mcy B), and optimized the reaction conditions for the real-time PCR. Each partial regions of two genes were cloned into the pUC57 vector to produce artificial positive control DNA in order to quantify gene copy numbers more simply and accurately. The detection limits for the real-time PCRs were 10^2 copies/reaction, and R^2=0.999 and 0.997, respectively. In the test using the field samples, the results from the K-water and NCKU (Taiwan)'s research groups were similar. These also showed that not only total *Microcystis* and toxin-producing *Microcystis* could be detected but also the ratio of toxin-producing *Microcystis* could be seen, indicating that the methods in this study could be used in general. Therefore, the real-time PCR method developed in this study could be used as a useful tool for detecting the harmful *Microcystis* and the potential microcystins in water.

Keywords: Cyanobacteria, Microcystin, Microcystis, Real-Time PCR, mcy B

B013

Evaluation of Biofilm Microbial Community Structure and Stability for Demonstration of Bio-stone Ball Contact Oxidation System

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The Bio-stone ball contact oxidation system is a method of fixing biofilm on a carrier having high porosity, which retains the typical advantages of the contact oxidation method in that the return sludge is not necessary and the treatment effect is stable. In this study, the microbial community structure and stability of biofilms attached to the carriers were analyzed to identify the mechanism of decontamination of domestic sewage and to secure the scientific basis for the introduction of the system. 16S rRNA sequencing was performed using a MiSeq system. As a result of analysis, an average of 324 genera were identified in the aerobic zone.
At the genus level, Gram-positive bacteria, *Mycobacterium* was most prevalent, followed by *Caldilineales* belonging to *Chloroflexi*, aerobic heterotrophs, *Parvibaculum*, and *Thiothrix*, an anaerobic sulfated bacterium. Analysis of heterotrophic bacterial counts by inoculation on PCA medium revealed that each Bio-stone ball collected from anaerobic zone contained about 2.1–3.3 x 10^9 CFU, and between 0.8–1.4 x 10^9 CFU per ball in aerobic zone. As a result of analysis of physiological activity of Bio-stone ball biofilm microorganisms using Biolog PM1 microplate, the number of substrates (S) used by microorganisms among 95 wells was as high as 89 per monthly average in the anaerobic zone, and the diversity of substrate utilization was found to be stable without significant change over time. [Supported by grants from Hallym University]

Keywords: Contact oxidation system, Biofilm, Bacteria

B014

Antibacterial Characteristics of Activated Carbon Coated with Silver Nanoparticles on *Escherichia coli*

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This study was conducted to evaluate the efficiency of antimicrobial activity of granulated activated carbon (GAC) coated with silver nanoparticles. The antibacterial properties of Ag/GAC were compared with a control GAC without silver nanoparticles in terms of growth and morphological change of *E. coli* observed under TEM. Comparisons between the GAC and Ag/GAC filters were conducted in a simulated water purification system by inoculating 100 ml of *E. coli* cell suspension (10^7 CFU/ml) onto the 80 L phosphate-buffered saline reservoir. Flow rate was set at 1.7 LPM and the filtration capacity of 80 L was filtered a total of 10 times. The final filtered water was inoculated on PCA medium, and the control and Ag/GAC filtrate showed 750 and 220 CFU/ml. The results were consistent with the extracellular enzyme activity of *E. coli* in filtrate tested using MUG-based analysis. The production of MUF from the Ag/GAC filter (17.3 RFU) compared to the control (25.0 RFU) suggested a higher enzyme activity in the control. Community-level physiological profile (CLPP) analysis using Biolog® GN microplate was also performed to measure metabolic potential index (MPI). As a result, MPI values were also higher in the control. After the completion of the test, biofilm sample retrieved from Ag/GAC filter revealed 86.7% inhibition compared to the control. Based on these results, Ag/GAC filter seemed to be an effective way to control microbiological contaminants and subsequently to improve the quality of treated water.
Keywords: Activated carbon, Silver nanoparticle, Biofilm, *E. coli*, Antibacterial

B015

Prevalence and Characterization of *Escherichia coli* Isolated from Groundwater in Nonsan City Area, S. Korea

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Groundwater is an important sources of water for drinking, industrial, and agricultural uses. So, contamination of water by fecal, pathogenic, or antimicrobial resistant bacteria is a major environmental and public health concern. In this study, we evaluated the groundwater chemical and biological quality in the Nonsan city area along the Noseong stream. We isolated *E. coli* from groundwater in Nonsan city area along the Noseong stream using mTEC agar at April and September, 2018. Total 169 isolates were characterized for phylogenetic groups using multiplex PCR approaches. As a result, 70/169 (41.42%) belonged to phylogroup A, 44/169 (26.04%) to phylogroup B1, 7/169 (4.14%) were B2 and 48/169 (28.40%) belonged to group D, respectively. Phylogroups A and D were the most predominant groups in study area. However, B2 groups possibly indicates the presence of pathogenic *E. coli*. Therefore, further studies are scheduled to assess risks associated with isolates harboring antibiotic resistance and/or pathogenic genes. Our results suggest that regular monitoring of rural and urban groundwater which used for drinking are required for public health.
[This research was supported by the Basic Research Project (GP2017-008) of the Korea Institute of Geoscience and Mineral Resources (KIGAM), funded by the Ministry of Science, ICT and Future Planning of Korea.]

Keywords: Groundwater, Multiplex PCR, *Escherichia coli*, Phylogroups

B016

Monitoring of Odor Material Produced by Cyanobacteria in North-Han River Watershed

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To effectively respond to the odor material (Geosmin, 2-MIB) produced by cyanobacteria, it is important to specify the odor-producing potential in the water column, in which cyanobacterial cells are distributed. This study detected the odor material producing potential and expression of cyanobacteria at the gene level in in the North-Han River watershed. Surface water samples were collected at all points during the cyanobacterial bloom period (July to September) in 2019 and the biofilm samples were scraped off from each stream site. DNA and RNA extract from all samples by a commercial kit. The potential and expression of odor synthesis genes (*gys1, mibC*) were analyzed by using the real-time PCR. In the North-Han River watershed, 2-MIB was the major odor materials produced by cyanobacteria, and the *mibC* genes were found at most sites. 2-MIB producing genes were observed not only in the surface sample but also in the biofilm samples. The 2-MIB synthesis gene copy number in the surface water increased in August from Sambong-ri to Jara Island. However, Geosmin synthesis genes were found only at the Paldang lake watershed in July. The trend of odor material synthesis genes caused by cyanobacteria in North-Han River watershed in this period was different from that of in 2015. This difference may be due to the change in the dominant cyanobacteria group from the *Dolichospermum* to *Pseudanabaena* because of the short retention time by heavy rainfall and change of hydrologic structures.

Keywords: North-Han River, Cyanobacteria, *mibC*, *gys1*, Odor material

B017

Isolation and Characterization of a Benzophenone-3-biodegrading Bacterium, *Rhodococcus* sp. S2-17

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Benzophenone-3 (BP-3) that has been widely used as a sunscreen agent is very recalcitrant to biodegradation and BP-3-biodegrading bacteria have not been reported yet. To isolate BP-3-biodegrading bacteria, an enrichment culture using BP-3 as a sole carbon source was established and subcultured four times. Community analysis of the final enrichment culture showed that *Rhodococcus*, *Achromobacter*, *Sphingobium*, and *Dyella* were dominant and bacterial strains belonging to the major genera were isolated. Biodegradation tests of the isolates revealed that only strain S2-17 belonging to the genus *Rhodococcus* had an ability to degrade BP-3 and it was able to grow using BP-3 as a sole carbon and energy source. The genome and transcriptome analysis of strain S2-17 showed that two operons containing cytochrome P450 and an unknown monooxygenase were highly upregulated by BP-3. Activity tests of cytochrome P450 and LC-QTOF-MS analysis revealed that cytochrome P450 had activity to convert BP-3 to BP-1. The unknown monooxygenase was also purified and its function will be characterized. In addition, we will propose the biodegradation pathway of BP-3 by strain S2-17 in more details in the poster section.

Keywords: Benzophenone-3, Biodegradation, Genomics, LC-QTOF-MS, Transcriptomics

B018

Colored Microbial Mats from Lava Tubes of the Jeju Island

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We have been evaluating microbial diversity of colored mats in Jeju volcanic caves. It is aiming to provide microbial diversity of the caves as part of the Cultural Heritage Administration project, which little is known. Samples were collected from 6 lava tubes including Manjang (MJ) and Yongcheon (YC) lava tube, while they may play roles in the subsurface ecosystem. It is to analyze microbial diversity found in lava tube from two different types of lava tubes whether the entrance is closed or open type. The management of the lava tubes should be careful, above all, the latter is extremely fragile with values. These studies will result in a multitude of new insights into the dynamics between microbes and environments and will have the potential to elucidate development of colorful wall mats. Sampling led to the characterization of members of a microbial community in wall mats of Lava Tubes. Samples were mainly taken from white or yellow mats forming on the surface of lava tubes. Preliminary procedures are ongoing for understanding whether the samples are microbial mat and what the source of wall color is. Metagenomic Next-Generation Sequencing (mNGS) will be performed using the single molecule, real-time (SMRT) sequencing that allows us whole metagenomic shotgun sequencing (Metagenomics). Further analyses of these data will provide insights into the roles they play in such lava tube environments.

Keywords: Lava tube, NGS, Sampling

B019

Fungal Diversity of Loose-flower Hornbeam (*Carpinus laxiflora*) Stand in Gwangneung Forest

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Gwangneung Forest (Pocheon-si, Korea) is one of the best natural forests in Korea and various plants, macrofungi, and animals have been recorded. From 2013 to 2014, investigation of fungal diversity was conducted in Loose-flower hornbeam (*Carpinus laxiflora*) stand of Gwangneung Forest. We have collected the macrofungi and soil samples while considering environmental parameters. Based on morphological characteristics, the collected macrofungi (130 specimens) were classified into 2 phyla, 5 classes, 13 orders, 32 families, 58 genera, and 102 species. Of those, *Omphalotus japonicus* belonging to Basidiomycota was a dominant species (3.08%). On the other hand, the soil-fungal communities were classified into 2 phyla, 12 classes, 30 orders, and 52 families 72 genera and 104 OTUs (14,936 sequence reads) using next generation sequencing (NGS). Although the diversity of genera collected from the macrofungi collections and the soil-fungal communities are totally different, they were detected 130 genera. In this study, we confirmed that species richness affects ecological community in both investigations. Therefore, further research needs to be addressed to fully understand the detailed environmental factors. This is the first study of fungal diversity as well as soil-fungal community of *C. laxiflora* stand in Gwangneung Forest.

Keywords: Abundance, Biodiversity, Mushroom, Species diversity

B020

Different Impact of Abiotic Factors on Fungal Communities in Arbuscular Mycorrhizal and Ectomycorrhizal Forests Soil (*Carpinus cordata* and *Fraxinus rhynchophylla*)

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Arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) forests are known to have different nutrient foraging strategies and soil properties, but their effect on soil fungal communities during seasonal change is less understood. Here, we examined the effect of a biotic factor (tree species) and abiotic factors (season, soil property) on fungal communities in soil of adjacent *Carpinus cordata* (ECM) and *Fraxinus rhynchophylla* (AM) forests in Mt. Jeombong (South Korea) with high-throughput Illumina MiSeq sequencing. Our analyses showed that effect of environmental factors on both fungal community composition and their ecological guilds were significantly different between AM and ECM forests. In *C. cordata* forest, ECM fungi such as *Inocybe*, *Sebacina*, or *Russula* were dominant, but in *F. rhynchophylla* forest, saprotrophic or pathogenic fungi such as *Mortierlla*, *Leohumicola*, or *Chaetomium* were mainly found. Overall, while seasonal and soil property effects were mostly significant in fungal communities in *F. rhynchophylla* forest soil, fungal communities in *C. cordata* forest were not distinguished by these factors. Collectively, our data provides an additional insight into fungal phylogeny, functionality and interactions in forests with different mycorrhizal associations.
[This study was supported by the research project for exploring potential fungal diversity in forest soil (KNA-18-C-09) from the Korea National Arboretum.]

Keywords: Arbuscular mycorrhizal (AM) fungi, Ectomycorrhizal (ECM) fungi, Plant-soil interactions, Mycorrhizal associations, Fungi

B021

Endophytic Fungi Isolated from Leaves of Various Plants in Ullengdo

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'Endophytic fungi' are the common name for fungi that live in plants and do not damage host plants. And they also do not transform appearance of hosts. They are known to encourage growth of plants and protect plants from herbivore. Studies needed to be conducted about endophytic fungi isolated from lots of plants, because endophytic fungi are essential for plant's life.
In this study, we isolated endophytic fungal strains from leaves of various plants in Ullengdo. The isolated strains were identified based on morphological characteristics and molecular analysis of ITS rDNA regions. As a result, we confirmed 41 fungal species from 23 genera from leaves. These results showed differences between endophytic fungi isolated from each host plant.

Keywords: Endophytic fungi, Fungi

B022

Effect of Orchid Roots Fungal Endophytes on Seedling Growth in a *Calanthe discolor* (an Endangered Terrestrial Orchid)

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Orchids, most diverse and widespread family of flowering plants, depend on specialized endophytic fungi from the Basidiomycota at some point in their lives. Orchid seeds are tiny and don't contain sufficient nutrients to support the growing embryonic plant, so they get what they need from the mycorrhizal association. Mycorrhizal and non-mycorrhizal endophytes directly or indirectly contribute to the growth and development of orchids as well as the production of valuable secondary metabolites. Many orchid species are threatened or even endangered all over the word, because of habitat destruction, excessive overcollection, and climate change. *Calanthe discolor* is a well-known native orchid species that is endangered in Korea. Since the majority of orchids are difficult to cultivate artificially, there is a need to find suitable fungal partners for the conservation and restoration of endangered orchids.
This study was performed to isolate the endophytic fungi, including mycorrhizal fungi, from surface-sterilized roots of wild orchids in Korea. The taxa of strains were classified based on morphological characteristics and molecular analysis. And for the estimation of the inoculation effects fungal strains to orchids, 150 *Calanthe discolor* were inoculated with 9 strains isolated from roots of wild orchids. There are needed more studies about investigation of another enhanced seedling growth parameter to by inoculation to assure the effect of the fungus on orchid growth.

Keywords: Fungal endophytes, Fungal inoculation, Orchid mycorrhiza, Plant growth

B024

Diversity of Macrofungi and a Record of New Species from Gayasan National Park

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Macrofungi are fungal species that form large fruiting body visible to the naked eye and play important roles in forest management as saprotrophs, symbiotrophs, and pathotrophs. Despite this importance, few studies on macrofungal flora have been carried out in Gayasan National Park. We surveyed macrofungi in Gayasan National park from 2017 to 2018 and identified them through molecular analyses of the Internal Transcribed Spacer region and their morphologies. A total of 189 species belonging to 124 genera and 47 families were identified. 20 macrofungi were confirmed to be new in Korea and their detailed morphological descriptions are provided in this study. Here, we provide the updated list of macrofungi from the Gayasan National Park.

[This work was supported by the project on the survey and excavation of Korean indigenous species of the National Institute of Biological Resources (grant number NIBR 201701104)]

B025

Comparative Analysis of Methane Consumption of Methanotrophic Bacteria by Pure or Mixed Culture with Heterotrophic Bacteria

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To isolate methane-oxidizing bacteria (MOB) from various types of soils (paddy field, wetland, garbage landfill, etc.), enrichment culture was performed for 2 weeks at 30℃ using nitrate mineral salts (NMS) medium with methane as sole carbon and energy source. Heterotrophic bacteria, co-cultured with MOB on NMS agar medium in the first subculture, were isolated from LB agar medium in the second subculture. MOB and heterotrophic bacteria were co-cultured in a NMS medium contained in the 160 mL gas tightly sealed vial and supplemented with methane/air gas mixture (50/50, v/v). Methane consumption rates were variable, but, in many cases, mixed cultures exhibited higher methane consumption rate than pure cultures of MOB. Depending on the strains, methane consumption rate (Δ% of methane / h) increased by 13–288% from 0.25–1.12 in pure cultures to 0.77–1.35 in mixed cultures. Higher methane consumption in mixed cultures was assumed due to growth promoting substances produced by co-cultured heterotrophs. Some pure MOB strains required vitamin for rapid methane consumption, but other MOB consumed methane rapidly when grown with heterotrophic bacteria. In conclusion, these results confirmed that some MOB has a symbiotic relationship with heterotrophic bacteria living in the same soil sample.

[This work was supported by a research grant of Ministry of Science and ICT (NRF-2018R1A2B2001006).]

Keywords: Methanotrophic bacteria, MOB, Heterotrophic bacteria, Symbiotic relationship

B026

Seasonal and Spatial Distribution of Male-specific and Somatic Coliphages from Major Aquaculture Areas in Republic of Korea

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Human or animal feces are important sources of various microbial contaminations in water. Especially, enteric viruses, the major agents of waterborne infection, can show long-term survival in water environments due to their strong resistances via various environmental factors including pH, salinity, and temperature. Coliphages have been suggested as the promising viral indicators for fecal contamination in water environments. Here, we investigated the seasonal and spatial distribution of male-specific and somatic coliphages in surface water and seawater at three major aquaculture areas including Gomso Bay, Goseong Bay and Aphae Island in Republic of Korea over a period of 1 year. We chose 6 surface water and 14 seawater sampling sites for each study area and collected a total of 480 water samples from March 2014 to February 2015. Overall, surface water samples showed higher occurrences of coliphages compared to seawater samples. The high coliphage concentrations were shown in spring to summer (May to August 2014). Moreover, environmental factors such as cumulative precipitation showed strong correlations with coliphage concentrations. Therefore, we suggested that the coliphages could be a good indicators for tracking fecal contamination in water.

[This research was supported by a grant (14162MFDS973) from Ministry of Food and Drug Safety in 2016 and a National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (no. 2018R1D1A1B07050085).]

Keywords: Male-specific coliphage, Somatic coliphage, Fecal indicator, Fecal source tracking, Enteric virus

B027

Analysis of Intestinal Microbiome in Bristle Worm *Cheilonereis cyclurus* Using Cultivation and Next Generation Sequencing

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Cheilonereis cyclurus is a bristle worm belonging to the family Nereididae in class Polychaeta. It harbors in the shell of the hermit crab as commensalism. *Cheilonereis cyclurus* has been studied as a symbiont in the crabs but its microbial diversity has not been studied well. Therefore, in this study, we investigated the microbial diversity of the intestine in *C. cyclurus*. Intestines were dissected into three fractions (forward, middle, and rear). Culture-dependent method and amplicon sequencing were used to analyze microbial groups in these intestinal fractions. In culture-dependent method, a total of about 300 strains were isolated and their phylogenetic positions were identified and dominant genera were *Bacillus, Vibrio*, *Microbacterium*, and *Shewanella*. Minorities were *Staphylococcus, Lysinibacillus*, and *Photobacterium*. Amplicon sequencing showed that most of *C. cyclurus* shared *Entomoplasmatales*, *Mycoplasmatales*, and *Pseudomonas* groups. In this study, core microbiome of *C. cyclurus* were revealed.

Keywords: *Cheilonereis cyclurus*, Bristle Worm, Polychaeta, Microbiome, Intestine

B028

Isolation and Characterization of Nitrogen-fixing Bacteria and Phosphate-solubilizing Bacteria from Oriental Melon Farm Soil

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In Korea, more than 95% of oriental melon is cultivated under structure. Although greenhouse growing is more producible, salt accumulation in greenhouse soil is major impediments for growing oriental melons. Salt accumulation reduces plant growth rate by impeding nutrient uptake of plants. Thus, using microbial fertilizer which contains nitrogen-fixing bacteria and phosphate-solubilizing bacteria as a substitute for chemical fertilizer for oriental melon farm can be a key for solving salt accumulation problems of cultivation under structure. The objective of this study is to isolate and characterize potential candidate bacteria for microbial fertilizer for oriental melon cultivation. Bacteria were isolated from six soil samples from two different oriental melon farms by using NFb, JMV, N2F agar and PVK, NBRIP-BPB agar for isolation of nitrogen-fixing bacteria and phosphate-solubilizing bacteria. After grouping isolates through REP-PCR pattern analysis, isolates were identified by 16S rDNA sequencing. Color changing observation of NFb semi-solid media and measurement of phosphate solubilization index (PSI) were used as qualitative assessment of nitrogen-fixing bacteria and phosphate-solubilizing bacteria respectively. 145 strains were isolated from nitrogen fixation selective media and among them 22 strains exhibited good nitrogen fixation activity. 21 strains among 36 strains isolated from phosphate-solubilizing selective media exhibited good P-solubilizing activity.

Keywords: Bacteria, Nitrogen fixation, Phosphate solubilization, Salt accumulation, Oriental melon

B029

Community of Endophytic Fungi of Subalpine Conifers in Mt. Seorak

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For subalpine conifers - *Abies nephrolepis*, *Pinus pumila*, *Taxus cuspidata* var. *nana*, *Thuja koraiensis* - were examine for endophytic fungi. A total 108 of endophytic fungi were isolated. They were 4 taxa in *A. nephrolepis*, 12 in *P. pumila*, 18 in *T. cuspidata* var. *nana*, and 17 in *T. koraiensis*. They were divided into 5 classes; Agaricomycetes (3.2%), Dothideomycetes (29%), Leotiomycetes (15%), Sordariomycetes (41.9%), Orbiliomycetes (1.6%). The most prevalent fungus was *Sydowia polyspora* (22.7%) and *Xylariaceae* sp. (22.7%) in *P. pumila*, *Phomopsis juglandina* (16.1%) in *T. cuspidata* var. *nana*, fungal endophyte (60.8%) in *T. koraiensis*. However, there is no dominant species in *A. nephrolepis*. Some of these host plant were analyzed by next generation sequencing (NGS) method. For *A. nephrolepis*, we obtained 4,618 reads and for *T. koraiensis*, we obtained 2,268 reads. At species level, *Cleosporium larixicola* (68%), *Sydowia polyspora* (20%) and Dermataceae (4%) was top three endophytic fungi in *A. nephrolepis*. *S. polyspora* (16%), *Pseudoveronaea* sp. (14%) and *Geastrumnia* sp. (10%) in *T. koraiensis*. Our results should show different community of endophytic fungi among different host plants even if host plants are flanked in each other. These ecological niches will have important meanings in terms of restoration of subalpine conifers.

Keywords: *Abies nephrolepis*, Endophytic fungi, *Pinus pumila*, *Taxus cuspidata* var. *nana*, *Thuja koraiensis*

B030

Evaluation of a Real-Time Reverse Transcription PCR Assay for Detection of Male-specific Coliphage

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Shellfish contaminated with norovirus could bring a large size of food and waterborne infections. In the United States, they have conducted safety management in seawater using the Male-Specific Coliphage (MSC), a virus that is specifically infecting *E. coli*, as a pollutant indicator species. The conventional MSC detection method, which is a double agar layer method, has the disadvantage of low detection sensitivity and long incubation hours to confirm the MSC levels. To improve the efficiency of MSC detection, this study was conducted to real-time RT-PCR using a shellfish by artificially infected with MSC. According to the results, primers for the gene encoding maturation protein (*mat*) showed the best detection sensitivity and efficiency as compared to the lysis protein (*lys*), the coat protein (*cp*), and the replicase protein (*rep*). Based on the results of MSC level detected in the mid-gut gland, we also found that artificial infected MSC was accumulated in the mid-gut gland site than other sites of the shellfish, suggesting mid-gut gland as indicator site.

B031

Isolation and Characterization of Compost Decomposing Bacteria from Duck Litter

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The objective of this study was to isolate and identify new compost decomposing bacteria from duck litter, evaluate the characteristics of these strains and determine the remove effects of odor materials in duck litter mixed excreata. Of 128 colonies, three strains derived from duck litter consisted of rice husk and sawdust were selected and clustered as RS 2 (*Bacillus licheniformis* ATCC 14580 AE017333, 99%), RS 5 (*Arthrobacter arilaitensis* Re117 FQ311875, 99%) and RS 15 (*Pseudomonas xiamenensis* C10-2 DQ088664, 98%). The growth rate to 80°C was higher and shown to produce inhibition zone against pathogenic bacteria in the duck farms. Also, all grew well up to 10% NaCl. The spray effect into the duck litter mixed manure samples with single or complex microbial agent were analyzed by modified Lee method (2008). The single microbial agent, RS 5 (*Arthrobacter arilaitensis* Re117, FQ311875), showed marked removal activity of ammonia gas (32.3%) and hydrogen sulfide gas (55.9%) ($P < 0.05$). In field test, it decreased overall by 6 wks and eliminated ammonia gas (60.5%), hydrogen sulfide gas (100%) and amines (66.1%). Consequently, the RS 5 plays a role in the reduction of odorous compounds and spray in the duck litter seems to have potential applicability for improving agent of duck-farm environment.
[Supported by a research grant from Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry]

Keywords: Compost decomposing bacteria, Duck litter, *Arthrobacter arilaitensis*

B032

A Report of Twelve Unrecorded Fungal Species of Jeju Island, Korea

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Twelve unrecorded fungal species were isolated and identified during a survey of soil samples obtained from rhizosphere of *Amanita rubescens, A. virosa, Cortinarius violaceus, Russula cyanoxanyha* mushroom at Jeju Island in Korea. This study reports the descriptions of the 12 unrecorded fungal species, *Arthrinium kogelbergense, Clonostachys rosea, Dendrothyrium longisporum, Neopyrenochaeta cercidis, Penicillium soppii, Phomatodes nebulosa, Pyrenichaeta nobilis, Saitozyma podzolica, Talaromyces kendrickii, T. qii, Tolypocladium album* and *Umbelopsis ramanniana.* For all the identified species, morphological characteristics including colony features formed on media, light microscopic images and molecular phylogenetic relationships based on nucleotide sequences of the ITS rDNA, 18S rDNA, 28S rDNA, β-tubulin gene, calmodulin gene, and TEF gene were described.

Keywords: Jeju Island, Wild mushroom, Fungal diversity

B033

Dissemination of Antibiotic Resistance Genes in Freshwater from the Wastewater of Livestock and Aquaculture Farm

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Antibiotics used in livestock and aquaculture farm are released into the environment and affect the accumulation of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB). This study identified the bacteial community and ARGs in the upstream, effluent and downstream of the 6 rivers in Korea. Total of 57 samples were obtained from the treated waste water of 10 livestock and waste water of 3 aquaculture farm. A total of 476 subtypes within 19 ARG types were detected and the most common types were Beta-lactam, Sulfonamide, Aminoglycoside, and Tetracycline. Metagenome-assembled genomes (MAGs) were reconstructed from co-assembled contigs. Total 1086 bacterial and 5 alcheal MAGs were obtained and 1018 of bacterial MAGs had ARGs in their genomes. Patescibacteria mainly had beta-lactamase class A, and Actinobacteria had class B. In Proteobacteria, class B was dominant but class A was also detected. 129 bacterial MAGs, including Patescibacteria, Bacteroidota, Proteobacteria and Actinobacteria, were found to remain downstream under the influence of effluents. These results indicate that the key bacteria, that have ARGs from the wastewater can affect the diffusion and accumulation of ARBs and ARGs in the downstream environment. We suggest that the intensive use of antibiotics would lead a continuous influx of ARGs from wastewater into the environment, which would have a significant impact on aquatic environmental contamination and further on human health.

Keywords: Antibiotic resistance genes, Metagenome, Metagenome assembled-genome, Wastewater

B034

Studies of Gene Expression of a Triton X-100 Degrader *Moraxella osloensis* Isolated from Human Skin

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Alkylphenol polyethoxylates (APEOn) as non-ionic surfactants are vastly used in industry for emulsification and wetting agents of industrial products. The fate and biodegradability of APEOn in the environment have received alerts because the recalcitrant intermediates with EO units of 1–2 have potentials for endocrine disruptors in aquatic animals. Here, we report the isolation of 10 strains of *Moraxella osloensis* from human skin with its ability to degrade Triton X-100 (*t*-octylphenol polyethoxylates). *M. osloensis*, a Gram-negative bacterium that is known to be saprophytic on skin and mucosa, rarely causes infections. Of the isolates, *M. osloensis* TT16 showed the fastest growth even with 5% Triton X-100. To further characterize the catabolic pathway and surfactant-resistance mechanism, we determined the complete genome sequence and compared the gene expression at proteomic and RNA expression levels. Our result showed some oxidoreductase genes such as threonine dehydrogenase and aldehyde dehydrogenase, and gene encoding formate acetyl transferase were highly expressed when grown with Triton X-100 as a carbon source. Our results of whole-genome sequencing data and comparative gene expression will help mining genes related to Triton X-100 catabolism and surfactant resistance.
[This research was granted by IRB and supported by the Basic Science Research Program through the National Research Foundation of Korea (No. 2016R1D1A1B01007775)].

Keywords: Triton X-100, Moraxella, Complete genome sequence

B035

Analysis of Microbial Community Attached to Waste Plastic Films Buried in Forest Soils for more than 40 Years

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Proper disposal of plastic wastes has been one of the most urgent global issues because of their non-biodegradability. Microbial degradation of plastics is a promising solution to this issue. To find efficient microorganisms to degrade plastics, we analyzed the microbial community attached to waste plastic films buried in forest soils for more than 40 years and tried to isolate the microorganisms detected more frequently in waste plastic films than in nearby soils. In the forest soils, the waste plastic films which had been seemingly degraded (D-PF), ones that were not degraded at all (ND-PF), and nearby soils (NS) were collected and total DNAs were extracted. The bacterial and fungal community structures were analyzed based on bacteria 16S rRNA genes and fungal ITS regions, respectively, using Illumina MiSeq Technology. Among the bacteria present in the samples, the members of genera Massilia and Arthrobacter were highly enriched in the D-PF samples (~9.2% compared to ~5.7% in ND-PF samples and ~0.9% in NS samples) while among the fungi the members of genus Mortierella were highly enriched in the D-PF samples (~18.7% compared to ~4.8% in ND-PF samples and ~1.3% in NS samples). We isolated strains belonging to the genera Massilia, Arthrobacter, and Mortierella, and are examining their degradation potential of polyethylene films.
[This work was supported with grants (PJ01450903) of National Institute of Agricultural Sciences, RDA, Republic of Korea.]

Keywords: Plastics, Biodegradation, Soil, Microorganisms

B036

Niche Differentiation of Pelagic Microbes in Artificially Warmed Lakes

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Zooplankton-associated microbes have been proposed to provide numerous services to their “host”, including nutrient acquisition, stress protection and detoxification thereby contributing to zooplankton’s fitness. Here, we provide insights into microbiome composition of zooplankton, phytoplankton and bacterioplankton in the water column of a series of lakes artificially warmed by cooling water discharge of two power plants. High-throughput amplicon sequencing of the 16S rRNA gene showed that diversity and composition of the bacterial community associated to copepods, cladocerans, phytoplankton, and bacterioplankton varied significantly from one another, grouping in different clusters indicating niche differentiation of pelagic microbes. Two phyla (e.g. *Proteobacteria* and *Bacteroidetes*) dominated in zooplankton microbiomes whereas *Actinobacteria* was the dominant phylum in the bacterioplankton. Indicator species analysis showed that 9%, 8%, 12%, and 21% unique OTUs were significantly associated with copepods, cladocerans, bacterioplankton, and phytoplankton, respectively. Surprisingly, some genera of methane oxidizing bacteria (MOB), methylotrophs and nitrifiers (e.g., *Nitrobacter*) significantly associated with microbiome of zooplankton and phytoplankton. Our study demonstrates that warming affects microbiome composition of pelagic microbial communities and microbes that harbour on zooplankton are significantly linked to C and N-cycling.

B037

To Reveal the Relationship between Host Lichen and Endolichenic Fungi

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Endolichenic fungi (ELF) are representative group of filamentous fungi living inside lichen thalli without desease symptoms. Several secondary metabolites from them receive attention for their bioactivities. In ecological viewpoint, however, actual niche of ELF remains still unclear. A large number of reported ELF are not host-specific. Therefore, it is urgent to reveal which strains are host-specific and what roles they play. We set up three steps to determine the subject of research. First step is establishing isolation method of ELF to secure diverse strains. Second step is investigation of host-specific strains through the Metagenome. The last step is identification of relationship between lichen and host-specifi ELF. Here, we show the result of first step-research. Using two kinds of media (PDA and BBM), the smallest segment (1 mm^2) of lichen host and moderate surface sterilization (90 sec of 0.4% NaOCl after 90 sec of 70% ethanol) showed the highest diversity of ELF in this research. Although isolation of diverse strains was successful compared to previous culture reports of ELF, results from culture and NGS approach were significantly different. T most frequent order was Xylariales (Sordariomycetes) in culture but Capnodiales (Dothideomycetes) in NGS, respectively. It reflects several limits of two approaches and implies that a combination of two kinds of methods is needed for more accurate diversity and further physiologic study.
[Supported by Korea National Arboretum]

Keywords: Lichen, Endolichenic fungi, Diversity, Culture, NGS

B040

Violacein Production in *Chromobacterium violaceum*

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Violacein is a purple hydrophobic molecule that act as antibiotic toward Gram-positive bacteria, produced by various Gram-negative strain. Though violacein is a hydrophobic molecule, it still can be delivered to other bacteria in hydrophilic environment. Here, we demonstrate the membrane vesicles (MVs) mediated violacein delivering strategy, using *Chromobacterium violaceum* ATCC 12472. *C. violaceum* is a Gram-positive bacteria which produces violacein as it enters to stationary phase.
We purified MVs from supernatant of *C. violaceum* and checked that violacein is contained in MVs and this is the way violacein being soluble in hydrophilic condition. We checked presence of violacein in cell and purified MVs while it is not in MVs filtered supernatant by measuring violacein concentration.
We constructed vioA Knock-out *C. violaceum* to disable violacein production. Colony of vioA KO *C. violaceum* was white while wild type was purple, indicating KO strain violacein does not produce violacein. We confirmed this fact by measuring violacein concentration within cell, culture, MVs. Violacein was not measured overall.
By purifying *C. violaceum* WT, vioA KO *C. violaceum's* MVs and added to *S. aureus* Xen8.1 to see antibiotic effect. Compared to purified violacein, *C. violaceum* WT MVs showed bioluminescence decreasing while vioA KO *C. violaceum* MVs didn't.
For conclusion, we here demonstrate *C. violaceum* uses MVs to deliver violacein in hydrophilic condition to kill other bacteria.
Keywords: Bacteria, *Chromobacterium violaceum*, Violacein, Membrane vesicle

B041

Reshaping of the Gut Microbiota in Small Heterodimer Partner Deficient Mice

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The small heterodimer partner (SHP) is an orphan nuclear receptor that can interact with various nuclear receptors and transcription factors related to lipids and bile acids metabolism. SHP is activated by farnesoid X receptor (FXR) and regulates bile acid biosynthesis by inhibiting cytochrome P450 7A1 (CYP7A1), which is the rate-limiting enzyme in synthesis of bile acid from cholesterols. Gut microbiota has profound effects on bile acid metabolism by promoting deconjugation of taurine and glycine, dehydrogenation and dihydroxylation of primary bile acids.
We investigated how SHP deficiency in mice affects gut microbiota profile through 16S rRNA gene amplicon sequencing. We found that microbial diversity (α-diversity) was significantly increased in SHP KO mice compared to WT mice ($p < 0.002$). Community structures of gut microbiota from WT and SHP KO were significantly different and clustered separately ($p < 0.001$). In detail, compared to SHP WT, the bacterial genera *Bacteroides*, *Butyricimonas* and *Rikenella* were decreased and *Lactobacillus*, *Allobaculum* and unclassified Peptostreptococcaceae were increased in SHP KO mice. As a result of dysbiosis in SHP KO mice, mice were more susceptible to dextran sulfate sodium (DSS)-induced colitis. We investigated specific taxa that differently represented in WT and SHP KO mice before DSS treatment. Especially, *Lactobacillus* increased in SHP KO mice before DSS treatment.
From these data, we conclude that SHP deficiency can modify the host gut bacterial community and regulate host sensitivity to DSS induced colitis. Through further study, we will figure out interaction of the genus *Lactobacillus* with SHP KO and DSS sensitivity.

Keywords: SHP, Gut microbiota, Colitis, *Lactobacillus*, Bile acid

C001

Regulation of Urease Affects Glutamate Biosynthesis and Biological Efficacy via Ca^{2+}/Calmodulin in the Entomopathogenic Fungus *Cordyceps militaris*

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Urease (EC 3.5.1.5) catalyses the hydrolysis of urea into carbon dioxide and ammonia and plays an important role in nitrogen metabolism by providing a biologically relevant nitrogen source. In this study, structural and biochemical analyses of *Cordyceps militaris* urease (*Cm*Ure) *in vitro* and *in vivo* showed that it contains a novel calmodulin (CaM)-binding motif (1-8-14 motif-type) in the N-terminal region. CaM binding directly inhibited *Cm*Ure activity. Similar results were obtained using a plant (jack bean) urease, indicating that inhibition of urease activity by CaM is most likely evolutionarily conserved as a common mechanism between plants and fungi. Treatment of *Cm*Ure with the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) significantly reduced glutamate content in *C. militaris*, suggesting that *Cm*Ure functions in biosynthesis of glutamate. This study suggests that *Cm*Ure plays a role in urea-mediated glutamate biosynthesis via the Ca^{2+}/CaM signalling pathway in *C. militaris*. Experiments with *C. militaris* extract showed that it has novel physiological activities for improving skin health, such as anti-elastase and anti-collagenase activity, and that *Cm*Ure is involved in maintaining these activities of *C. militaris*.
[This study was supported by the Individual Basic Research Support Project of the National Research Foundation of Korea (NRF-2018R1D1A1B07051052).]

Keywords: Calcium, Calmodulin, CaM target protein, Entomopathogenic fungus, Glutamate biosynthesis

C002

A Unique Domain of *Polaribacter irgensii* KOPRI 22228 CspB is Responsible for the Extraordinary Freeze-tolerance

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Psychrophilic organisms must possess a mechanism to survive extremely cold temperatures. A small number of proteins, including cold-shock proteins (Csps), are induced selectively upon temperature downshifts. Overexpression of *cspB*$_{Pi}$ from the Arctic bacterium *Polaribacter irgensii* KOPRI 22228 greatly increased the freezing tolerance of its host. This protein consists of a unique *N*-terminal domain and a well conserved *C*-terminal cold shock domain (CSD). To elucidate the detailed mechanisms involved in the extraordinary freeze-tolerance conferred by CspB$_{Pi}$, we introduced mutations in the CSD that are crucial for binding RNA or single-stranded DNA. These mutations did not impair the ability of the host to survive freezing stress. When the effects of domain-deletion and domain-shuffling of CspB$_{Pi}$ were analyzed, all CspB$_{Pi}$ variants containing the *N*-terminal domain retained the ability to confer the unprecedented cold-resistance. Slow electrophoretic mobility and far-UV circular dichroism spectra suggested that the *N*-terminal domain is intrinsically disordered. The *N*-terminal domain bound to lipid vesicles *in vitro*. This lipid vesicle binding characteristic is shared with other intrinsically disordered proteins, known to confer cold-tolerance when overexpressed, suggesting a mechanism for cold-survival through membrane binding.
[This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2015R1D1A1 A01058206).]

Keywords: Cold-shock protein, Intrinsically disordered protein, Lipid vesicle binding, Freeze-tolerance

C003

The Active Site Residue of Peptidyl Prolyl Isomerase Cpr7p is not Essential for Freeze-tolerance in Yeast

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Exposure to low temperatures may disturb proteome stasis due to cold denaturation and subsequent aggregation of proteins. Deletion of some chaperone genes, particularly peptidyl-prolyl *cis-trans* isomerases, rendered yeast cells more vulnerable to freeze-thaw treatment. Overexpression of the identified yeast PPIases (*cpr1*, *cpr3*, *cpr7*, and *fpr2*) in *Escherichia coli* increased the viability of the new host upon freeze-thaw treatment. To elucidate their mode of action, an active site mutation was introduced into an identified peptidyl-prolyl isomerase, *cpr7*. Expression of Cpr7p R64A also recovered freeze survival in *cpr7Δ* yeast significantly. Extensive protein aggregates were formed in *cpr7Δ* yeast cells upon freeze-thaw treatment, and introduction of either wild-type or *cpr7 R64A* mutant significantly mitigated protein aggregation. Translation elongation factor 2 (EF-2) was predominantly found in the aggregated fraction in *cpr7Δ* yeast. Purified Cpr7p facilitated the refolding of unfolded Z-type antitrypsin proteins *in vitro*. Our results suggest that Cpr7p protects cells from freeze-induced protein aggregation and is potentially involved in the biosynthesis and/or folding of new proteins during recovery from freezing damage.
[This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2015R1D1A1 A01058206).]

Keywords: Cold stress, Chaperone, Freezing stress, Peptidyl-prolyl isomerase, Protein folding

C004

Processing of *Mycobacterium tuberculosis* MoaD/E Fusion Protein by JAMM, Metalloprotease

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Ubl (ubiquitin-like protein), they are SAMP (small archaeal modifier proteins) in *Haloferax volcanii* and MoaD in *Deinococcus radiodurans*. SAMP and MoaD exist in the fusion form with MoaE. These are involved in the synthesis of molybdopterin. In order to play that role, they have to cleaved by zinc-dependent metalloprotease JAMM (JAB1 / MPN / Mov34) which is known to process SAMP-MoaE or MoaD-MoaE fusion at c-terminal di-Gly residues of SAMP or MoaD. *Mycobacterium tuberculosis*, also contains MoaX, a fusion form of MoaD-MoaE. We confirmed that *M. tuberculosis* and *M. smegmatis* which is belonging to the genus Mycobacterium, also had JAMM homologues. Then, we overexpressed and purified JAMM homologues and MoaX to determine if JAMM homologue can actually process the MoaX. So, we can checked that *M. smegmatis* JAMM ($JAMM_{MS}$) was able to process *M. tuberculosis* MoaX ($MoaX_{MT}$) by SDS-PAGE. In addition, we confirmed that EDTA prevented processing of $MoaX_{MT}$ from $JAMM_{MS}$. It means $JAMM_{MS}$ is also a metalloprotease. Interestingly, unlike *D. radiodurans* JAMM ($JAMM_{DR}$), almost all $MoaX_{MT}$ is processed without addition of Zn ions, suggesting the possibility that the $JAMM_{MS}$ may have stronger metal ion affinity than $JAMM_{DR}$. In conclusion, we have indirectly confirmed that $MoaX_{MT}$ is processed by $JAMM_{MS}$ and further study will confirm processing by *M. tuberculosis* JAMM.
[This work was supported by Science Research Center grant (NRF-2018R1A5A1025077)]

Keywords: Ubl (ubiquitin-like protein), MoaD-MoaE fusion, JAMM, Zinc-dependent metalloprotease

C005

Medium Composition Changes for the Improvement of Mycelial Growth in Liquid Cultures of *Lentinula edodes*

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Lentinula edodes (Shiitake) is the most popular edible mushroom in Asia. Sawdust spawn has been mainly used in cultivation, but it spent long time until mushroom harvest. In this study, we investigated liquid medium composition for reducing cultivation period of liquid spawn. Various carbon, nitrogen, potassium and ammonium sources were mixed with basic medium (pH 6.0). Liquid media were autoclaved at 121°C for 15 min and inoculated with strains of *L. edodes*. They were incubated at 25°C for 21 days and then measured in biomass by mycelial dry weight were measured. Trehalose and raffinose were useful carbon source to improve spawn growth. Usually mycelial biomass increased when nitrogen sources. However, our study didn't show the relationship between mycelial biomass and ammonium sources.

Keywords: Composition, *Lentinula edodes*, Liquid culture

C006

Neurite Outgrowth Activity of FK506 Derivatives Containing Structural Modification

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FK506 is macrocyclic polyketide and has been used as immunosuppressant drug to prevent the organ transplant rejection. A hybrid polyketide synthase-nonribosomal peptide synthetase (PKS/NRPS) system is responsible for the biosynthesis of the linear polyketide chain. L-pipecolate derived from l-lysine by FkbL is attached to the terminal end of the linear chain of FK506. Subsequent cyclization catalyzed by NRPS FkbP forms the macrolactone ring. The ring of FK506 requires final post-PKS modification steps, C9-oxidation catalyzed by cytochrome P450 hydroxylase (FkbD), and C31-O-methylation by S-adenosylmethionine (SAM)-dependent methyltransferase (FkbM). In this study, seven new FK506 derivatives are biosynthesized by structural modification. Moreover, we tested immunosuppressive activity and *in vitro* neurite outgrowth activity of the nine FK506 derivatives. These derivatives showed significantly lower immunosuppressive activity than FK506. However, neurite outgrowth activity was maintained *in vitro* compared to FK506. The results suggested the potential of FK506 derivatives as neurodegenerative drugs without immunosuppressive side-effect. Overall, this study demonstrated the usefulness of the metabolic engineering approaches to generate new bioactive molecules.

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Keywords: Polyketide, Biosynthesis, Neurite outgrowth activity

C007

Molecular Characterization of a Novel Family V Esterase (*Ha*Est) from *Halocynthiibacter arcticus* Stain PAMC 20958

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A novel family Ⅴ esterase from *Halocynthiibacter arcticus* stain PAMC 20958 was identified, purified, and characterized by biochemical and biophysical methods. *Ha*Est is composed of 252 amino acids and the molecular mass of *Ha*Est was estimated to be 29.4 kDa. Biochemical characteristics of *Ha*Est were investigated by SDS-PAGE, Native-PAGE, acetic acid release, biochemical assays, enzyme kinetics, mass spectrometry, and intrinsic fluorescence. Sequence analysis of *Ha*Est indicated that *Ha*Est belongs to esterase family Ⅴ. Furthermore, sequence analysis revealed a conserved motif of Gly^{63}(G)–X-Ser^{65}(S)–X-Gly^{67}(G) and Gly^{99}(G)–X-Ser^{101}(S)–X–Gly^{103}(G). Mutation analysis found that Ser^{65} is not functional. Finally, immobilization by cross-linking methods were examined to investigated potential biotechnological applications.

Keywords: Bacteria, Esterase, *Halocynthiibacter arcticus*

C008

Biochemical Characterization of a Novel HSL Family, Alpha/Beta Hydrolase from *Halocynthiibacter arcticus* Stain PAMC 20958

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A novel hormone sensitive lipase, *Ha*HSL from *Halocynthiibacter arcticus stain PAMC 20958* was identified, purified, and characterized by biochemical and biophysical methods. *Ha*HSL is composed of 300 amino acids and the molecular mass of the *Ha*HSL was estimated to be 34.0 kDa. Biochemical characteristics of *Ha*-HSL were investigated by SDS-PAGE, Native-PAGE, acetic acid release, biochemical assays, enzyme kinetics, mass spectrometry, and intrinsic fluorescence. *Ha*-HSL sequence analysis found Gly^{145}(G)-X-Ser^{147}(S)-X-Gly^{149}(G) that is a conserved motif of bacterial esterase family Ⅳ, indicating that *Ha*HSL belongs to bacterial esterase family IV. Finally, immobilization by cross-linking methods were examined to investigated potential biotechnological applications.

Keywords: Bacteria, Esterase, HSL, *Halocynthiibacter arcticus*

C009

Identification and Characterization of a Novel Esterase (*Lc*Est) from *Leuconostoc citreum*

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A novel esterase (*Lc*Est) from *Leuconostoc citreum* KM20 was identified, expressed in *Escherichia coli,* characterized, and immobilized for industrial applications. Recombinant (*Lc*Est) protein containing a C-terminal His tag was overexpressed in *Escherichia coli* and purified. Biochemical characteristics of *Lc*Est were investigated by performing SDS-PAGE, mass spectrometry, enzyme assays, and intrinsic fluorescence. Finally, immobilization by cross-linking methods were examined to investigated potential biotechnological applications.

Keywords: Bacteria, Esterase, *Leuconostoc citreum*

C010

Isolation and Characterization of Fibrinolytic Enzyme-producing *Bacillus subtilis* subsp. *subtilis* BYSnat3

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The Gram-positive, endospore-forming and fibrin-degrading bacterium was isolated from honey bee. Based on 16S rDNA sequences analysis, the results of API CHB50 and some morphological-physiological characteristics, the isolate was identified and named as *Bacillus subtilis* subsp. *subtilis* BYSnat3. Among the media tested, trypticase soy broth (TSB) was best for the cell growth. For the cell growth in TSB, temperature and pH was 25°C and 7–8 was excellent, respectively. Using the fibrin plate method for fibrinolytic enzyme activity, the optimum temperature and pH was 37–40°C and 7–8, respectively. The fibrinolytic enzyme activity of *B. subtilis* subsp. *subtilis* BYSnat3 was increased after 24 h of cultivation time.

Keywords: *Bacillus subtilis* subsp. *subtilis*, Characterization, Fibrinolytic enzyme activity, Identification, Isolation

C011

Inhibitory Effect of H_2O_2 and Benzalkonium on Cell Growth of Methicillin-resistant *Staphylococcus aureus*

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This study was carried out to determine the antibacterial activity of disinfectant H_2O_2 and some surfactants against pathogenic bacteria, 4 strains of *Staphylococcus aureus* and 6 strains of methicillin-resistant *S. aureus* (MRSA). When 400 μg/disk of H_2O_2 was tested, the inhibition size of *S. aureus* and MRSA ranged in 14.8–29.4 mm and 13.6–33.9 mm, respectively. Minimal inhibitory concentration (MIC) of *S. aureus* ranged in 150–250 μg/ml and that of MRSA did 150–200 μg/ml, Among the detergents tested, benzalkonium showed excellent antibacterial activity. As 400 μg/disk of benzalkonium was applied, the inhibition size of *S. aureus* and MRSA ranged in 14.8–29.4 mm and 13.6–33.9 mm, respectively. MICs of *S. aureus* and MRSA ranged from 700 to 850 μg/ml. These results suggest that these compounds could be useful to preventive-control against antibiotic-resistant MRSA.

Keywords: Antibacterial activity, Benzalkonium, H_2O_2, MIC, MRSA

C012

Enforcement of Unfolded Protein Response Diminishes Z-Type α_1-Antitrypsin-induced Cytotoxicity in Yeasts

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Extremely retarded protein folding of human Z-type α_1-antitrypsin variant causes accumulation of folding intermediates prone to aggregation in endoplasmic reticulum (ER), and leads to subsequent cell death. When the misfolded proteins are persistent in the ER, ER stress is detected by an intracellular signal transduction pathway, termed the unfolded protein response (UPR). The contribution of UPR elements in Z-type α_1-antitrypsin-induced cytotoxicity was investigated. Deletions of individual UPR elements exacerbates diminished growth of Z-type α_1-antitrypsin-expressing yeast cells. Overexpression of UPR elements, except *kar2*, alleviated the slow growth phenotype of Z-type α_1-antitrypsin-expressing cells. Accumulation of misfolded Z-type α_1-antitrypsin in the ER provoked oxidative stress, monitored by dichlorofluorescein assays. Antioxidant treatment alleviated Z-type α_1-antitrypsin-induced oxidative stress. Our results would provide further information on therapeutic strategies to deal with protein folding diseases.

[This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2015R1D1A1 A01058206).]

Keywords: αAntitrypsin, Chaperone, Misfolded protein, Protein folding, Unfolded protein response

C013

Comparison of Bioactivity from Cultivated Five Wood-decay Fungi

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Fungi are known to contain various physiologically active substances and are highly available biological resources. In this study, we investigated the growth characteristics such as temperature and media for 5 strains (*Laetiporus cremeiporus, Lycoperdon perlatum, Phaeolus schweinitzii, Piptoporus betulinus, Postia alni*). The utilization of carbon sources was analyzed by BIOLOG. In addition, the bioactivity of 5 strains cultured under various conditions were investigated. The optimum temperature of 4 strains except *L. perlatum* was 25–30°C, and the growth rate of each medium did not show any significant difference. D-Ribose and D-Xylose were used by all five strains. But 9 sources including Amygdalin and Sebacic acid were not used. The bioactivity results showed antimicrobial activity from *P. betulinus* and *L. cremeiporus.* Both species showed activity against Gram-positive and negative bacteria. *P. schweinitzii* cultured in DY medium was confirmed antioxidant effect of 97.65% in comparison with the positive control (Ascorbic acid 10^3 ppm). As a result of this study, the growth of 5 species was greatly influenced by the culture temperature. However, the biological activity is different depending on the medium components, so both the temperature and the medium conditions should be considered when using as a biological resource.
[Supported by grants from National Institute of Biological Resources, under the Ministry of Environment of the Republic of Korea (NIBR201902113).]

Keywords: Wood-decay fungi, Antimicrobial activity, Antioxidant activity

C014

Structural Study of Antibacterial Agent Development Targeting UDP-glucose Pyrophosphorylase (UGPase) from *Acinetobacter baumannii*

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Acinetobacter banumannii is a Gram-negative bacterium and becomes increasingly important due to hospital-derived, nosocomial, infections over the past decades. UDP-glucose pyrophosphorylase (UGPase) is the enzyme for the synthesis of uridine diphosphate glucose (UDP-glucose) from a D-glucose 1-phosphate (Glu-1P) and uridine triphosphate (UTP) in an Mg^{2+}-dependent reaction while releasing pyrophosphate. UGPase has a key role in capsule and lipopolysaccharide synthesis in bacteria and there is no homology between eukaryotic and prokaryotic UGPases, although it exists in both eukaryotes and prokaryotes, which makes bacterial UGPase a promising antibacterial drug target. We expressed, purified, and crystallized UGPase from *Acinetobacter banumannii* (AbUGPase) for the structural study to develop a putative antimicrobial agent. I chose pET system for the expression of AbUGPase in *E. coli*, and purified with a Ni-NTA column for the 1st purification, and an anion column of XXXX column name for the 2nd purification. The X-ray diffraction data of AbUGPase was collected up to 2.6 Å resolution l try to attach substrates and inhibitors to a crystal to find where they are bindingThe crystal structure of AbUGPase will be useful for the development of a new AbUGPase inhibitor.

Keywords: UDP-glucose pyrophosphorylase (UGPase), Drug target, Antibiotics, *Acinetobacter baumannii*, X-ray crystallography

C015

Effect of NaCl Treatment on Growth Characteristics of *Pleurotus ostreatus* Fruits Body

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There are about 10 kinds of edible mushrooms grown in Korea, and among them, *Pleurotus ostreatus* (PO) is produced 62,467 million tons as of 2015, which is the most produced in Korea. Recently, due to the imbalance in supply and demand of nitrogen sources in sawdust medium and the increase in import cost, various studies have been conducted to replace nitrogen sources. The study was conducted as a basic study for the possibility of replacing nitrogen source of mushroom sawdust medium with domestic food waste, and it was considered that NaCl would inhibit the growth of mushrooms when nitrogen source was replaced.
In this study, PO 'Heuktari' (ASI 0665) was used. Sawdust medium was made of poplar sawdust: beet pulp: cottonseed foil at a 5: 3: 2 ratio, and NaCl was 0.5%, 1.0%, 1.5%, and 2.0%. Treated with (W/W). After 42 days of incubation, the growth characteristics of PO were 225.2 ± 34.3 g, fresh weight 44.6 ± 9.0 mm, thickness of pileus 3.2 ± 1.3 mm, and NaCl 0.5% treatment. The total weight was 243.6 ± 20.3 g, the width of pileus was 47.6 ± 2.9 mm, and the thickness of the pileus was 4.0 ± 1.3 mm. The total weight was 18.4 g, 3 mm, and the thickness of the pileus was 0.8mm. In the other treatments, the total weight, width of pileus, and pilues of thickness were decreased compared to the control.
The results showed that the total weight, width of pileus, and pileus of thickness decreased when the NaCl content in sawdust medium increased above 1.0%.

Keywords: NaCl, *Pleurotus ostreatus*, Heuktari, Growth character, Product yield

C016

Physiological Characteristics of a Wild Edible *Giant Puffball* Mushroom (*Calvatia gigantea*)

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Calvatia gigantea is one of the edible mushrooms which are potentially important for human health as well as human alimentation. Many people are interested in this mushroom because of its various bioactivities (e. g. anti-cancer, anti-inflammatory, and analgesic effects). Nevertheless, wild *C. gigantea* is rarely distributed in Korea, and basic research for artificial cultivation of it has not been actively conducted. The aim of this study is to provide basic information for its artificial cultivation by identifying the physiological characteristics. Two strains of *C. gigantea* (NIFoS 3270 and 3852) were collected from the National Institute of Forest Science in 2016 and confirmed as *C. gigantea* by transcribed spacer region sequence analysis. The mycelial growth characteristics of them were investigated under different culture media and temperatures. After 21 days of incubation, NIFoS 3270 showed the higher mycelial growth on sabouraud dextrose agar (SDA) than on PDA. The optimal culture media for NIFoS 3852 were both PDA and SDA. The optimal growth temperatures for NIFoS 3270 and NIFoS 3852 were 30°C and 25–30°C on PDA, respectively. The maximum biomass of NIFoS 3270 and NIFoS 3852 in liquid culture media (PDB) was observed at pH 5.0–6.0. Carboxymethylcellulase activity was significantly higher in NIFoS 3270 than NIFoS 3852. Laccase activity of NIFoS 3270 was similar to that of NIFoS 3852.
[Supported by a grant from National Institute of Forest Science (FP 0801-2010-01)]

Keywords: *Calvatia gigantea*, Carboxymethylcellulase, Laccase, Mycelial growth

C017

Comparison of Mycelial Growth Characteristics and Wood-decay Extracellular Enzyme Activities between *Rhizina undulata* Strains with Different Isolation Origin

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Rhizina undulata is a soil borne pathogen causing a Rhizina root rot of conifers and it has also been reported to cause severe damage to coastal pine forests in Korea. However, there is no prevention method or control drugs have been developed to date. Therefore, it is necessary to establish prevention strategy based on the physiological characteristics of *R. undulata*. Here, we compared the mycelial growth characteristics under different culture conditions and wood-decay extracellular enzyme activities of *R. undulata* strains. Two *R. undulata* strains with different isolation origin (spore-derived NIFoS 2527 and vegetative tissue-derived NIFoS 2529) were collected from *Pinus thunbergii* coastal forests in the western region of Korea. After 15 days of incubation, the mycelial growth rates on both PDA and Sabouraud dextrose agar (SDA) were the same in two strains, whereas the growth rate of NIFoS 2529 on malt extract agar (MEA) was slightly higher than NIFoS 2527. The growth rate of them was the same in the range of 20-30°C on PDA, but the growth rate of NIFoS 2529 was higher than that of NIFoS 2527 at 10 and 15°C. The maximum cell biomass of both strains in liquid culture media (PDB) was obtained at pH 4.0 after 21 days of incubation. They showed also a high level of carboxymethylcellulase activity, whereas they did not degrade the laccase substrate ABTS at all.
[Supported by a grant from National Institute of Forest Science (FP 0801-2010-01)]

Keywords: Carboxymethylcellulase, Laccase, Mycelial growth, Rhizina root rot, *Rhizina undulata*

C018

Transcriptomic Identification and Biochemical Characterization of HmpA, a Nitric Oxide Dioxygenase, Essential for Pathogenesis of *Vibrio vulnificus*

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Nitric oxide (NO) and its derivatives are important effectors of host innate immunity, disrupting cellular function of infecting pathogens. Transcriptome analysis of *Vibrio vulnificus*, an opportunistic human pathogen, identified a set of genes induced upon exposure to NO. Among them, *VvhmpA* (*V. vulnificus hmpA*), encoding a multidomain NO dioxygenase, was the most greatly induced upon exposure to NO and was thus further characterized. Absorption spectra demonstrated that *Vv*HmpA is a heme protein in which the heme iron can exist in either reduced, NO-bound, or oxidized state. Biochemical studies revealed that *Vv*HmpA is a flavohemoglobin containing equimolar amounts of heme and FAD as cofactors. The K_M and k_{cat} values of *Vv*HmpA for NO at 37°C, the temperature encountered by *V. vulnificus* in the host, were greater than those at 30°C, indicating that *Vv*HmpA detoxifies high levels of NO effectively during infection. Compared with the wild type, the *VvhmpA* mutant exhibited a lower NO-decomposition activity and impaired growth in the presence of NO *in vitro*. Also, the cytotoxicity and survival of the *VvhmpA* mutant infecting the NO-producing murine macrophage cells were lower than those of the wild type. Furthermore, the mouse lethality of the *VvhmpA* mutant was reduced compared to that of the parental wild type. The combined results revealed that *Vv*HmpA is a potent virulence factor that is induced upon exposure to NO and important for the survival and pathogenesis of *V. vulnificus* during infection.

Keywords: *Vibrio vulnificus*, Gene expression profiling, Nitric oxide, Flavohemoglobins, Virulence factors

D001

Membrane Engineering via *trans*-Unsaturated Fatty Acid Synthesis Increased Succinic Acid Production in *Mannheimia succiniciproducens*

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Engineering of microorganisms to produce desired bio-products with high titer, yield, and productivity is often limited by product toxicity. This is also true for succinic acid (SA), which is a four carbon dicarboxylic acid of industrial importance. Acidic products often cause product toxicity to cells through several different factors, membrane damage being one of the primary factors. In this study, *cis-trans* isomerase from *Pseudomonas aeruginosa* was expressed in *Mannheimia succiniciproducens* to produce *trans*-unsaturated fatty acid (TUFA) and to reinforce the cell membrane of *M. succiniciproducens.* The engineered strain showed significant decrease in membrane fluidity as production of TUFA enabled tight packing of fatty acids, which made cells to possess more rigid cell membrane. As a result, the membrane-engineered *M. succiniciproducens* strain showed higher tolerance toward SA and increased production of SA compared with the control strain. The membrane engineering approach employed in this study will be useful for increasing tolerance to, and consequently enhancing production of acid products.

[This work was supported by the C1 Gas Refinery Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2016M3D3A1A01913250)]

Keywords: Succinic acid, Membrane engineering, *trans*-Unsaturated fatty acid, *Mannheimia succiniciproducens*

D002

Development of Metabolically Engineered *Mannheimia succiniciproducens* for the Production of Succinic Acid from Formic Acid as a Carbon Source

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To solve environmental problem, much effort has been exerted to reduce one carbon (C1) gas emission. As one promising way to more conveniently utilize C1 gas, several technologies have been developed to convert C1 gas into useful chemicals such as formic acid (FA). In this study, *Mannheimia succiniciproducens* was engineered using systems metabolic engineering method to efficiently utilize FA. ^{13}C isotope analysis of *M. succiniciproducens* showed that FA could be utilized through formate dehydrogenase (FDH) reaction and/or the reverse reaction of pyruvate formate lyase (PFL). FA assimilation via FDH was found to be more efficient than the reverse reaction of PFL. Four different FDHs from *M. succiniciproducens*, *Methylobacterium extorquens*, and *Candida boidinii* were amplified in the LPK7 strain to find suitable FDH for enhancing FA assimilation. As a result, this strain produced 76.11 g/L SA with the yield and productivity of 1.28 mol/mol and 4.08 g/L/h, respectively, using sucrose and FA as dual carbon sources. The strategy employed here will be similarly applicable in developing microorganisms to utilize FA and to produce valuable chemicals and materials from FA.

[This work was supported by the C1 Gas Refinery Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2016M3D3A1A01913250)]

Keywords: Formic acid, Succinic acid, *Mannheimia succiniciproducens*, C1 gas refinery

D003

Engineering of an Oleaginous Bacterium for the Efficient Production of Fatty Acid and Fuels

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Production of free fatty acids (FFAs) and derivatives from renewable non-food biomass by microbial fermentation is of great interest. Here we report the development of engineered *Rhodococcus opacus* strains producing FFAs, fatty acid ethyl esters (FAEEs) and long-chain hydrocarbons (LCHCs). Culture condition is optimized to produce 82.9 g/L of triacylglycerols from glucose. An engineered strain with acyl-CoA synthetases deleted, overexpressing three lipases with lipase specific-foldase produces 50.2 g/L of FFAs. Another engineered strain with acyl-CoA dehydrogenases deleted, overexpressing lipases, foldase, acyl-CoA synthetase, and heterologous aldehyde/alcohol dehydrogenase and wax ester synthase produces 21.3 g/L of FAEEs. A third engineered strain with acyl-CoA dehydrogenases and alkane-1 monooxygenase deleted, overexpressing lipases, foldase, acyl-CoA synthetase, and heterologous acyl-CoA reductase, acyl-ACP reductase and aldehyde deformylating oxygenase produces 5.2 g/L of LCHCs. Metabolic engineering strategies and strains developed here will help establish oleaginous biorefinery platform for the sustainable production of chemicals and fuels.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)]

Keywords: Metabolic engineering, Oleoginous, Biorefinery, Fuel

D004

Antibacterial Activities of Meroindenon and Merochlorins C, E, and F, Produced by a *Streptomyces* sp. Isolated from a Marine-derived Sediment

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Over the last decades, the effectiveness of antibiotics is facing serious clinical concerns due to the outbreak of antibiotic-resistant bacteria. Natural products have been crucial sources of antibacterial agents historically, and they are still worthy as they provide novel compounds with the potent antibacterial activity. Especially, marine actinomycetes are recognized as prolific sources of structurally unique secondary metabolites with diverse biological activities. In this study, antibacterial activities of four meroterpenoids, meroindenon (**1**) and merochlorins C (**2**), E (**3**), and F (**4**), isolated from a marine *Streptomyces*, CNH-189, were tested by broth microdilution method. Compounds **3** and **4** exhibited strong antibacterial activities against *Bacillus subtilis*, *Kocuria rhizophila*, and *Staphylococcus aureus*, with a range of MIC values from 1 to 2 μg/ml.
[Supported by grants from the National Research Foundation (NRF).]

Keywords: *Streptomyces* sp., Antibacterial activity, Minimum inhibitory concentration

D005

Co-immobilized Bio-capsules for Use in Lignocellulosic Ethanol Production

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Bio-capsule formation and its utilization were tried for the simultaneous saccharification and fermentation (SSF) process in lignocellulosic bioethanol production. Firstly, bio-capsules were formed using cellulase producing fungal strains with ethanol producing yeast strains. Secondly, pellet formation was done to select the optimal condition for bio-capsule formation, because pellet formation is the very first requisite for bio-capsule formation. The optimum conditions for pellet formation were determined to be 28°C and 120 rpm. The optimal nonionic surfactant and initial pH for pellet formation and enzymatic saccharification were also measured. Finally, ethanol yields of about 4.9% (v/v) were achieved using cellulase producing fungal strains with ethanol producing yeast strains. The results provide valuable insights into the bio-capsule formation for SSF process of lignocellulosic bioethanol and the practical use of bio-capsule for the lignocellulosic bioethanol production.

Keywords: Bio-capsule, Lignocellulosic bioethanol, Cellulase producing fungus, Simultaneous saccharification and fermentation, Yeast

D006

Effects of Different Feeding Systems on Ruminal Fermentation Characteristic and Microbial Community Changes

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Enteric fermentation methane has become an issue to resolve in the world because they can affect the global warming. This study was conducted to identification of various microbial community changes in rumen according to the different feeding systems. The experiment was conducted using five Korean native cattle Hanwoo steers, 1.2 kg of forage (rice straw 0.6 kg + timothy hay 0.6 kg), 3.0 kg of concentrates feed were fed twice a day. Feeding systems was Treat 1) feeding of concentrate 1 h later feeding of forage, Treat 2) feeding of forage 1 h later concentrate, Treat 3) as a total mixed ration and performed in 3 x 3 Latin square design. In the results, pH value was significantly higher in Treat 2 group than Treat 3 group ($p < 0.05$). Total VFA and Individual VFA did not differ between groups. Ammonia nitrogen concentrate was higher in 1 h after feeding Treat 1 group ($p < 0.01$). The highest abundant phyla were Bacteroidetes and Firmicutes shown 44% and 39% of the total sequences respectively, in all 3 dietary groups. Prevotellaceae (28%) was the most dominant family and the sequences of Lachnospiraceae (9%), Barnesiellaceae (6%), Ruminococcaceae (5%), and Acidaminococcaceae (5%) showing high abundance with significantly different abundances among the dietary groups; particularly Treat 2 and Treat 3 showing high abundance of fermentative related bacteria. Therefore, we speculate that various feeding systems can change the ruminal fermentation characteristic and microbial community.

Keywords: 16S rRNA gene, Rumen bacterial communities, Hanwoo

D007

Competition Test of Probiotic Candidates Using Korean Gut Microbiota *in vitro* Incubation System

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Several health-promoting lactic acid bacteria (LAB) are known to have beneficial effects on the human gut microbiome. However, there was a limitation to identify the effects of LAB on Korean gut microbiome. Therefore, we have constructed *in vitro* Korean gut microbiota incubation model to screen the effect of LAB on Korean gut microbiome. We collected the fecal samples of Korean donors (n=12, female=6, male=6). To construct *in vitro* Korean gut microbiota incubation model, inoculated fecal samples were incubated with a basal medium in anaerobic conditions. To analyze microbiota changes, we used 16S rRNA gene-based sequencing technique (V4 region; Illumina MiSeq) based on the standardized protocol by Earth Microbiome Project (EMP). We found that the enterotypes of subjects were separated by gut microbiome structure. And one of LAB candidates derived from Korean traditional fermented foods showed higher remained LAB contents after incubation, in relation to other LAB strains. These results revealed that the screening of LAB which has higher stability in Korean gut environment will have the potential to be developed as novel probiotics specialized for Korean gut microbiome. In addition, these results showed that this incubation system could be used to screen LAB which has higher stability in Korean intestinal environment.

Keywords: *In vitro* gut incubation system, Microbiome, LAB, Enterotype, Korea traditional fermented foods

D008

A Comparative Study on the Physiochemical Properties of Rice Beer Manufactured from Pure Selected Yeast and Commercial Yeast

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Seoul Venture University Brewing Technology

This study compares the quality characteristics of rice beer between five types of yeast separated from various traditional yeast and commercial brewing yeast. Five *Saccharomyces cerevisiae* yeasts selected (KCCM 90316, KCCM 90299, KCCM 90317, KCCM 90300, KCCM 90301) were isolated in whole wheat, rice, and Ehwa grains, and were fermented continuously over three consecutive periods at 20°C to compare their fermentation and quality characteristics with commercial yeast. The six types of yeast represented 4.3 to 5.2% alcohol content after the first 10 days of fermentation, while the sugar content was 7 to 8 brix, the pH was 4.2 to 4.5 and the yeast was 3–5 × 10^6, showing the five types of yeast, which were generally separated, showed suitable brewing characteristics as compared to commercial yeast. After the second and third fermentation, similar patterns such as the first fermentation were seen, but the second and third fermentation made it possible to observe the fermenting activity in all yeast. In addition, the analysis to determine the physiochemical characteristics of selected yeasts showed that the concentration of ester affecting aroma of rice beer was similar to that of commercial (21.2 ppm).

Keywords: Rice beer, Commercial brewing yeast

D009

Selection and Optimization of Putative Probiotic *Lactobacillus plantarum* Strain D2-1 for Alfa-hydroxy-isocaproic Acid (HICA) Production

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The loss of muscle mass and strength in an aging society is a major public health issue. Alpha-Hydroxyisocaproic acid (HICA) is a leucine metabolite by the reduction reaction of hydroxy acid dehydrogenase (HicDH). According to previous study, HICA increases protein synthesis with muscle mass. Fermented foods such as kimchi and geotgal are rich in HICA content via protein fermentation of the lactic acid bacteria (LAB). In this study, various LAB strains were isolated from fermented foods for HICA producing putative probiotics. *Lactobacillus plantarum* strain D2-1 having HicDH gene and probiotic properties was selected as a HICA producing putative probiotic strain. The optimal condition of HICA expression were profiled by UPLC-MS-MS compared with *Lactobacillus plantarum* strain WCFS1. The results showed environmental conditions which are temperature, pH, osmotic pressure, and culture medium components can influence the HICA production. In several culture condition, HICA amount of strain D2-1 was similar or higher than WCFS1. Thus, the probiotic candidate can be expected to have functional aspects of sarcopenia preservation and treatment. As a further study, it desires to demonstrate muscle synthesis due to mTOR activity among the functions of HICA with muscle cell line and *in vivo* mouse experimental model.
[Supported by "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01354402)" Rural Development Administration, Republic of Korea.]

Keywords: *Lactobacillus plantarum*, HICA, Muscle synthesis, Probiotics, Kimchi

D010

Transcriptome-based Identification of a Gene Cluster for the Biosynthesis of Biruloquinone, a Phenanthraquinone with Purple Pigment, Produced by the Lichen-forming Fungus *Cladonia macilenta*

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Sunchon National University

Lichens are renowned for production of novel types of polyketide-derived secondary metabolites. Lichens form a stable symbiotic association between mycobionts and photobionts. The purple pigment biruloquinone, first identified in the lichen thallus of *Parmelia birulae*, inhibit acetylcholinesterase, one of the key enzymes involved in Alzheimer's disease progression. Although biruloquinone has not been found in any *Cladonia* species in nature, we previously identified a morphological variant derived from a mycobiont of *Cladonia macilenta*, which produces biruloquinone *in vitro*. Thus, we compared the transcriptional profile of the biruloquinone producer (purple strain) and the original mycobiont (white strain) to identify genes responsible for biruloquinone biosynthesis and its associated gene cluster. Among the 33 polyketide synthase (PKS) genes in *C. macilenta*, a non-reducing PKS gene was highly expressed in the purple strain, while the PKS gene expression was negligible in the white strain. Genes adjacent to the PKS gene appear to be co-expressed, suggesting these genes are involved in biosynthesis of biruloquinone. The five neighboring genes include genes encoding a transcription factor, an oxidoreductase, an *O*-methyltransferase and two transporters. This study highlights the effectiveness of applying transcriptome data for identification of biosynthetic gene clusters, especially in lichen-forming fungi recalcitrant for genetic transformation.
[Supported by grants from KRF]
Keywords: Lichen, Secondary metabolites, Polyketides, *Cladonia*, Gene cluster

D011

Anti-adipogenic Effect of Korean Lichen Extracts

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Lichens possess various kinds of secondary metabolites known as lichen substances, which have been reported to have bioactive functions including anti-microbial, anti-oxidant, anti-tumor and anti-inflammatory activities; however, the anti-adipogenic activity remains to be studied. This study examined the anti-adipogenic effect of 64 extracts from 32 Korean lichens samples using two kinds of solvent, acetone and methanol. The screening results showed that some of the extracts decreased the oil red O stained 3T3-L1 adipocytes. 2 lichen substances significantly down regulated mRNA expression of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT element binding protein α (C/EBPα) which are key adipogenic transcriptional factors in adipogenesis pathway. 2 lichen species, *Nipponoparmelia laevior* and *Parmotrema cristiferum*, both of them contain salazinic acid. It was proved that salazinic acid could suppress the expression of adipocyte. The results indicated that lichen compounds are capable of inhibiting the differentiation process and lipids accumulation in 3T3-L1 preadipocytes, suggesting its potential function of anti-obesity.

[Supported the Forest Science & Technology Projects (Project No. 2017024A00-1720-BA01) provided by the Korea Forest Service.]

Keywords: Lichen substances, Salazinic acid, Adipocyte, Anti-adipogenic effect, Anti-obesity

D012

Enzymatic Activities of Breeding Strains between Sanbaeghyang and Sanjo 707 ho, *Lentinula edodes*

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In order to develop superior varieties of *Lentinula edodes*, we cultivated new breeding strains and investigated their culture characteristics. Extracellular enzyme activities of new breeding 36 strains and parental strains of *L. edodes* were tested on CMC (carboxymethyl cellulose) agar plates and ABTS agar plates. NIFoS 4072, NIFoS 4086, NIFoS 4088, NIFoS 4089, NIFoS 4099 and NIFoS 4103 had the highest cellulase activities (19.5 cm^2) among them, followed by NIFoS 4093 (19.2 cm^2), NIFoS 4078, NIFoS 4091 (18.9 cm^2), NIFoS 4082, NIFoS 4097 (18.6 cm^2) and NIFoS 4095 (18.5 cm^2). The cellulose activities of parent al strains Sanbaekhyang (15 cm^2) and Sanjo 707 (14.4 cm^2) were below the average (16.1 ± 3.2).

NIFoS 4099 (30.5 cm^2) had the highest laccase activities (19.5 cm^2) among them, followed by NIFoS 4097 (27.9 cm^2), NIFoS 4089 (27.6 cm^2), NIFoS 4095 (27.1 cm^2) and NIFoS 4094 (27 cm^2). The parent al strains Sanbaekhyang (27.2 cm^2) and Sanjo 707 (26.7 cm^2) showing more than average activity (25.1 ± 2.2 cm^2).

Keywords: Breeding, Cultural characteristics, Extracellular enzyme, *Lentinula edodes*

E001

Cloning of 9-cis β-Carotene Isomerase from *Oryza sativa* Japonica in *E. coli*

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Beta-carotene (BC) is an essential pro-vitamin A to humans. Some critical products are derived from Beta-carotene such as 11-cis retinal and retinoic acid (RA), They play important roles in human body controlling differentiation and also act as an antioxidant that provides protection against degenerative diseases, associated with immune systems, energy metabolism, and cardiovascular systems.
Dunaliella bardawil and *Dunaliella salina* Teodoresco are known as the most abundant natural sources of beta-carotene, which is equal to 10 percent of the dry weight.
The biological synthesis path of beta-carotene in *Dunaliella* is established but cis-beta-carotene isomer formation mechanism in plants and algae is not fully revealed. Previous studies have not clearly identified the mechanism of 9-cis-beta-carotene formation in *Dunaliella*. It has recently been described that an all-trans/9-cis beta-carotene isomerization protein named DWARF27 (D27) in part of the biosynthesis path of stegolactone in *Oryza sativa*.
In this study, the DWARF27 (D27) was cloned into pET28a vector and over-expressed for catalyzing 9-cis/all-trans beta-carotene isomerization.
The study shows that the isomerase actually catalyzes 9-cis/all-trans beta-carotene isomerization, indicating that they are closely involved in the formation of 9-cis beta-carotene in *Oryza sativa*.
[supported by grants from the Ministry of Oceans and Fisheries, Korea.]

Keywords: Cloning, DWARF27, Beta carotene, *Oryza sativa*, *Dunaliella*

E002

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

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

Keywords: *Cryptococcus neoformans*, Developmental process, Mating

E003

e-Membranome: A Database for Genome-wide Analysis of *Escherichia coli* Outer-membrane Proteins

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Enteric bacterial pathogens such as pathogenic *Escherichia coli* (*E. coli*) bind to glycans of host intestine by lectin-like adhesins during the infection. Therefore, lectin-like adhesins are popular target for vaccine or drug development. However, a database for lectin-like adhesins of *E. coli* has not been developed yet. In this study, we developed a database, e-Membranome, for genome-wide analysis of putative adhesins of *E. coli*, using several software. PSORTb was used for predicting outer-membrane embedded proteins that could be adhesion from the annotated genes of *E. coli* strains and TMHMM version 2.0 (v2.0), SignalP v5.0 and LipoP v1.0 were used for classifying the data. Further analysis was performed by Interproscan and String database. The candidate protein will be investigated for epitope region (ABCpred), homology modeling of the 3D structure (I-TASSER v5.1) and affinity information (glycan array) and stored into e-Membranome database. e-Membranome is implemented using Django v2.2.5 framework. As a web application server, Apache Tomcat 6.0 is employed in the platform on Ubuntu Linux v16.04 and MySQL v5.7 is used as a database engine. The platform, e-Membranome, will be a good resource for the research of outer-membrane embedded proteins and the construction of lectin-glycan interaction network of *E. coli*.
[This work was funded by Korea Centers for Disease Control and Prevention, Funding Number: 4845-300-210-13.]

Keywords: Pathogenic *Escherichia coli*, Genome-wide analysis, Lectin-like adhesins, Lectin-glycan interaction, Database platform

E004

Identification and Functional Characterization of Essential Transcription Factors in Human Fungal Pathogen

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Cryptococcus neoformans is an opportunistic human fungal pathogen that causes cryptococcosis and fatal meningoencephalitis. Due to its clinical importance and lack of effective, selective and safe antifungal agents to treat cryptococcosis, it is crucial to identify and validate novel antifungal drug targets. Among these, essential proteins can be valuable antifungal targets as they directly affect the growth of a fungal pathogen. According to our previous report (Jung et al 2015 *Nat Comm*), 23 transcription factors are suspected to be essential for the growth of *C. neoformans* as they could not be deleted from the genome. For these genes, we constructed promoter-replaced mutant strains to control their expression levels. Among 19 transcription factors tested thus far, 9 of them were found to be required for growth, whereas the remaining 10 were dispensable for the growth of *C. neoformans*. By phenotypic analysis, we confirmed that the growth-required TFs were highly related to DNA damage response and membrane stability. To examine the essentiality of the 9 growth-required transcription factors, we constructed heterozygous mutants in an engineered diploid strain of *C. neoformans*, and are analyzing basidiospores from these strains to explore the role of these genes in viability. The outcome of this study is to identify and functionally characterize transcription factors essential for the growth and pathogenicity of *C. neoformans*.
Keywords: *Cryptococcus neoformans*, Essential gene, Transcription factor

E005

Systematic Functional Analysis of Phosphatases Networks in *Cryptococcus neoformans*

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Cryptococcus neoformans causes fatal cryptococcal meningoencephalitis mainly in immunocompromised patients. Despite its clinical importance, comprehensive understanding of its pathobiological signaling networks is far from completion and therapeutic options are highly limited. Here, to further elucidate complex signaling networks regulating the virulence of *C. neoformans*, we aimed to identify and functionally characterize the 139 putative phosphatases, which are major signaling components. We selected putative phosphatases based on annotation in the *C. neoformans* var. *grubii* genome database provided by NCBI and performed a BLAST search with their protein sequences to identify any corresponding orthologs in *S. cerevisiae*, *A. nidulans*, *C. albicans*, *F. graminearum* and human. We classified putative phosphatases into 16 groups based on InterPro phosphatase domain annotation. Thus far, we have successfully constructed 230 signature-tagged gene-deletion strains representing 114 putative phosphatases through homologous recombination methods. We are in the middle of examining their phenotypic traits under 30 different *in vitro* conditions and *in vivo* virulence potential in insect and mammalian hosts. Along with our previous functional genetic studies for *C. neoformans* transcription factors and kinases, this study will provide a comprehensive insight into the fungal pathobiological signaling networks.

Keywords: Cryptococcus, Phosphatase, Fungal pathogen

E006

Systematic Dissection 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 global fungal meningoencephalitis. 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. We performed NanoString-based *in vivo* transcription profiling of 183 kinases, 178 transcription factors, and 139 phosphatases during the whole infection process. Here we focused on 23 transcription factors which *in vivo* expression were highly induced during host infection and its deletion mutant exhibits decreased pathogenicity in signature-tagged mutagenesis score. To elucidate their *in vivo* functions, the expression level of 23 genes were measured under *in vitro* host mimic condition (HMC). To classify which host factor causes the increased expression of gene during infection, HMC signals were further dissected into four major host factors which are defined *in vivo* condition including altered temperature, glucose level, carbon dioxide and nitrogen source. We discovered virulence-regulated TFs were differently regulated by each cue, especially *PDR802, HOB5, and FZC39* were upregulated in body temperature. However, the expression of *PDR802* was highly induced by carbon starvation, where else the expression of *HOB5* and *FZC39* were further induced in cell culture media. In conclusion, this study provides further insight into complex signalling pathways modulating the host-pathogen interactions of *C. neoformans*.

Keywords: *Cryptococcus neoformans*, Transcription factors, Host-pathogen interaction, Host mimic condition

E007

Restoration of Genomic Injury by Transposition of Insertion Sequences in Radiation Resistant Bacterium *Deinococcus geothermalis*

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Radiation resistant bacterium *Deinococcus geothermalis* genomic DNA contains 19 types insertion sequences (ISs) in total 93 ISs. ISs actively moved to other sites resulting in gene inactivation and disruption by in case of oxidative stress as well as radiation and other stressors. In this work, we extracted all ISs from *D. geothermalis* genomic DNAs containing main chromosome and two mega plasmids. Total size of 78.8 kb DNA length was reduced, 14 genes were recovered as 13 functional ORFs and 1 promoter region. There are mainly typical structure of IS elements with conserved direct repeat (DR) sequence and terminal inverted (TIR) sequences and several unique IS structures for examples, IS*Dge2* with counter direct repeat sequences and IS*Dge4* with completely different terminal inverted sequences as well as IS200/IS605 family members, IS*Dge10*, IS*Dge18*, and IS*Dge19*, with specially single DNA intermediates involved transposition mechanisms. From several ISs integration hot places, we also determined the order of ISs integration resulting in might be integration of IS1 members was done relatively later. This work is not simplified bioinformatic handles for genetic element extraction and even more exciting analysis for genetic evolutionary traits for recovery of disrupted structural genes and action modes of specific transposases, and understanding of genomic plasticity in bacteria.
[This work was supported by grants from NRF (2015R1D1A1A02062106) and KHU (20151262).]

Keywords: Genomic plasticity, Transposition, Insertion sequences, Restoration, *Deinococcus geothermalis*

E008

The Roles of *vidC* in Fungal Growth and Development in *Aspergillus* spp.

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In the model organism *Aspergillus nidulans*, the VosA-VelB hetero-complex acts as a transcription factor that regulates mRNA expression of the genes related to sporogenesis. In this study, we characterized one of VosA/VelB-inhibited developmental gene *vidC* (*AN4206*) in *A. nidulans* and *A. flavus*. The *vidC* gene encodes a DnaJ domain containing protein, an ortholog of *Saccharomyces cerevisiae* XDJ1 involved in the DNA damage repair system. The *AnividC* deletion mutant strains exhibited defect conidiation and cleistothetia production. The *AnividC* deletion mutant conidia contain less amount of trehalose compared to wild-type. In addition, the *AnividC* deletion mutant conidia were more sensitive to thermal stress compared to wild-type conidia. These results suggest *vidC* plays crucial role in fungal development in *A. nidulans.* Similar to the role of *vidC* in *A. nidulans* development, deletion of the *vidC* ortholog gene (AFLA_050040) caused decreased conidial production and sclerotia production in *A. flavus*. In secondary metabolite production, deletion of *vidC* resulted in increased sterigmatocystin and aflatoxin production in *A. nidulans* and *A. flavus*, respectively. Overall, these results demonstrate that VidC plays a similar role in *Aspergillus* spp. that VidC is essential for appropriate fungal development and regulation of secondary metabolites in *Aspergillus* spp.

Keywords: *Aspergillus nidulans*, *vidC*

E009

Characterization of the Homeobox Genes in *Aspergillus nidulans*

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The homeodomain-containing transcription factors play an important role in growth, development, and secondary metabolism in fungi and other eukaryotes. In this study, we characterized the roles of the homeobox transcription factors in the model organism *Aspergillus nidulans*. We characterized 4 homeobox genes among 8 homeobox genes in *A*. *nidulans* by genetic analysis and generated mutants for each gene. The deletion of *AN1217* caused defect growth, conidiation and sexual development. Absence of *AN2020* resulted in less conidiation but more sexual development. Overall, these results propose that the homeobox genes have effect in sexual and asexual development in *A. nidulans*.

Keywords: *Aspergillus nidulans*, Homeobox domain

E010

Functional Analysis of *vosA* in *Aspergillus nidulans* Sexual Spores

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VosA plays significant roles in asexual sporulation in the model filamentous fungus *Aspergillus nidulans*. In the present study, we characterize the roles of *vosA* in sexual spores. During ascospore maturation, deletion of *vosA* causes rapidly decreased spores viability. In addition, absence of *vosA* results in lack of trehalose and decreased in tolerance to thermal and oxidative stresses. Genome wide analysis demonstrated that loss of *vosA* induces expression of sterigmatocystin biosynthesis genes and slightly increases sterigmatocystin contents in ascospores. In the *vosA* deletion mutant ascospores, expression of other secondary metabolite gene clusters including asperthecin, microperfuranone, and monodictyphenone increased, but mRNA expression of genes involved in primary metabolite processes was decreased. Moreover, deletion of *vosA* results in alters mRNA expression of genes associated with cell wall integrity and trehalose biosynthesis. Overall these results demonstrate that VosA is a key regulator for sporogenesis in both asexual and sexual spores in *A. nidulans*.

Keywords: *Aspergillus nidulans*, *vosA*, Ascospore

E011

Unraveling the Role of the Casein Kinase II Complex 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. 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. In previous studies, we found Cka1, a serine/threonine eukaryotic kinase, is involved in regulation of cell growth, cell cycle, cellular morphology and pathogenicity of *C. neoformans*. In this study, we aim to figure out the regulatory mechanism of Cka1 in *C. neoformans.* We found one catalytic subunit Cka1 and two regulatory subunits which are Ckb1 and Ckb2 as a putative complex subunit. To confirm the protein localization and the complex formation of CK2, we constructed tagged or co-tagged mutants of Cka1-GFP, Ckb1-mCherry, and Ckb2-mCherry. We also constructed single and double knockout mutants of regulatory subunits. The regulatory subunits were involved in antifungal drugs susceptibility, oxidative stress and DNA damaging responses. Interestingly, when *CKA1* was overexpressed in *ckb2*Δ *ckb1*Δ, it restored the sensitive phenotypes of *ckb2*Δ *ckb1*Δ. We also constructed *cka1*Δ *ckb1*Δ *ckb2*Δ and it showed severe growth defect like *cka1*Δ mutant. As a result, Cka1 plays major roles and Ckb1, Ckb2 have minor roles in CK2 complex. This study will provide a comprehensive Cka1 cellular mechanism to develop an antifungal drug.

Keywords: Fungal pathogen, Casein kinase 2, Antifungal drug target

E012

Unwinding the Role of Pseudouridylation in an Opportunistic Fungal Pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans is a fungal pathogen that causes systemic cryptococcosis and meningoencephalitis mainly in immunocompromised individuals. Due to its clinical importance, revealing the factors that can affect its life cycle is critical for development of novel 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 *S. cerevisiae*, eight proteins exist as stand-alone pseudouridylases, and each protein has specific pseudouridylation sites and roles. To unravel the functions of pseudouridylases in *C. neoformans*, we identified six putative enzymes in *C. neoformans* by performing BLAST search in the FungiDB database with protein sequences of the known *S. cerevisiae* pseudouridylase genes. To characterize the function of the enzymes, we constructed more than two independent strains for 5 putative pseudouridylase genes and examined their phenotypic traits under various *in vitro* and *in vivo* 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.*

Keywords: *Cryptococcus neoformans*, Fungi, Pseudouridylation, Transcript

E013

Crosstalk among Hog1, Mpk1, and Cpk1 MAPK Pathways Governs Cell Wall Integrity in Human Fungal Pathogen

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Mitogen-activated protein kinases (MAPK) are central key kinases of multifunctional complex pathway in model pathogenic fungi *Cryptococcus neoformans* which causes meningoencephalitis to immunocompromised patients. There are three major MAPK pathways including Hog1, Mpk1 and Cpk1 pathway in *C. neoformans*. Even though the study of individual MAPK pathways have progressed extensively, the research concerning the crosstalk between different MAPK pathways have yet to be elucidated. In this study, we aim to understand the crosstalk among three major MAPKs and find the key regulatory subunits to explain the intricate signals regulating the virulence of *C. neoformans*. To confirm how Hog1, Mpk1, and Cpk1 MAPK crosstalk regulate the downstream signaling factors, we constructed MAPK deletion mutants (*mpk1*Δ *hog1*Δ, *cpk1*Δ *hog1*Δ, *mpk1*Δ *cpk1*Δ, *mpk1*Δ *cpk1*Δ *hog1*Δ). Through phenotypic analysis, we discovered that all three MAPKs have roles in thermosensitivity, osmotic stresses, cell wall, membrane stresses and ER stresses. Specifically, Mpk1 is known to play key roles in cell wall stress response, and we discovered that Hog1 and Cpk1 cooperatively contribute to cell wall integrity (CWI). To identify the regulatory mechanism in CWI with the crosstalk of MAPK, we observed the changes of pHog1 and pMpk1 in double MAPK mutants (*mpk1*Δ *hog1*Δ, *cpk1*Δ *hog1*Δ, *mpk1*Δ *cpk1*Δ) under basal and cell wall disturbing conditions. In conclusion, we aim to elucidate comprehensive interaction of MAPKs.

Keywords: *Cryptococcus neoformans*, MAPK pathway, Crosstalk, Phosphorylation

E014

Identification and Characterization of Clustered Ecumicin Biosynthetic Genes in *Nonomuraea* sp. MJM5123

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Drug-resistant tuberculosis (TB) has lent urgency to finding new drug leads with novel modes of action. A high-throughput screening campaign of >65,000 actinomycete extracts for inhibition of *Mycobacterium tuberculosis* viability identified ecumicin, a macrocyclic tridecapeptide that exerts potent, selective bactericidal activity against *M. tuberculosis in vitro*, including non-replicating cells. Ecumicin retains activity against isolated multiple-drug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis*. Ecumicin is a kind of cyclic peptide synthesized by NRPS (Non-Ribosomal Peptide synthase) gene cluster of *Nonomuraea* sp. MJM5123 isolated from Korean soil. In this study, we described the cloning, sequencing, characterization, and application of membrane transport-related genes in *N.* sp. MJM5123, whose detailed results will be further discussed.
[This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01319102)" Rural Development Administration, Republic of Korea.]

Keywords: Tuberculosis, Ecumicin, Biosynthetic gene cluster

E015

Purification and Characterization of a Putative DNA-Protection Protein (Dps) Originated from *Deinococcus geothermalis*

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The ferritin-based putative DNA protection protein from starved cells (Dps) of *Dinococcus geothermalis*, Dgeo_0257, was heterogeneously expressed and extracted from *Escherichia coli*. The 194 aa-length of DNA-bound protein revealed more than 90% identity with a hypothetic protein from *D. apachensis*, *D. metallilatus*, *D. aerius*, and *D. phoenicis* genomes. From RNA-Seq results of wild-type *D. geothermalis* and Δ*dgeo*_0257 strain, it is revealed that Dgeo_0257 could play an important role in the specific gene regulation, for examples, strong induction of IS*Dge5* insertion sequences and down regulation of iron transporters as well as DNA stabilization. Predominantly, Dps needs a conservative N-terminal tail to tightly correlate with the formation of DNA-Dps complex. Early Dps samples containing His-tags in the N-terminal region expressed in the pQE80L_Dgeo0257 vector interfered with DNA binding and also prevented purification of the expressed protein pool due to self-aggregation structure. Therefore, we recombined the target gene using the pCS19_Dgeo0257 vector with His-tags in C-terminus and succeeded to extract the pure target protein. By subsequent electrophoretic mobility shift assay (EMSA), Dgeo_0257 showed dominant DNA binding affinity under the presence of metallic ion such as Fe(II) and Mn, Zn, Mg, Ni, Ag, Cr, etc. With all results, we claim Dgeo_0257 is a novel Dps protein in *D. geothermalis*.

Keywords: Bacteria, *Deinococcus geothermalis*, Dps, Ferritin, Protein purification

E016

Comparison of Targeted Next-Generation Sequencing for Comprehensive Analysis of Hantaan Orthohantavirus in *Apodemus agrarius*

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Hantaan orthohantavirus (HTNV), harbored by *Apodemus agrarius* (the striped field mouse), is the causative agent of hemorrhagic fever with renal syndrome in humans. Endemic infections of hantaviruses are responsible for annual 150,000 clinical cases with mortality rates from 1–35% around the world.
Next-generation sequencing (NGS) is a potent method to sequence the viral genome, using molecular enrichment methods, from clinical specimens containing low virus titers. Hence, a comparative study on the target enrichment NGS methods is required for whole-genome sequencing of hantavirus in clinical samples.
In this work, we prepared the RNA samples from 14 *A. agrarius* lung tissues and enriched the viral RNA using sequence-independent, single primer amplification, target capture, and amplicon-based methods prior to the NGS library preparation. We analyzed the coverage of the HTNV genome based on the viral RNA copy number, which is quantified by real-time PCR. Target capture and amplicon NGS demonstrated a high coverage rate of HTNV in *A. agrarius* lung tissues containing up to 10^3–10^4 copies/μl of HTNV RNA. Furthermore, the amplicon NGS a 10-fold (10^2 copies/μl) higher sensitivity than the target capture NGS. Thus, this study provides useful insights into target enrichment NGS for the rapid identification and characterization of hantaviruses during endemic outbreaks.
[Supported by grants from ADD and NRF]

Keywords: *Apodemus agrarius*, Hantaan orthohantavirus, Next-generation sequencing, Whole-genome sequencing

E017

Development of an *Agrobacterium tumefaciens*-mediated Transformation Method in the Lichen-forming Fungus *Cladonia macilenta*

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Despite the fascinating biology of lichens, such as symbiotic association of lichen-forming fungi (LFFs) with photosynthetic partners and their growth in extreme environments, studies on the genetic mechanisms underpinning the lichen biology have been hindered by lack of genetic tools for the manipulation of LFFs and their slow growth rate. Previously, we developed an *Agrobacterium tumefaciens*-mediated transformation (ATMT) method in the yeast-like growth form of the LFF *Umbilicaria muehlenbergii*. However, it is currently not known if the ATMT can be applied to the vast majority of LFFs that grow in hyphae. Cladoniaceae is one of the largest and most diverse families of lichens. Thus, we established an ATMT method in the LFF *Cladonia macilenta* that exhibited relatively faster growth rate in culture media. We generated a total of 192 transformants, among which about 80% of the transformants were confirmed to have a single T-DNA copy in their genome. We performed TAIL-PCR to determine T-DNA insertion sites in the eight selected transformants. TAIL-PCRs were successful with four transformants: one had T-DNA insertion within a coding sequence, and the other three had T-DNA insertion in intergenic regions. Determination of T-DNA insertion sites for the remaining transformants is ongoing. This ATMT approach will offer unique opportunities to study the genetic mechanism behind the lichen symbiosis, stress tolerance, and secondary metabolite biosynthesis.

Keywords: Lichen, *Agrobacterium*, Transformation, *Cladonia*

E018

Iron Uptake and Antifungal Susceptibility are Modulated by pH in *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic fungal pathogen which causes fatal meningitis in immunocompromised hosts. *C. neoformans* can invade by inhalation, but host's immune system militates against infection of the pathogen by phagocytosis. In that process, phagocytosed *C. neoformans* resides in phagosome which matures to form phagolysosome that results in acidification. However, in *Candida albicans* which is a fungal pathogen, it was showed that susceptibility of *C. albicans* against to fluconazole was reduced at acidic pH while it was high susceptible at neutral pH. In *C. neoformans*, deletion of *CFO1* and *CFT1*, which encode ferroxidase and iron transporter, respectively, each mutant showed reduced susceptibility at acidic pH. Based on this discovery, we investigated the interaction of pH, antifungal susceptibility and iron metabolism. To investigate relations of pH and iron uptake, western blotting, phenotypic analysis and growth curve analysis were performed. MICs analysis and phenotypic analysis were carried out to investigate interaction of pH and antifungal susceptibility. The results showed antifungal susceptibility of *C. neoformans* against to fluconazole were more reduced at acidic pH than neutral pH. Additionally, *cfo1Δ* and *cft1Δ* recovered growth defect in low iron media at acidic pH while each mutant showed growth defect at neutral pH. It suggested that iron uptake mechanism was altered by pH. Overall, our results suggested pH of media modulated survival mechanisms of *C. neoformans* such as cell wall integrity, antifungal susceptibility and iron uptake.

Keywords: *C. neoformans*, pH, Antifungal, Fluconazole, Iron

F001

A High ATP Concentration Enhances the Cooperative Translocation of the SARS Coronavirus Helicase nsP13 in the Unwinding of Duplex RNA

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Severe acute respiratory syndrome coronavirus nonstructural protein 13 (SCV nsP13), a superfamily 1 helicase, plays a central role in viral RNA replication through the unwinding of duplex RNA and DNA with a 5′ single-stranded tail in a 5′ to 3′ direction. Despite its putative role in viral RNA replication, nsP13 readily unwinds duplex DNA by cooperative translocation. Herein, nsP13 exhibited different characteristics in duplex RNA unwinding than that in duplex DNA. nsP13 showed very poor processivity on duplex RNA compared with that on duplex DNA. More importantly, nsP13 inefficiently unwinds duplex RNA by increasing the 5′-ss tail length. As the concentration of nsP13 increased, the amount of unwound duplex DNA increased and that of unwound duplex RNA decreased. The accumulation of duplex RNA/nsP13 complexes increased as the concentration of nsP13 increased. An increased ATP concentration in the unwinding of duplex RNA relieved the decrease in duplex RNA unwinding. Thus, nsP13 has a strong affinity for duplex RNA as a substrate for the unwinding reaction, which requires increased ATP hydrolysis to processively unwind duplex RNA. Our results suggest that duplex RNA is a preferred substrate for the helicase activity of nsP13 than duplex DNA at high ATP concentrations.

Keywords: Severe acute respiratory syndrome coronavirus, Helicase, RNA unwinding, ATP concentration

F002

Effects of Tear Solution on Biofilm Formation of *Staphylococcus epidermidis* in Two Soft Contact Lens Materials

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Staphylococcus epidermidis is common causative organism of bacterial keratitis, especially in contact lenses users. The ability to form biofilm of the organisms contributes to the virulence and is affected by several environmental factors including tear in eye.
In this study, we investigated the effects of tear solution on the biofilm formation of *S. epidermidis* using two different materials of soft contact lenses, etafilcon A and hilafilcon B.
The tear solution was prepared in 100 ml of 0.01M phosphate buffer saline with 0.18 g of lysozyme, 0.18 g of globulin, and 0.54 g of albumin. Biofilm formation was compared using XTT assay and dry weight measurement. The expression levels of biofilm formation-related genes were compared using reverse transcription PCR and real time PCR.
The formation of biofilm was higher in etafilcon A than hilafilcon B. After exposure to tear solution, protein deposition was significantly higher in etafilcon A than in hilafilcon B. Pre-treatment with tear solution inhibited the biofilm formation on both soft lens materials. Moreover, the expression levels of biofilm formation-related genes including icaA, icaB, icaC, icaD and arcA were also decreased by exposure to tear solution in both soft lens materials. These results indicate that tear protein may inhibit biofilm formation and prevent eye infection. Therefore, maintaining the physiological condition of the eyes is helpful to prevent ocular infection in contact lens users.

Keywords: *Staphylococcus epidermidis*, Tear, Etafilcon A, Hilafilcon B, Contact lens

F003

First Report of Anthracnose of Chili Pepper Fruit Caused by *Colletotrichum truncatum* in Korea

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Chili pepper is a popular vegetable crop valued worldwide for the color, flavor, spice, and nutritional aspects that imparts many dishes. Several fungi affect this plant and anthracnose caused by *Colletotrichum* spp. is an economically important disease, affecting both fruit and seed quality of chili. In September 2015, anthracnose symptoms were observed affecting approximately 20 to 30% of the fruit (Manita) growing in a field of Goesan county, Chungcheongnam-do province, Korea. Fruit infected with anthracnose exhibited small circular brown spots that progressed into sunken zones with concentric rings of orange conidial masses. Consequently, the isolated fungi (CNU180001, CNU180011, and CNU180021) were characterized and identified based on morphological characteristics, pathogenicity test and nucleotide sequence data of the internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and β-tubulin (TUB2). Based on the combined data set of morphological and molecular analysis, isolated fungi were identified as *Colletotrichum truncatum*. Pathogenicity test also revealed that these three isolated fungus showed the circular spots of decay with sunken area symptoms similar to those observed in the field and the control fruit remained asymptomatic. Remarkably, the isolated fungus (CNU180001, CNU180011, and CNU180021) were noted for the first time as the causes of chili anthracnose disease caused by *C. truncatum* in Korea.

Keywords: Anthracnose, Chilli, *Colletotrichum truncatum*, Molecular analysis, Morphology

F004

Generation and *in vitro* Characterization of Recombinant Severe Fever with Thrombocytopenia Syndrome Virus Isolated from Ticks Using a Reverse Genetics System

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Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne *banyangvirus* that causes severe disease in humans. The absence of licensed vaccines and therapeutics for SFTSV emphasizes the need for developing countermeasures against SFTSV infection. In this regard, the reverse genetics system is one of powerful tools to achieve this goal.
In this study, we developed a T7 RNA polymerase-driven reverse genetics system to rescue infectious clones of tick-derived Korean SFTSV strain entirely from cDNA. To develope a reverse genetics system, we cloned cDNAs encoding the three antigenomic segments into transcription vectors in which each segment was transcribed under the control of T7 promoter and HdvRz sequence. We also constructed two helper plasmids expressing the NP or viral RdRp under the control of T7 promoter and EMCV IRES. After co-transfection into BHK/T7-9 cells with three transcription and two helper plasmids and passaged in Vero E6 or Huh-7 cells, we confirmed that the recombinant SFTSV was efficiently rescued. By various infectivity tests *in vitro*, including growth kinetics, plaque morphology, and pattern of viral protein synthesis, our results indicated that recombinant and parental viruses possessed indistinguishable growth properties.
This reverse genetics system for SFTSV could be used to explore viral replication and pathogenesis and to facilitate the development of vaccine and therapeutic strategies.

Keywords: SFTSV, Reverse genetics system

F005

A Rapid and Specific Molecular Diagnosis of Mosquito-borne Viruses

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Mosquito-borne viral diseases are those that are transmitted to people through the bite of an infected mosquito by Zika virus (ZIKV), West Nile virus (WNV), Japanese encephalitis virus (JEV), Dengue virus (DENV), and Chikungunya virus (CHIKV). Mosquito-borne diseases outbreaks include changes in the environment such as global warming, changes in human behavior such as increasing overseas travel, and social changes such as unbanization.
In this study, we designed specific primers and probes of ZIKV, WNV, JEV, DENV, and CHIKV for a rapid and correct diagnosis to mosquito-borne diseases. New primers and probes for each virus were designed in areas with maximal conservation. The primers and probes were confirmed by conventional PCR, real-time PCR and Fast RT real-time PCR using the one-step RT-PCR Taq-polymerase. In order to establish a diagnosis method in the laboratory, primers and probes with high sensitivity and specificity for each virus will be selected by combining the results of three respected experiments. Therefore, the specific primers and probes are considered as a rapid and useful molecular diagnosis method of mosquito-borne viruses.

Keywords: Mosquito-borne viral diseases, ZIKV, WNV, JEV, DENV, CHIKV

F006

Genome-wide Analysis of the Putative G-quadruplex-forming Sequences in Varicella-zoster Virus

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G-quadruplex (G4), a non-canonical nucleic acid structure formed by repetitive guanosine-rich sequences, has been known to play multiple regulatory roles in living cells. It has been shown that putative G4-forming sequences (denoted as GQs) are presented in several viral genomes. The location of viral GQs appears to be non-random and preserved in gene promoters, replication origins and genome ends. We recently performed a genome-wide analysis of G4 formation in human cytomegalovirus and showed that the G4 formation in viral promoters negatively regulates viral gene expression. In this study, we also performed a genome-wide analysis of GQs present in Varicella-Zoster virus (VZV). We found that GQs are distributed throughout the VZV genome and highly enriched in ORF14 and ORF61-62-OriS. Interestingly, GQs in ORF14 were found in the repeated region and seemed to be associated with sequence variation. We confirmed the G4 formation in the ORF14 and ORF61-62-OriS regions using Circular Dichroism analysis of GQ oligomers. For ORF14 GQs, all 9 GQs showed anti-parallel structures. In ORF61-ORF62-OriS GQs, 15 showed parallel structures and 7 formed mixed conformations. The G4 formation in OriS was further confirmed by analysis of mutation sequence. Importantly, the VZV growth in human fibroblast cells was reduced by 1,000-fold by treatment of a G4 stabilizing ligand, NMM. Our results suggest that the G4 formation in the VZV genome has regulatory roles in productive viral infection.

Keywords: VZV, G-quadruplex

F007

Downregulation of SAMHD1 Expression by the CUL2-associated CRL Complex during Human Cytomegalovirus Infection

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SAM domain and HD domain-containing protein 1 (SAMHD1) acts as a restriction factor by limiting the intracellular pool of dNTPs or playing as a nuclease. However, its regulation by viruses is not fully understood. In this study, we show that SAMHD1 suppresses the growth of human cytomegalovirus (HCMV), but its expression is downregulated at late stages of viral infection. The HCMV growth in human fibroblast cells was increased by 8.5-fold in SAMHD1 knock-down cells, demonstrating the antiviral activity of SAMHD1 in HCMV infection. We found that the expression of SAMHD1 was decreased at late times in HCMV-infected cells and that this loss of SAMHD1 was blocked by treatment of MG132, a proteasome inhibitor, or MLN4924, an inhibitor of Nedd8-activating enzyme (NAE) that blocks the formation of the cullin-RING-E3 ligase (CRL) complex. Furthermore, SAMHD1 was reduced in CUL2-overexpressing cells, compared to cells overexpressing other cullins. Consistently, HCMV-induced SAMHD1 degradation was blocked in cells transfected with CUL2 siRNA. Taken together, our results demonstrate that SAMHD1 inhibits the growth of HCMV, but HCMV induces degradation of SAMHD1 at late stages of infection in a manner involving the CUL2-associated CRL complex.

Keywords: HCMV, SAMHD1, CRL complex

F008

Novel Bacteriophage Lytic to Colistin-resistant *Acinetobacter baumannii* Clinical Isolates: Characterization of *in vitro*, *in silico,* and *in vivo*

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Increasing prevalence of multidrug-resistant (MDR) *A. baumannii* in intensive care units has been a major public health concern; especially, the emergence of colistin-resistant *A. baumannii* in a clinical setting has caused significant problems worldwide. Bacteriophages, viruses that infect bacteria, have been reviewed as a potential alternative therapeutic agent for controlling these MDR pathogens. Colistin-resistant *A. baumannii* clinical strains were isolated from patients and phage Bϕ-R410 was isolated from sewage samples in a university hospital, South Korea. Newly isolated Bϕ-R410, belonging to the family *Myoviridae*, has the genome of 44,597 bp linear ds DNA (GC%: 38.28) and 79 putative ORFs. Antimicrobial activities of Bϕ-R410 accessed *in vitro* tests of the host range (90%, 29 of 32 strains) and bacterial lysis assay (OD_{600} at 6 h: MOI 10=0.062, 1=0.129, 0.1=0.194, uninfected=1.017). In the evaluation of therapeutic potential of phage against colistin-resistant *A. baumannii* using *G. mellonella* larvae *in vivo* models, Bϕ-R410 increased the survival rate (from 0% to 90% with MOI 100 at 24 h) of infected larvae. This study strongly suggests that the novel *A. baumannii* phage Bϕ-R410 could be an alternative antibacterial agent to control colistin-resistant *A. baumannii* infections.
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Keywords: Bacteriophage, Colistin-resistant *Acinetobacter baumannii*, *G. mellonella*

F009

Characterization for Serotypes, Genotypes, Toxins and Antibiotic Resistance of *Escherichia coli* Resources in the National Culture Collection for Pathogens

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Background: The purpose of this study is to enhance the utilization of infectious diseases, medicine and food industry by analyzing the characteristics of Escherichia coli resources collected and listed in NCCP.
Methods: The characteristics of 142 *E. coli* resources collected in NCCP for 10 years were analyzed. O serotype analysis was performed by the microplate agglutination method. Antibiotic susceptibility testing was performed by the broth microdilution method using Sensititre's panels and minimal inhibitory concentration was confirmed. Multilocus sequence typing (MLST) analysis was performed using seven house keeping genes. Alleles of the seven genes were analyzed at the MLST website to provide the sequence type (ST).
Results: O serotypes showed 30 types of single serotype such as O6, O26, O55, O104, and O157. There were 60 sequence types of MLST analysis such as ST 11, ST 131, and ST 410. Antibiotic susceptibility characteristics were divided into 45 different types depending on the type of resistant antibiotics, including carbapenemase production *E. coli*. It is possible to provide researchers with *E. coli* resources with various kinds of characteristic information for each resistant antibiotic.
Conclusions: These resources characteristics are expected to be of great help to infectious diseases, food and medical research. All of these *E. coli* resources will be released on the homepage (http://nccp.cdc.go.kr) and some resources are being distributed to researchers.

F010

Prevalence of Different Mechanisms of Fusidic Acid Resistance in *Staphylococcus aureus*

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Fusidic Acid (FA) is used as a topical agent to treat skin infection. FA works by interfering with bacterial protein synthesis, specifically by preventing the translocation of the elongation factor G (EF-G) from the ribosome. FA resistance determinants are alteration of binding site (*fusA* or *fusE=rlpF*) or acquired FA resist We investigated the prevalence of FA resistance in clinical isolates *S. aureus*. Also, we examed distribution of FA resistance determinants. From 2014 to 2018, *S. aureus* were collected in Asan Medical Center in Seoul. A total of 380 *S. aureus* (239 MRSA and 141 MSSA) isolates, the rate of FA resistance was 41% in MRSA and 57% in MSSA. Of these, 92 of 120 ST5-MRSA isolates were resistant to FA, whereas 44 of 52 ST72-MSSA were resistant to FA. Among 99 FA-resistant MRSA, 91 possessed fusA mutation conferring high-level resistance to FA (MIC of ≥ 128 μg/ml), 7 had fusC conferring low-level resistance to FA (MIC of ≤ 32 μg/ml). Among 87 FA-resistant MSSA, 49 had fusA mutation, and 32 had fusC. For 140 isolates (91 MRSA and 49 MSSA) in *fusA* mutations, amino acid substitutions in EF-G were detected, L461K alteration was predominant and only 5 isolates showed other single amino acid substitutions (V90I,N463S,P404L,R483C and S488Q). In this study, FA resistance was dominant in ST5-MRSA and ST72-MSSA with predominance of *fusA* mutation in high resistance. Also, FA resistance was common in ST1-MSSA with predominance of *fusC* acquisition in low resistance.

Keywords: Fusidic acid, Mechanism, *Staphylococcus aureus*

F011

Prevalence of Rifampin Resistance in *Staphylococcus aureus* Isolates

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Antibiotic resistance remains an important problem around the world and the frequency of *Staphylococcus aureus* resistant to rifampin (RIF) has increased dramatically. Mutations in the rifampin resistant determining region (RRDR) of *rpoB* have been shown to confer resistance to RIF in *S. aureus*. This study aimed to investigate the prevalence of RIF resistance in clinical isolates, and to analyze mutation of *rpoB* in 1615 *S. aureus*. The samples obtained from Asan Medical Center, Seoul, Republic of Korea from 2008 to 2017. The MIC of RIF was carried out by using the broth microdilution method following standards recommended by the CLSI. The molecular typing of the isolates was performed MLST, *spa* and *SCCmec* and tested for *rpoB* mutation by PCR. Of the 843 MRSA isolates, 52 (6.2%) were resistant to RIF and among 772 MSSA isolates, 5 (0.6%) were resistant to RIF ($p < 0.001$). Of the 52 isolates, 51 (98.1%) were high-level RIF resistant (MIC ≥ 8 mg/L) while only one (1.9%) has a low-level resistance to RIF (MIC4 mg/L). We identified 19 different types of mutations in the *rpoB* gene mutation analysis. The most common single mutation is A477D (17/48, 35.4%) and the most common spa is t2460 (27/52, 51.9%). We found ST5-MRSA-Ⅱ-spa t2460 (26/52, 50%) molecular type with high resistance to RIF. In conclusion, RIF R of *S. aureus* is closely associated with mutations in the *rpoB* gene and these data suggest that ST5-MRSA-II-spa t2460 confers resistance to RIF.

Keywords: *Staphylococcus aureus*, Rifampin resistant, β-Subunit of RNA polymerase, *rpoB* Gene, Mutation

F012

Isolation and Characterization of Bacteriophages for Various of Bacterial Species from Environmental Samples

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Phage therapy is presented as an alternative to antimicrobial therapy in human and veterinary medicine. The aims in this study were to isolate and characterized for bacteriophages against animal pathogens. A total of eight wastewater collected form six chicken slaughterhouses and two dairy farms. Isolation of phages was done by double agar layer methods using nine different animal pathogens and one antimicrobial resistant bacteria. A total of 51 phages were isolated from slaughterhouse wastewater : 22 for *Salmonella* spp., 11 for pathogenic *Escherichia coli,* 5 for *Klebsiella pneumoniae,* 7 for *Proteus mirabilis*, 2 for *Pseudomonas aeruginosa,* and 6 for methicillin resistant *Staphylococcus aureus.* Phages were grouped by Randomly Amplified Polymorphic DNA (RAPD) using four different primers. The host range were examined using 5 to 39 different strains for each species. Finally, a total of 29 phages were grouping by RAPD and host range test : 12 for *Salmonella* spp., 8 for pathogenic *E. coli,* 3 for *K. pneumoniae,* 2 for *P. mirabilis*, 2 for *P. aeruginosa,* and 2 for MRSA. The isolated phages showed difference in plaque size, turbidity, and host ranges. These phages could be used as candidates for phage cocktails to control bacteria associated animal diseases.

Keywords: Bacteriophage, Phage therapy, Animal pathogens

F013

Epstein Barr Virus Processivity Factor EA-D Induces Proteasomal Degradation of Poly (ADP-ribose) Polymerase-1

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Poly (ADP-ribose) polymerase-1 (PARP-1) is a nuclear protein and transfers ADP-ribose moieties to a target protein by consuming NAD+, called poly (ADP-ribosyl)ation (PARylation). It is involved in important cellular processes such as DNA damage response, apoptosis, cell cycle and gene expressions. In EBV infection, PARP-1 acts as a negative regulator of virus lytic reactivation by PARylating the CCCTC-binding factor (CTCF) insulator protein to keep the lytic promoter from activating and restricts EBV lytic replication by binding to the BZLF1 promoter. Here, we showed downregulation of PARP-1 upon EBV reactivation. PARP-1 transcripts were not changed upon EBV reactivation, suggesting that PARP-1 downregulation was at the post-transcriptional stage. We previously showed that the viral processivity factor PF-8 of Kaposi's Sarcoma-associated herpesvirus (KSHV) was a key factor to induce the degradation of PARP-1 in a ubiquitin-dependent manner, thereby promoting KSHV lytic replication. EBV viral DNA processivity factor, Early Antigen-Diffuse (EA-D) encoded by BMRF1 gene, also interacted with and poly-ubiquitinated PARP-1. The results suggest a conserved molecular mechanism by which oncogenic gammaherpesviruses counteract against a host restriction factor, PARP-1 to facilitate viral lytic replication.
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Keywords: Epstein Barr Virus (EBV), Poly (ADP-ribose) Polymerase-1 (PARP-1), Early Antigen Diffuse (EA-D), Ubiquitination

F014

The Evaluation of Immunogenicity and Efficacy in the Mouse Model after Development of Recombinant Vaccines Using Vaccinia Viral Vector against the Zika Virus

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Zika virus (ZIKV) belong to flavivirus family and genus, a mosquito-borne virus, has been accompanied with Guillain-Barré syndrome (GBS), neurological defect, and microcephaly. However, there are not yet approved vaccines for ZIKV. In the study, we designed the novel ZIKV vaccine candidates using KCDC vaccinia viral vector with the genomic sequence of isolated ZIKV at the Brazil in 2015. Five constructs of vaccine candidates have encoded including Envelope domain Ⅰ/Ⅱ (ED Ⅰ/Ⅱ), domain Ⅲ (ED Ⅲ), full-length envelope without transmembrane (EΔTM), full-length pre-membrane with envelope tagged-His (prME-His), and full-length removed-transmembrane (prMEΔTM) as the composition of ZIKV. C57BL/6 mice were inoculated with 10^7 or 10^8 PFU of 2 out of the 5 candidates (prME-His and ED Ⅲ) by subcutaneous injection. Immunized-mice with 10^7 PFU of prME-His showed highest T cell response inducing interferon-γ in the splenocyte using the ELISPOT assay. In 10^8 PFU, the KCDC vaccinia viral vectors expressing prME-His and ED Ⅲ showed higher total IgG, subclass IgG1, and IgG2b levels than GFP (mock vector). However, neutralizing effects of ZIKV were induced by ED Ⅲ and PIV candidates. *In vivo*, prME-His and ED Ⅲ vaccine candidates reduced ZIKV RNA levels in brain and heart, and both candidates showed protective effects against Asian lineage (PRVABC59). Therefore, we will further study optimal dose and route in order to support a prime-boost vaccination strategies of KCDC viral vaccines with other platforms of ZIKV vaccine candidates.
[This study was supported as part of Internal Research Project No. 2017-NI48001-00 and 2019-NI096-00 by a grant from the Korea National Institute of Health]

Keywords: Zika virus, Recombinant vaccines, Vaccinia virus, Efficacy evaluation

F015

Characterization of ESBL-producing Strain Isolated from Foodborne Illness in Gyeonggi-do

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The 135 strains of pathogenic *Escherichia coli* were isolated from patients with foodborne illness in Gyeonggi province from January to December 2017. In this study, the ESBL - producing pathogenic *Escherichia coli* was screened for antibiotic susceptibility test and ESBL gene. The ESBL-producing pathogenic *Escherichia coli* was isolated from 16 strains of EAEC, 4 strains of EPEC and 20 strains of ETEC. After then, phylogenetic group analysis and MLST analysis were performed to investigate the characteristics of the isolates. In 39 strains, CTX-M and TEM were found to be overlapping, and only one strain was found in TEM. As a result of antimicrobial susceptibility test, all ESBL - producing pathogenic *Escherichia coli* were resistant to 5 antibiotics. Phylogenetic group analysis showed that 94% of EAEC strains were classified as D group and 95% of ETEC strains were classified as A group. MLST analysis showed that it was mainly ST40(EAEC), ST949(ETEC), ST775(EPEC) type.

Keywords: Pathogenic *Escherichia coli*, Foodborne illness, ESBL

F016

Molecular Characteristics of Fluoroquinolone-resistant *Campylobacter* Species Isolated from Korean Livestock during 2010-2016

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Campylobacter is a normal intestinal flora in animals and one of the main sources of food-poisoning in humans. The aim of this study was to investigate the recent trends of fluoroquinolone-resistance (FQ) and molecular characteristics of *Campylobacter* isolated from Korean livestock. A total of 604 *C. jejuni* and 933 *C. coli* were isolated from fecal and carcass samples during 2010–2016. FQ of isolates was determined by broth dilution. Mutations of quinolone resistance determining region (QRDR) and presence of virulence factors were investigated by PCR and sequencing. FQ was high both in *C. jejuni and C. coli* regardless of sample source. The resistance reached to 65% in *C. jejuni* and 100% in *C. coli.* Furthermore, high resistance rate was maintained for a study period. The selected 65 *C. jejuni* isolates had mutations with one to four in GyrA and all FQ isolates had Thr-86-Ile mutation. The analyzed FQ *C. jejuni* were genetically diverse suggest that clonal expansion in not involved in dissemination of FQ *C. jejuni*. Among 12 virulence factors tested, *flaA*, *cdtA*, *cdtB*, *cdtC*, and *dnaJ* genes were detected in more than 50% of isolates, however, *virB*, *pldA*, and c*euE* genes were none or rare. Our results showed that FQ was constantly high in *Campylobacter* species. Furthermore, most of FQ *Campylobacter* isolates carried one more virulence factors. Therefore, it needed the continuous surveillance in food-producing animals to prevent the transmission to humans of antibiotic resistant *Campylobacter* spp. via the food chain.
[Supported by a grant from the Animal and Plant Quarantine Agency (N-1543081-2017-24-0103)]

Keywords: *Campylobacter*, Fluoroquinolone-resistance

F017

Innate Immune Scavenger Receptor MARCO Enhances Binding and Entry of Influenza Virus and Varicella Zoster Virus

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Macrophage receptor with collagenous structure (MARCO) is a cell surface receptor that is thought to bind and recognize numerous bacterial pathogens. However, the role of MARCO during viral entry and binding remains to be determined. Here, we describe the role of MARCO in the promotion of entry and replication of influenza virus (IFV) and varicella zoster virus (VZV), including both clinical (YC01) and vaccine (SuduVax) strains of VZV. Expression of MARCO was found to be increased upon IFV entry in differentiated human monocytic THP-1 cells and blocking MARCO resulted in a significant reduction of influenza nucleoprotein expression. Meanwhile, MARCO overexpression in VZV-infected MeWo melanoma cells led to increased transcript levels of VZV genes, including open reading frame 14 and 63, and higher numbers of infectious progeny virus. Both ELISA and co-immunoprecipitation data showed that MARCO specifically interacts with VZV glycoprotein E. The expression of a naturally occurring transcript variant lacking the C-terminal scavenger receptor cysteine-rich domain (SRCR) (MARCO-II), resulted in suppression of VZV entry and replication, compared with that of full-length MARCO (MARCO-I)-expressing cells. Altogether, the data here suggest that MARCO promotes the entry of both IFV and VZV and further highlights the importance of scavenger receptors during viral entry.

Keywords: MARCO, Influenza virus, Varicella-zoster virus, Viral entry

F018

Development and Efficacy Evaluation of Vaccine Candidate for ZIKV Using Baculoviral Gene Delivery System

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Zika virus (ZIKV) is a mosquito-borne flavivirus, infection of pregnant women can cause a wide range of congenital abnormalities, including microcephaly in the infant. However, there is no vaccine available yet. In this study, we designed to use PrM/E, which is the main target gene of neutralizing antibodies, for the development of DNA vaccine for ZIKV. To enhance the gene delivery, a recombinant baculovirus whose surface was modified to express human endogenous retrovirus (HERV) envelope was constructed. Baculovirus with HERV envelope (AcHERV) showed distinguished higher gene delivery than wild type. Using the AcHERV as a delivery vector, we constructed major antigen (prM-E)-encoding DNA under the CMV promoter, AcHERV-ZIKA. Transducing of prM/E gene in a mammalian cell was confirmed by western blot. Immunization in mice with 10e7 of AcHERV-ZIKA elicited high IgG and neutralizing antibodies. In the challenge test, AcHERV-ZIKA immunized A129 mice showed perfect protection. These results suggest that AcHERV-ZIKA could be a potential vaccine candidate for human application.

Keywords: Zika virus, Vaccine, Baculovirus system, HERV

F019

Secretome Analysis in Pepper Anthracnose Pathogen *Colletotrichum scovillei*

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The occurrence of *Colletotrichum* sp. on pepper (*Capsicum annuum* L.) is a major problem in many countries over the world. *Colletotrichum acutatum* is a species complex causing anthracnose disease in a wide range of host plants. We isolated a *Colletotrichum* sp. from an infected pepper fruit in Gangwon Province of South Korea and it was identified as *C. scovillei* using combined sequence analysis. Isolated *C. scovillei* KC05 developed appressorium on pepper fruit and penetrated cuticle layer of pepper surface through penetration peg. From the penetration peg, a highly branched dendroid structure was formed in the cuticle layer of pepper fruit. Thin and branched hyphae were observed inside dendroid structure and the hyphae grew along with dendroid structure to the wall of the infected cell. Swollen biotrophic hyphae were formed in the neighboring cells. We hypothesized that small secreted proteins participate in *C. scovillei* infection on pepper fruit. We found a total of 39 proteins as *C. scovillei*-secreted protein candidates by SignalP4.1 and small secreted protein prediction pipeline. Targeted gene deletion was carried out on some genes among the candidates and we found *SSP16* (CSP_001584) is related to the disease development of *S. scovillei*. We constructed a SSP16:dsRED fusion construct and SSP16:dsRED proteins were localized inside dendroid structure. Collectively, our results indicate that *SSP16* might play an important role in *C. scovillei* infection on pepper fruit.

Keywords: *Colletotrichum scovillei*, Infection, Pepper, Secreted protein

F020

Biological Control of Pepper Anthracnose Pathogen *Colletotrichum scovillei* by Antagonistic Bacteria *Burkholderia cepacia* KF1

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Pepper (*Capsicum annuumm* L.) is an economically important crop in many countries over the world. Pepper anthracnose fungus *Colletotrichum scovillei* is one of the major problems in pepper production. Since no biological control agents are available to control *C. scovillei* in South Korea, we isolated bacteria form Cheonma mountain and evaluated antagonistic activities of the isolates against mycelial growth and germination of *C. scovillei*. The highest percent of the mycelial growth inhibition (66%) was obtained by *Burkholderia cepacia* KF1. We treated *B. cepacia* KF1 at concentration of 1×10^5, 1×10^6, 1×10^7, and 1×10^8 CFU/ml on *C. scovillei* conidia and the inhibition rates on the germination of *C. scovillei* conidia were 7.8%, 4.3%, 1.2%, and 0%, respectively. Our study showed that *B. cepacia* KF1 is an effective biological control agent against *C. scovillei*.

Keywords: Biological control, *Colletotrichum scovillei*, Pepper anthracnose

F021

Shedding and Transmission Modes of Severe Fever with Thrombocytopenia Syndrome Virus in a Ferret Model

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Background: Although human-to-human transmission of severe fever with thrombocytopenia syndrome virus (SFTSV) via direct contact with body fluids has been reported, the role of specific body fluids from SFTSV-infected hosts has not been investigated in detail.
Methods: To demonstrate the virus transmission kinetics in SFTSV-infected hosts, we adapted the ferret infection model and evaluated the virus shedding periods, virus titers, and transmission modes from various specimens of infected ferrets.
Results: Large amounts of infectious SFTSV are shed through nasal discharge, saliva, and urine from SFTSV-infected ferrets. Virus could be detected from 2 dpi and persisted until 12 dpi in these specimens, compared with the relatively short virus-shedding period in sera. Further, transmission studies revealed that SFTSV can be transmitted to close direct and indirect contact naive animals through various mediums, especially through contact with serum and urine. Further, ferrets contacted with human urine specimens from SFTSV-positive patients were successfully infected with SFTSV, suggesting that urine specimens could be a source of SFTSV infection in humans.
Conclusions: Our results demonstrate that the SFTSV can be shed in various body fluids for more than 12 days and that these specimens could be a source for direct or indirect transmission through close personal contact.

Keywords: Body fluids, Ferret, Indirect transmission, SFTSV, Virus shedding

F022

Tor1 and Sch9 Coordinately Regulate Hyphae Specific Genes or Ribosomal Protein Genes in *Candida albicans*

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Candida albicans is an opportunistic fungal pathogen. In immunocompromised individuals, it can cause bloodstream infections with high mortality rates. *C. albicans* reversibly switches between yeast and hyphal morphologies, with hyphae being associated with virulence. Here, we present the first evidence showing that the transcription of RP genes in *C. albicans* is associated with hyphae formation via the Tor1 and Sch9 signaling pathways. We have observed a decrease in gene transcription of ribosomal proteins (RP) during hyphae formation. Also, morphogenesis-dependent reduction of RP genes transcription was confirmed in constitutive yeast or filamentous growing strains. We also observed that Tor1 and Sch9 kinase activity was reduced in hyphae growing cells compared with in yeast growing cells. Five residues in c-terminus of Sch9 play a role in the regulation of RPG and HSG expression and cell–cell adhesion. Interestingly, the major portions of hyphae are composed of vacuoles and we showed that the filamentous growth of *C. albicans* requires vacuolar H^+-ATPase function. Based on these findings, Vma4 and Vma10 are not only involved in vacuole biogenesis and hyphal formation, but also are good targets for antifungal drug development in *C. albicans*. Our new findings provide evidence to show that the Tor1-Sch9 kinase cascade stimulates RP transcription, and V-ATPase activity plays an important role in morphological changes of *C. albicans*.

F023

Development of a DNA Vaccine for SFTSV that Confers Complete Protection against Lethal Infection in Ferrets

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Severe fever with thrombocytopenia syndrome (SFTS) is a newly emerging tick-borne infectious disease caused by the SFTS virus (SFTSV) belonging to the *Phenuiviridae* family. The majority of SFTS cases have been identified in East Asia, primarily in China, Korea, and Japan. The incidence of SFTSV infection has increased from its discovery with a mortality rate of 10–20% and the major clinical symptoms of SFTSV infection are fever, vomiting, diarrhea, thrombocytopenia, leukopenia and multiple organ failure. However, no effective vaccines are currently available for SFTSV. Here, we describe the development of a SFTSV vaccine using DNA vaccine-based platform, its immunogenicity, and its protective efficacy. Vaccine candidates induced both a neutralizing antibody response and multifunctional SFTSV-specific T cell response in mice and ferrets. To investigate the vaccine efficacy i*n vivo*, we applied a recently developed ferret model of lethal infection that can accurately mimic SFTS progression in humans. Immunization of ferrets with SFTS vaccine candidates conferred complete protection against lethal-dose SFTSV challenge without any clinical symptoms. Moreover, we found that anti-envelope antibodies play an important role in protective immunity and non-envelope-specific T cell responses also can contribute to protection against SFTSV infection. This study provides a valuable insight to the design of preventive vaccines for SFTSV, as well as corresponding immune parameters, to control SFTSV infection.

Keywords: Severe fever with thrombocytopenia syndrome (SFTS), SFTS virus (SFTSV), DNA vaccine, Protective immunity

F024

Whole Transcriptome Analyses Reveal Differential mRNA and microRNA Expression Profiles in Primary Human Dermal Fibroblasts Infected with Clinical or Vaccine Strains of Varicella Zoster Virus

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Licensed live attenuated vaccines have been developed to prevent Varicella zoster virus (VZV) infection, which causes chickenpox and shingles. The genomic sequences of both clinical- and vaccine-derived VZV strains have been analyzed previously. To further characterize the molecular signatures and complexity of virulent (clinical) versus nonvirulent (vaccine-derived) VZV-mediated host cellular responses, we performed high-throughput next generation sequencing to quantify and compare the expression patterns of mRNAs and microRNAs (miRNAs) in primary human dermal fibroblasts (HDF) infected with virulent (*YC01-low*) and nonvirulent (*YC01-high, SuduVax, VarilRix*) VZV strains. 3D multidimensional scaling of the differentially expressed genes demonstrated the distinct grouping of virulent and nonvirulent strains. In particular, we observed that HDFs infected with nonvirulent strains had an elevated expression of DEGs involved in retinoic acid inducible gene–I-like receptor and interferon-mediated signaling pathways compared with virulent strains. Additionally, miRNA expression patterns were profiled following the infection of HDFs with VZV. Small RNA sequencing identified that several miRNAs were upregulated, including miR-146a-5p which has been associated with other herpes virus infections, whereas let-7a-3p was downregulated in both virulent and nonvirulent VZV-infected cells. This study identified genes and miRNAs that may be essential in VZV pathogenesis.

Keywords: Varicella zoster virus, *SuduVax*, RNA-seq, miRNA

F025

Development of Bivalent Vaccines for Poultry

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Although public health measures and drug interventions are absolutely necessary tools in our efforts to combat virus outbreaks, vaccines are equally crucial to countering such threats. In fact, vaccination has proven to be the single best weapon against infectious diseases in animals. It is thus believed that vaccination is a rational and important strategy for protecting the animal against infectious diseases. Even though current animal vaccines are effective in hampering the spread of disease, they need to be improved for the preparation of unpredicted virus outbreaks by technology such as reverse genetics system. This system can allow company to shorten the lengthy process of preparing vaccine seed viruses since the rapid development of new and better vaccines against future virus outbreak appears to be imperative. Live attenuated-vaccines have proven to be effective against a variety of viral diseases, and they have been shown to induce strong and long lasting immunity. By using reverse genetics system, we can rapidly develop live-attenuated vaccines that would be safer, optimized against unexpected viral outbreak. In recent years, outbreaks of high pathogenicity avian influenza have been reported in Asia, Africa, and Europe. These outbreaks involving H5N1 or H7N7 influenza viruses resulted in lethal infections in domestic poultry, and the death of a limited number of human cases. Vaccination against avian influenza in poultry can play an important role in the reduction of virus shedding and in raising the threshold for infection and transmission. Therefore, poultry vaccination with a high quality vaccine against avian influenza virus can be part of an effective control program. For this, a recombinant bivalent vaccine was constructed by reverse genetics system generating lentogenic Newcastle disease virus strain with insertion of the hemagglutinin gene from avian influenza virus.

Keywords: Newcastle disease virus (NDV), Avian influenza virus (AIV), Bivalent vaccine

G001

Development of Engineered Antibodies for Effective Diagnostics and Immunotherapy

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Antibody engineering has made a considerable impact on biological and biotechnological researches as well as on its clinical and environmental applications. Among them, immunosensor based on a change in a fluorescent property when an antigen binds to antibody has been extensively studied by virtue of its ease of use, yet with high specificity and sensitivity. We developed unique and powerful fluoroimmunosensor named Quenchbody (Q-body) that works on the novel principle of antigen-dependent removal of quenching effect on fluorophores. The outstanding advantage of Q-body assay is its simplicity, which can be carried out by just mixing the Q-body with antigen and measuring its fluorescence. Using this convenient method, we have successfully quantified various target antigens including phosphorylated serine residues in vimentin, narcotics such as morphine, influenza hemagglutinin, and hen egg lysozyme. Moreover, using this Q-body, cellular imaging of osteocalcin produced by differentiated osteoblast cells or claudin proteins on tumor cells was successfully accomplished. Due to its simplicity and versatility, Q-body-based assay is expected to have a range of applications, from *in vitro* diagnostics to imaging of various targets *in situ*, and also expand our knowledge on various biological phenomena.
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Keywords: Recombinant antibody, Fluoroimmunosensor, Immunotherapy, Pancreatic cancer

H001

Effecient Separation and Purification of Three, Four, and Five Carbon Diamines from Fermentation Broth

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1,3-Diaminopropane (1,3-DAP), 1,4-diaminobutane (1,4-DAB), and 1,5-diaminopentane (1,5-DAP) are important chemicals due to their wide industrial use such including bionylon synthesis. Over the last decade, several studies on bio-based production of diamines by metabolically engineered microorganisms have been published. However, development of efficient methods for the recovery of 1,3-DAP, 1,4-DAB, and 1,5-DAP from fermentation broth has not been reported. In this study, an efficient process for the separation and purification of 1,3-DAP, 1,4-DAB, and 1,5-DAP from fermentation broth without using highly flammable or toxic solvents was developed. The optimal process for the recovery and purification of these diamines from fermentation broth comprises several unit operations including removal of cell debris, decolorization of fermentation broth, product concentration, deprotonation of diamines, product separation, and final polishing to obtain pure 1,3-DAP, 1,4-DAB, and 1,5-DAP with yields of 87 ± 3%, 86 ± 4%, and 81 ± 2%, respectively. The strategy reported here could be similarly applicable in developing downstream processes to recover and purify other diamines and related chemicals from fermentation broth.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea.]

Keywords: Separation, Purification, 1,3-Diaminopropane, 1,4-Diaminobutane, 1,5-Diaminopentans

H002

RecET-Based Markerless Knockout and Integration of Genes in *Pseudomonas putida*

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Pseudomonas putida is a promising engineering host for producing value-added natural products. While gene integration is critical to construct stable industrial strains producing heterologous bioproducts, engineering of *P. putida* currently relies on time-consuming homologous recombination techniques and transposon-mediated random insertions. Here we developed a RecET recombineering system for integrating heterologous genes to the *P. putida* chromosome without residual antibiotic marker. The capacity of the RecET recombineering system was firstly demonstrated by knocking out various chromosomal loci spanning 0.6-101.7 kb. Subsequently, four biosynthetic gene clusters were successfully integrated to the target locus of *P. putida* chromosome as proof-of-concept examples using the RecET recombineering. Cre/*lox* system and efficient plasmid curing system were coupled to the RecET recombineering system to complete the markerless recombineering system for generating marker- and plasmid-free strains. This RecET-based markerless recombineering system will expedite metabolic engineering of *P. putida*, a bacterial host strain of increasing academic and industrial interest.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) of the Ministry of Science and ICT of Korea and the Novo Nordisk Foundation (CFB core funding and NNF 160C0021746).]

Keywords: *Pseudomonas putida*, Recombineering, RecET, Gene knockout, Gene integration

H003

Metabolic Engineering of *Escherichia coli* for Production of Free Heme Secreted to Medium

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Heme has wide applications in healthcare and food supplement industries. *Escherichia coli* engineered to harbor the C4 pathway has been used to produce small amount of heme accumulated inside the cell, requiring subsequent extraction for applications. Here we metabolically engineered *E. coli* to produce extracellular free heme. The biosynthesis of heme was firstly enhanced by optimizing the endogenous C5 pathway and the downstream heme biosynthetic pathway. Next, the *ldhA*, *pta* and also *yfeX*—encoding a putative heme-degrading enzyme—genes were knocked out, resulting in production of 7.88 mg/L of total heme with 1.26 mg/L of extracellular heme in flask cultivation. Moreover, a heme exporter CcmABC in the engineered *E. coli* strain was overexpressed, enabling secretion of 73.4 mg/L extracellular heme (63.5%) out of 115.5 mg/L total heme from glucose in fed-batch fermentations. Furthermore, supplementation of L-glutamate further enhanced secretion of heme to 151.4 mg/L (63.3%) out of 239.2 mg/L total heme produced. The engineering strategies we report here will be useful for microbial production of free heme.

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

Keywords: *Escherichia coli*, Heme, C5 pathway, Secretory production, Metabolic engineering

H004

Production of Terephthalic Acid from *p*-Xylene Using Metabolically Engineered *Escherichia coli*

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Terephthalic acid (TPA), an important building block chemical, is currently converted from *p*-xylene (*p*X) through an energy intensive and potentially hazardous oxidation process. Here we metabolically engineered an *Escherichia coli* strain for biotransformation of *p*X into TPA. The engineered *E. coli* strain harbors a synthetic metabolic pathway converting *p*X to TPA, which consists of the upstream pathway converting *p*X to *p*-toluic acid (*p*TA) and the downstream pathway transforming *p*TA to TPA. The synthetic TPA pathway was optimized by adjusting expression levels of upstream and downstream modules. The engineered strain converts 8.8 g *p*X into 13.3 g TPA in a two-phase partitioning fermentation, which corresponds to a conversion yield of 96.7 mol%. The engineered *E. coli* strain and the fermentation strategy developed in this study will provide a promising alternative for the large-scale biotechnological production of TPA with sustainability.

[This work was supported by the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) of the Ministry of Science, ICT & Future Planning through the National Research Foundation of Korea.]

Keywords: *Escherichia coli*, Terephthalic acid, *p*-Xylene, Biotransformation, Two-phase fermentation

H005

Coupleing CRISPR/Cas9 and Recombineering Systems for Scarless Gene Knockout in *Corynebacterium glutamicum*

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Corynebacterium glutamicum is an important workhorse for producing amino acids in industries. Genome of *C. glutamicum*, however, has been engineered using random mutagenesis and inefficient double crossover methods. Here we developed a rapid and iterative genome engineering system enabling scarless knockout of multiple genes in *C. glutamicum*. In this system, a recombinase RecT incorporates synthetic single-stranded oligodeoxyribonucleotides into the *C. glutamicum* chromosome and Cas9-sgRNA ribonucleoprotein complex counter-selects unedited cells by introducing double-stranded break to unedited target locus. Subsequently, the CRISPR/Cas9 and RecT vectors are cured to generate final strains free of plasmids and antibiotic markers. The performance of the system was demonstrated by generating seven different mutants within two weeks. The resulting strains were used to study effects of deleting three different genes on the production of γ-aminobutyric acid. This scarless genome engineering system will facilitate development of high-performing *C. glutamicum* for industrial applications.

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Keywords: *Corynebacterium glutamicum*, CRISPR/Cas9, Recombineering, Gene knockout, Scarless

H006

Reconstructing C1 Assimilation Pathway in *Escherichia coli* for the Assimilation of Formic Acid and CO_2

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Gaseous one-carbon (C1) compounds are largely responsible for global warming and climate change. With attention for solving this problem, biological conversion of C1 compounds has emerged as potential solution. Here,efficient assimilation of Formic acid(FA) and CO_2 in *Escherichia coli* was allowed by reconstructing C1 assimilation pathway. The *Methylobacterium extorquens* ftl, fch, and mtd genes were expressed to allow FA assimilation. The gcv reaction was reversed by knocking out gcvR gene and overexpressing the gcvTHP genes. The pyruvate-forming flux from FA and CO_2 could be increased to 14.9% by knocking out gcvR, pflB, and serA, expressing gcvTHP under trc, and overexpressing the reconstructed THF cycle, gcvTHP, and lpd genes. To reduce glucose usage required for redox generation, the *Candida boidinii* formate dehydrogenase (Fdh) gene was expressed. The resulting strain showed specific glucose, FA, and CO_2 consumption rates of 370.2, 145.6, and 14.9 mg·g dry cell weight $(DCW)^{-1}\cdot h^{-1}$, respectively. The C1 assimilation pathway consumed 21.3 wt% of FA. Furthermore, cells sustained slight growth using only FA and CO_2 after glucose depletion, suggesting that combined use of the C1 assimilation pathway and *C. boidinii* Fdh will facilitate cell growth without additional carbon source such as glucose.

[This work was supported by the C1 Gas Refinery Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT Grant NRF-2016M3D3A1A01913250]

Keywords: Tetrahydrofolate cycle, Reverse glycine cleavage pathway, Formic acid, CO_2, Formate dehydrogenase

H007

Metabolic Engineering Strategy for One-step Production of Aromatic Polyesters from Glucose in *Escherichia coli*

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Currently, Aromatic polyesters are widely used and produced from petroleum. Here, we engineered *Escherichia coli* strains for the production of aromatic polyesters from glucose by one-step fermentation. Poly(52.3 mol% 3-hydroxybutyrate [3HB]-*co*-47.7 mol% D-phenyllactate) can be produced from glucose and sodium 3HB by overexpressing the *Clostridium difficile* isocaprenoyl-CoA:2-hydroxyisocaproate CoA-transferase (HadA) and evolved polyhydroxyalkanoate (PHA) synthase genes in a D-phenyllactate-producing strain. Also, when we induce additional expression of *Ralstonia eutropha* β-ketothiolase (*phaA*) and reductase (*phaB*) genes, various poly(3HB-*co*-D-phenyllactate) polymers having 11.0, 15.8, 20.0, 70.8, and 84.5 mol% of D-phenyllactate are produced. Fed-batch culture of engineered strain produces 13.9 g/L of poly(61.9 mol% 3HB-*co*-38.1 mol% D-phenyllactate). Furthermore, different aromatic polyesters are produced from glucose when feeding the corresponding monomers. For one-step fermentative production of aromatic polyesters from renewable resources, our engineered *E. coli* will be useful bacterial system.

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Keywords: One-step fermentation, Aromatic polyesters, PHA synthase, CoA-transferases

H008

Engineered *Escherichia coli* Strain for the Production of Four-, Five- and Six-carbon Lactams

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Production of useful chemicals from renewable sources and biological pathway is becoming important for sustainable and environmental chemical industry. In this research, we developed engineered Escherichia coli for the production of four-carbon (butyrolactam), five-carbon (valerolactam) and six-carbon (caprolactam) lactams using new and efficient platform metabolic pathway. Constructed pathway uses ω-amino acids as precursors and comprises two steps. Activation of ω-amino acids catalyzed by the *Clostridium* propionicum β-alanine CoA transferase (Act) followed by spontaneous cyclization. The pathway can be operated *in both vitro* and *in vivo* situation. We developed three types of metabolically engineered *Escherichia coli* strains for production of butyrolactam, valerolactam and caprolactam from renewable carbon source. In this procedure, we used systems level optimization for constructing new metabolic pathway. As a results, our Final Engineered *E. coli* strain produced 54.14 g/L of butyrolactam in a glucose minimal medium in fed-batch fermentation which demonstrate the high efficiency of the novel lactam pathway developed.

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Keywords: Metabolic engineering, Butyrolactam, Valerolactam, Caprolactam, β-Alanine CoA transferase

H009

Development of Type III Polyketide Synthase-based Malonyl-CoA Biosensor for Metabolic Engineering of Bacteria

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Malonyl-CoA is an important central precursor for production of value-added malonyl-CoA derived natural compounds such as polyketides and phenylpropanoids groups. In this research, a new colorimetric malonyl-CoA sensor biosensor has been developed for application in three industrially important bacteria; *E. coli*, *Pseudomonas putida*, and *Corynebacterium glutamicum*. A type III polyketide synthase (RppA) which produces red-colored flaviolin was repurposed as malonyl-CoA biosensor in *E. coli*. The strains with enhanced malonyl-CoA accumulation showed parallel increase in red color intensity. For enhanced production of malonyl-CoA in *E. coli*, a 1858 synthetic small regulatory RNA library was conducted and applied to knockdown 14 gene targets that are responsible for increasing malonyl-CoA levels. The following knockdown of the genes alone or in combination, allowed rapid development of engineered strains being able to produce enhanced production of 6-methylsalicylic acid, aloesone, resveratrol, and naringenin to 440.3, 30.9, 51.8, and 103.8 mg/L, respectively.

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Keywords: Metabolic engineering, Malonyl-CoA biosensor, Type III polyketide synthase (RppA)

H010

Production of Putrescine and L-Proline by Gene Expression Knockdown Using Synthetic Regulatory RNA in *Escherichia coli*

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Escherichia coli gene expression knockdown using synthetic small RNA (sRNA) can be fine-tuned by altering sRNA sequences to modulate target mRNA-binding ability, but this requires thorough checking for off-target effects. Here, we present a synthetic small RNA (sRNA) gene expression knockdown system fine-tuned by using different promoters to modulate synthetic sRNA abundance. We use *in-silico* flux response analysis to select knockdown target genes and those related to production of target material. After that, we screen engineered strains with a library of synthetic sRNA promoter combination. In fed-batch culture, our engineered two Escherichia coli strains utilizing fine-tuned repression of argF and glnA gene produced putrescine (42.3 ± 1.0 g/L) and L-proline (33.8 ± 1.6 g/L). Fine-tuned gene knockdown by controlling sRNA abundance will be useful for rapid design of microbial strains through simultaneously optimizing expression of multiple genes at a systems level. Our results will present the solution for the difficulties of constructing and testing many different sRNAs and checking their cross-reactivity.

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Keywords: Synthetic small RNA, Knockdown, Putrescine, L-propline

H011

Metabolic Engineering of *Escherichia coli* for Improved Production of Astaxanthin

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Astaxanthin is a reddish keto-carotenoid, having a powerful antioxidant activity compared to other carotenoids such as β-carotene, lutein, lycopene. In this research, *Escherichia coli* was metabolically engineered to produce astaxanthin in high concentration and high productivity. First, the heterologous crt genes (from *Pantoea ananatis* and the truncated *BKT* gene [*trCrBKT*] from *Chlamydomonas reinhardtii* were introduced for astaxanthin synthesis. Then, eight different signal peptides were examined by attaching them to the N-terminus or C-terminus of the trCrBKT membrane protein to allow more stable expression and transport to the *E. coli* membrane. To further increase the production ability, *in silico* flux variability scanning based on enforced objective flux (FVSEOF) was performed to identify gene overexpression targets. With further optimization of culture conditions, it led to further astaxanthin concentration increase up to 432.82 mg/L (7.12 mg/gDCW) with a productivity of 9.62 mg/L/h. The following developed strategies reported in this research will be helpful for enhanced production of astaxanthin as well as related carotenoid products by engineered *E. coli* strains.
[Supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea.]

Keywords: Metabolic engineering, *Escherichia coli*, Astaxanthin

H012

Metabolic Engineering of *E. coli* for Various Single- and Multi-element Nanomaterials

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Previous nanomaterial (NM) synthesis methods, either chemical or physical, can be replaced by biological synthesis methods. However, the mechanisms for biological producibility of NMs such as crystalline versus amorphous, are not yet understood. In this research, we report biosynthesis of 60 different NMs by using recombinant *Escherichia coli* co-expressing metallothionein, a metal-binding protein, and phytochelatin synthase that synthesizes a metal-binding peptide phytochelatin. *In vivo* (live cells) and *in vitro* (cell extracts) are used to synthesize NMs. The periodic table was scanned to select 35 different metal elements, followed by biosynthesis of their NMs. Nine types of crystalline single-elemnts of Mn_3O_4, Fe_3O_4, Cu_2O, Mo, Ag, $In(OH)_3$, SnO_2, Te, and Au were biologically synthesized. This strategy was further applied to biosynthesize multielement NMs such as $CoFe_2O_4$, $NiFe_2O_4$, $ZnMn_2O_4$, $ZnFe_2O_4$, Ag_2S, Ag_2TeO_3, Ag_2WO_4, Hg_3TeO_6, $PbMoO_4$, $PbWO_4$, and $Pb_5(VO_4)_3OH$. The strategy described in this research provides a platform for manufacturing various NMs in an environmentally friendly manner.
[Supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science and ICT through the National Research Foundation of Korea]

Keywords: Metabolic engineering, Nanomaterial, Metal-binding peptide

H013

Metabolic Engineering of *Escherichia coli* for One-step Production of Poly(lactate-co-glycolate)

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In this research, we report one-step fermentative production of PLGA in engineered *Escherichia coli*. The following strain harbors an evolved polyhydroxyalkanoate (PHA) synthase that polymerizes D-lactyl-CoA and glycolyl-CoA into PLGA. For further engineering, Dahms pathway was introduced which enabled production of glycolate from xylose. In addition, deletion of *ptsG* gene enables simultaneous utilization of glucose and xylose was carried out. An evolved propionyl-CoA transferase was able to convert D-lactate and glycolate to D-lactyl-CoA and glycolyl-CoA, respectively. Additional deletion of *adhE*, *frdB*, *pflB* and *poxB* prevented by-product formation. We have also demonstrated modulating the monomer fractions in PLGA by overexpressing *IdhA* and deleting *dld* to increase the proportion of D-lactate. *aceB*, *glcB*, *glcD*, *glcE*, *glcF* and *glcG* were also deleted to increase the proportion of glycolate. Incorporation of 2-hydroxybutyrate was also prevented by deleting *ilvA* or feeding the strains with L-isoleucine. By producing copolymers containing 3-hydroxybutyrate, 4-hydroxybutyrate, and 2-hydroxyisovalerate, diverse types of PLGA were formed.

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Keywords: PLGA, *Escherichia coli*, Metabolic engineering, PHA synthase

H014

Antibacterial Activity of Axakacin (1-*N*-AHBA-kanamycin X)

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We had reconstructed the entire biosynthetic pathway through the heterologous expression of combinations of putative biosynthetic genes from *Streptomyces kanamyceticus* in the non-aminoglycoside-producing *Streptomyces venezuelae* to reveal the previously undescribed kanamycin biosynthetic intermediates. Co-expression of heterologous aminoglycoside genes with selected sets of kanamycin genes led to the *in vivo* biosynthesis 1-*N*-[S-4-amino-2-hydroxybutyric acid] (AHBA)-conjugated kanamycin X (axakacin).

The antibiotic activities of axakacin, amikacin, gentamicin complex, and kanamycin A were tested against several Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*). Amikacin, gentamicin complex, and kanamycin A were inactive (minimal inhibitory concentration [MIC] > 128 μg/ml) against these strains. Axakacin had enhanced activity against *E. coli*, *A. baumannii* and *P. aeruginosa* and, in particular, retained potency (MIC: ~4 μg/ml) against an amikacin-resistant clinical isolate of *P. aeruginosa* 2178 that was insensitive to amikacin.

[This work was supported by grant from "Cooperative Research Program for Agriculture Science & Technology Development (Project title: Development of semi-synthetic manufacturing process of axakacin combined with biological / chemical manufacturing process and acquisition of drug characterization data, Project No. PJ01316002)" Rural Development Administration, Republic of Korea]

Keywords: Axakacin, Aminoglycoside, Antibacterial activity, Kanamycin X

H015

Application of Anammox Bacteria for Treatment of Wastewater Containing High Concentrated Salt

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GS E&C

Wastewater containing high concentrations of ammonia is suitable for treatment by the Anammox process. However, anaerobic digester liquid for food wastes contains high concentrations of salts in addition to ammonia, which is known to affect the activity of Anammox microorganisms. In this study, we analyzed the feasibility for application of Anammox process using Anammox microorganisms cultivating in a lab scale reactor.
As a result, when the substrate was diluted (1/5 of raw water) in order to adjust the salinity concentration of anaerobic digested liquid component of food wastes to 1.1 g/L, the ammonia treatment efficiency was about 70%, but the salinity concentration of 2.2 g/L was used, which was diluted 1/3 of the raw water, the ammonia treatment efficiency decreased drastically. The analysis of Anammox microorganisms with NGS revealed that *Ca. Brocadia* and *Ca. Jettenia* have been analyzed as the dominant Anammox species in this process. However, these two species among Anammox species are reported to be inhibited even at low salt concentrations (under 5 g/L). In cases of *Ca. Kuenenia* and *Ca. Scalindua*, these Anammox species are known active even at high salt concentration (about 30 g/L). According to the related studies, *Ca. Brocadia* and *Ca. Jettenia*, they are reported that some activity is maintained even at high salinity after a sufficient period of acclimatization continuously at low salinity concentration.
[This research is supported by GS E&C.]

Keywords: Anammox bacteria, Ammonia treatment, Salinity concentration

H016

Preparation of Synthetic Viral RNA Reference Materials

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Virus-positive clinical samples or reference materials are essential for the development, validation, and verification of molecular diagnostics-based virus detection assays. Since obtaining virus-positive clinical samples is difficult, well-characterized synthetic viral RNA/DNA products could be used as an alternative. In this study, we attempted to fabricate synthetic norovirus RNAs that can be used as reference materials. To this end, nucleotide sequences of noroviruses of GI and GII genogroups registered with GenBank were downloaded and checked for variable or consensus sequence among norovirus genes using alignment tools. Noroviruses were observed to have diverse genotypes and have large variations in nucleotide sequences across the entire gene. Only the ORF1-ORF2 junction regions were found to include the consensus sequences required for real-time PCR assays. Norovirus GI and GII RNAs including these ORF1-ORF2 junction regions were synthesized using *in vitro* transcription techniques. The synthetic norovirus GI and GII RNAs prepared this way responded positively to six commercialized norovirus detection kits. These synthetic RNAs could be useful as reference materials for the validation, verification, and quality control of molecular diagnostics-based virus detection assays.
[This research was supported by a grant (19173MFDS334) from Ministry of Food and Drug Safety in 2019.]

Keywords: Reference material, Norovirus, Synthetic RNA

H017

An Effective and Rapid Method for Preparation of High Quality RNA from Non-conventional Yeast Species

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The increased use of high-throughput RNA-based analysis has spurred the demand for rapid and simple preparation of high quality RNA. RNA preparation from non-conventional yeasts having diverse cell wall and morphological characteristics is often inefficient using current methods adapted for the model yeast, *Saccharomyces cerevisiae*. We report a simple RNA preparation method based on glass bead-mediated breakage in a formamide/EDTA solution. High quality RNA is generated within 15 min from various non-conventional yeasts species. The obtained RNA can be directly used for experimentation without further RNA purification and buffer exchange.
[This work was supported by the National Research Foundation of Korea, Grant No. NRF-2017M3C1B5019295 (STEAM Research Project) and by the Korean Ministry of Agriculture, Food, and Rural Affairs, Grant No. 918010042HD030 (Strategic Initiative for Microbiomes in Agriculture and Food).]

Keywords: RNA extraction, non-Saccharomyces yeast, Cell wall, Hyphae

H018

Characterization of the Diverse Biosynthetic Pathways for Gentamicin B

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Gentamicin is an aminoglycoside antibiotic produced from *Micromonospora echinospora* and widely used to treat bacterial infections. The gentamicin B is the starting material for a semi-synthetic aminoglycoside antibiotic, but it is produced in trace amounts by wild-type producers. We have characterized the biosynthesis of gentamicin B through *in vitro* reconstitution and discovered unknown minor intermediates. Gentamicin B can be biosynthesized *via* three independent pathways; C4″-methylation of 6′-amino-2′-deamino-6′-deoxy-2′-hydroxygentamicin A by the flexible *C*-methyltransferase GenD1, GenQ-GenB1-catalyzed 6′-amination of the 2′-deamino-2′-hydroxygentamicin X2, or 2′-deamination of JI-20A catalyzed by GenJ-GenK2 enzymes. Our results establish the hidden biosynthetic pathways and intermediates to gentamicin B, providing genetic and biochemical insights to improve this valuable minor aminoglycoside congener, and demonstrate a new strategy for mining novel aminoglycosides from the nature.
[Supported by National Research Foundation of Korea (NRF) grants funded by the Ministry of Science and ICT (2019R1A2B5B03069338) and the Ministry of Education (2019R1I1A1A01062130)]

Keywords: Aminoglycoside, Gentamicin B, Biosynthetic pathway, *In vitro* reconstitution

H019

Development of a TaqMan Probe Real-Time PCR Detection Kit for the Validation of Minute Virus of Mice (MVM) Safety during the Manufacture of CHO Cell Culture-Derived Biopharmaceuticals

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Validation of viral safety is essential in ensuring the safety of mammalian cell culture-derived biopharmaceuticals, because numerous adventitious viruses have been contaminated during the manufacture of the products. Mammalian cells, especially CHO cells, are highly susceptible to MVM, and there are several reports of MVM contamination during the manufacture of CHO cell culture-derived biopharmaceuticals. In order to establish the testing system for the MVM safety, a TaqMan probe-based 'MVM-Sure MVM Real-Time PCR Detection Kit' was developed for quantitative detection of MVM in cell lines, raw materials, manufacturing processes, and final products as well as MVM clearance validation. The LOD (limit of detection) of the kit was calculated to be 10 copies/reaction. The kit was proven to be reproducible, very specific to MVM, and robust. In addition, the performance of the kit was more than equivalent to than of a foreign commercial kit. The established real-time PCR assay was successfully applied to the validation of Chinese hamster ovary (CHO) cell artificially infected with MVM. MVM DNA could be quantified in CHO cell as well as culture supernatant. Therefore, it was concluded that this rapid, specific, sensitive, and robust assay kit could replace infectivity assay for detection and clearance validation of MVM.

Keywords: TaqMan Probe Real-Time PCR Detection Kit, Minute Virus of Mice, Safety

H020

Development of NGS-Based Virus Detection Method for Adventitious Virus Contamination Testing from Biopharmaceuticals Manufacturing Process

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Manufacturing processes for the mammalian cell cultures (MCC)-derived biopharmaceuticals have the risk of viral contamination. Viral contamination can originate from contaminated cell lines, contaminated raw materials, or a GMP breakdown in the production and purification process. To ensure safety, government regulations require manufacturers to demonstrate that MCC-derived biopharmaceuticals are free of adventitious agents. Conventional virus testing methods such as *In vivo* and *In vitro* virus tests, retrovirus specific tests, electron microscopic evaluation, and PCR-based tests have been developed to detect contaminants of pre-established interest. However, these methods may not be sufficient to detect very low levels of viral contamination as well as viruses outside the scope of detection assays, or to identify the species of the contaminant. In this study, we developed NGS-based virus detection method for adventitious virus testing. Sample pre-treatment methods were established to minimize mammalian cell-derived DNA or RNA from the test articles. The bioinformatic pipeline were made up of quality control processes of read data, filtering process of reads originated from host cells, and identification of filtered reads using read-mapping and homology search strategies. This method may be an innovative tool to detect and identify virus contaminants not directly targeted and identify the species and variants of the contaminant with a high degree of sensitivity.

Keywords: NGS-based virus detection, Adventitious virus, Biopharmaceuticals safety

H021

Recombinant Protein Expression in *Escherichia coli* Using Synthetic Stationary-phase Promoters

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Genes that ensure cell survival under the stressful conditions at the stationary phase are expressed under the influence of the stationary-phase promoters. Their promoters could be used in auto-inducible production of recombinant proteins. Though this has the advantage of reduced monitoring of cell culture and elimination of toxicity due to chemical inducers, these natural stationary phase-promoters are weaker compared to conventional promoters. In this study, synthetic stationary phase promoters were exploited and screened for improved expression of a model recombinant gene, mNeonGreen (mNG), at the stationary-phase of *Escherichia coli* BL21(DE3) cell culture. Three selected synthetic stationary-phase promoters and their hybrid promoters of part natural and part synthetic promoters were cloned along with the mNG gene into an expression vector and time-course measurement of O.D and fluorescence were done in 36-hour period batch cultures. There was a marked increase in mNG gene expression under the control of the synthetic stationary phase promoters compared to the natural ones. The hybrid promoters showed significant improvement in mNG expression over both the natural and synthetic stationary phase promoters.

Keywords: Recombinant protein, *Escherichia coli*, Synthetic promoter, Stationary-phase, mNeonGreen

H022

Expression of Truncated HA2 Variants Containing Fusion Peptide of Influenza A Virus in *Escherichia coli*

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Cell disruption is an essential step in the purification of target protein and this could be achieved by conventional methods, such as lysozyme or detergent treatment, using sonication or the French press. Though this step is feasible at the lab scale, it would require a large energy cost and may not be efficient in industrial applications. In addition, there is a risk of denaturing sensitive recombinant proteins. Influenza A virus hemagglutinin2 (HA2) is responsible for membrane fusion of the viral membrane to an endosomal membrane of the target host cell triggered by conformational change at low pH. The insertion of the N-terminal fusion peptide (FP) of HA2 reduces the integrity of the membrane by disrupting of phospholipid bilayer. HA2 and its truncated variants containing fusion peptide might provide a way to improve both cell disruption process and protein yield. Two conventional promoters were tested for their expression in *Escherichia coli* XJb(DE3) or BL21(DE3) strain in this work.

Keywords: HA2, Fusion peptide, Fusion protein, Influenza A virus, *Escherichia coli*

H023

Screening of Natural Stationary-phase Promoters for Recombinant Protein Expression in *Escherichia coli*

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Due to their efficiency in the over-expression of recombinant protein, vectors having chemically-induced promoters are popular despite some of its key drawbacks including the requirement of expensive and sometimes toxic inducers, unequal rate of inducer uptake by cells (heterogeneous uptake) and regular monitoring of cell growth and culture conditions. Triggering induction without chemical inducers can address these drawbacks. The onset of stationary-phase can act as agents to effect induction of recombinant proteins through stationary-phase promoters and would require no extra chemical inducers. Using *Escherichia coli* BL21(DE3) cell culture to express the recombinant protein, mNeonGreen (mNG) gene at the stationary-phase, this research takes advantage some of these naturally-occurring promoters. Five selected natural stationary-phase prompters were cloned along with the mNG (as the reporter gene) into a p15A ori expression vector. A time-course measurement of O.D and fluorescence was done in a 36-hour period. Generally, a marked increase in the level of mNG from 7 to 12 h peaking at 36 h was noted indicating early stationary-phase activity. These promoters could be fit for use upon further improvement on their strength.

Keywords: *Escherichia coli*, Natural stationary-phase promoters, Auto-inducible, Stationary-phase

H024

***De novo* Biosynthesis of a Monophosphoryl Lipid A Adjuvant**

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The paradigm in the modern vaccine industry has shifted to safe and effective vaccine developments along with the demand for effective immuno-stimulatory agents (adjuvants) such as Monophosphoryl lipid A (MPLA). MPLA including MPL is a derivative of lipopolysaccharide (LPS). MPL and its synthetic analog, GLA are developed as adjuvants in vaccines and allergy treatments as well as treatments for cancer immunotherapy by GlaxoSmithKline and Immune Design, respectively. MPL is produced by Acid/Base hydrolysis of LPS extracted from *Salmonella Minnesota* R595. Despite of its safety profile and high efficacy, its usages remain within the premium vaccine or high cost treatments because of high production cost. To overcome this limitation, we here develop a *de novo* biosynthetic pathway and a simple purification strategy for MPLA production using an engineered *Escherichia coli* strain. We also demonstrated that MPLA purified from this strain has the similar immuno-stimulating activity to those of GLA and MPL. Our system, employing the first engineered *E. coli* strain that directly produces MPLA, could transform the current standard of industrial MPLA production.
[Supported by grants from KIST intramural grants, NRF, and the Ministry of Trade, Industry & Energy (MOTIE)]

Keywords: Adjuvant, Monophosphoryl lipid A, Lipopolysaccharide biosynthesis, Lipid A 1-phosphatase, Vaccine adjuvant

H025

Effect of Inducer Contained Medium on Laccase and Antioxidants Production from *Galactomyces reessii*

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Laccase has received attention because of its efficacy in various bioindustries such as food, pulp, nano biotechnology and soil bioremediation. In this study, lignin-containing inducers such as sawdust and rice straw were added to the culture medium for laccase production from *Galactomyces reessii*. In addition, the production of laccase and antioxidants according to the culture conditions were investigated and the most effective production conditions by *G. reessii* were determiend. The culture of *G. reessii* (4 day) using sawdust and rice straw was achieved the laccase activity of 2.95 ± 0.04 and 7.09 ± 0.34 U/L, respectively, and the activity was about 9.2% and 30.1% higher than the control group. In the culture using sawdust, antioxidants content was measured by DPPH radical scavenging activity, total phenol and flavonoid content, and it was found to be 369.14 ± 0.01 g/L, 0.54 ± 0.06 g/L and 0.15 ± 0.08 g/L, respectively. The antioxidants content of *G. reessii* cultures using rice straw was found to be 68.73 ± 28.96 g/L, 0.61 ± 0.06 g/L, and at 0.19 ± 0.09 g/L, respectively, measured by DPPH radical scavenging activity, total phenol and flavonoid content.

Keywords: *Galactomyces reessii*, Laccase, Sawdust, Rice straw, Lignin

H026

Effect of Culture Conditions on Laccase Production by *Phanerochaete chrysosporium* Using Lignocellulosic Biomass

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White-rot fungi are known to break down the lignin in wood, leaving lighter-colored cellulose, because it can produce enzymes such as laccase, which are needed to break down lignin and other complex organic molecules. In particular, laccase is attracting attention in the food and cosmetic industry for its antioxidant activity. In this study, lignin-containing materials such as sawdust and rice straw were added in the medium to produce laccases from two strains of *Phanerochaete chrysosporium*, and the conditions for maximum production of laccases were investigated. The use of rice straw in all cultures was found to be more effective in the production of laccases and antioxidants. Cultivation time for the maximum production of laccase was different for each strain, and the highest activity from A and B strains was 41.33 ± 1.13 U/L (4 day) and 49.51 ± 19.04 U/L (7 day), respectively. Total polyphenol and DPPH radical scavenging activity (IC_{50}) of strain A were 0.46 ± 0.03 g/L and 40.82 ± 0.64 g/L, respectively, and strain B were 0.50 ± 0.15 g/L and 42.44 ± 0.00 g/L, respectively.

Keywords: *Phanerochaete chrysosporium*, Laccase, Antioxidant, Rice straw

H027

Possibility of Invasive Plant Resources for Saccharification and Ethanol Fermentation

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Sicyos angulatus, an annual vine in gourds, is that uses up costs and labor to eliminate annually because it disrupts domestic ecosystems and damages cropland. This study is a concept of biorefinery which produces bioethanol using *S. angulatus* as raw material, and confirmed that bioethanol was produced by *Saccharomyces cerevisiae* using the hydrolysates. To improve the enzymatic digestion of *S. angulatus*, alkali pretreatment was carried out at 121°C using 6% NaOH for 30 min, and the sugars conversion was 5-fold improved compared to the control group. Both experimental (*S. angulatus*) and control group (glucose) medium contained about 10 g/L of reducing sugars, and the fermentation result confirmed that both experiments produced bioethanol. However, about 40% inhibition of bioethanol fermentation was found in the experimental group than in the control group. It can be inferred that fermentation inhibitors are produced during pretreatment or saccharification.

Keywords: *Sicyos angulatus*, *Saccharomyces cerevisiae*, Pretreatment, Saccharification

H028

Combination of Three Plant Extracts with Synergistic Antifungal Effect on *Candida albicans*

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Candida albicans causes fungal infection. Rose flower extract (A), Moutan Radicis cortex extract (B), and Scutellariae Radix extract (C) were known as antibiotics and anti-virus efficacy. In this study, their synergistic antifungal activities evaluated according to CLSI M27-A2 method using the checkerboard assay. When *C. albicans* cultures were treated with 0.20–0.23% (A) extract and 0.63–0.65% (B) extract, the values of GI_{A+B} / GI_A and GI_{A+B} / GI_B were 3.26–5.09 and 8.36–12.83, respectively. This results showed that there was a synergistic inhibitory effect on the growth of *C. albicans* by the combination of the two extracts because both numbers have a value of 2 or greater. When *C. albicans* cultures were treated with 0.20–0.28% (A) extract and 2.13–2.50% (C) extract, the values of GI_{A+C} / GI_A and GI_{A+C} / GI_C was 2.52–19.47 and 4.15–27.40, respectively. The combination of the two extracts also shows a synergistic inhibitory effect on the growth of *C. albicans.* Based on the ratio of synergistic concentrations, the mixture of three plant extracts was proposed at the ratio of 1 (A) : 2.89 (B) : 9.5 (C). The three plant extract mixtures inhibited the growth of *C. albicans* in a concentration dependent manner. In this study, we propose a method to enhance the inhibitory effect of natural compounds on the growth of fungi.

[Supported by grants from the Next-Generation BioGreen21 Program (PJ01389603), Rural Development Administration, Republic of Korea.]

Keywords: *Candida albicans*, Antifungal activity, Plant extracts, Synergistic effect

H029

Biosynthesis of Rosmarinic Acid Derivatives Using Engineered *Escherichia coli*

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Rosmarinic acid is one of the conjugated phenolic compounds that several biological effects including anti-inflammatory, antiviral, and neuroprotective effect. Rosmarinic acid is a conjugation of caffeic acid and salvinic avid. It can be synthesized from salvinic acid and caffeoyl-CoA by rosmarinic acid synthase (*RAS*). We have cloned three *RASs* from *Coleus blumei*, *Salvia miltiorrhiza*, and *Lavandula angustifolia* and tested them to synthesize three rosmarinic acid derivatives, caffeoyl-4-hydroxyphenyllactic acid, p-coumaroyl-3,4-dihydroxyphenyllactic acid and Isorinic acid. And then, we synthesized these derivative from glucose by introducing several genes for the synthesis of substrates of rosamaric acid derivatives. In order to synthesize caffeic acid, we used tyrosine amino lyase (*TAL*) from *Saccharothrix espanaensis and* 4-hydroxyphenyllactate 3-monoxygenase BC (*hpaBC*) from *Escherichia coli.* And, then caffeic acid was converted by 4-coumarate-CoA ligase (*4CL*) from *Oryza sativa.* Salvinic acid was synthesized from 4-hydroxyphenyl-pyruvate by lactate dehydrogenase (*LDH*) from *Cupriavidus necator* or *Lactobacillus plantarum* and hpaBC. Using various genes, we could successfully synthesize caffeoyl-4-hydroxyphenyllactic acid, p-coumaroyl-3,4-dihydroxyphenyllactic acid, and isorinic acid in *Escherichia coli.*

Keywords: Rosmarinic acid synthase, Caffeoyl-4-hydroxyphenyllactic acid, p-Coumaroyl-3,4-hydroxyphenyllactic acid, Isorinic acid, *Escherichia coli*

H030

Characterization of a Novel Cold-active Epoxidase, *Rru*EPH1 from *Rhodospirillum rubrum* ATCC 11170

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A novel cold-active epoxidase, *Rru*EPH1 from *Rhodospirillum rubrum* ATCC 11170 was identified, purified, and characterized by biochemical and molecular simulation. Multiple sequence alignment revealed that *Rru*EPH1 has a conserved penta-amino acid (Asp333-Trp334-Tyr381-Tyr465-His523), which is critical for the catalysis of epoxide hydrolases. *Rru*EPH1 was purified in denatured condition because *Rru*EPH1 was expressed in inclusion body in *E. coli*. Refolding of *Rru*EPH1 was confirmed by circular dichroism, gel filtration, native-PAGE, and enzymatic assay. *Rru*EPH1 showed the higher enzymatic activity at low temperature than room temperature and exhibited a good durability against the repeated freezing-thawing cycle, which indicate that *Rru*EPH1 is a cold-active enzyme.

Keywords: Epoxidase, Cold-active enzyme

H031

Characterization of a Novel Family VIII Carboxylesterase from *Caulobacter crescentus CB15*

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A novel family VIII carboxylesterase (*CcEstA*) from *Caulobacter crescentus CB15* was identified, purified, and characterized by biochemical and molecular simulation methods. Multiple sequence alignment of *CcEstA* with other related enzymes confirmed a putative catalytic serine (Ser68) and the three conserved motifs. Based on the crystal structure of *CcEstA,* Phe229, Ser235, Trp244, and Trp331 were chosen for the mutation to change the enzyme activity of *CcEstA*. All the mutation have shown the increased activity toward nitrocefin. Interestingly, Ser68 that works as a nucleophile, and Phe229 were found to be critical for esterase activity but not for β-lactamase activity. Disc diffusion assay with *CcEstA* and the mutants indicates β-lactamase activity is tunable property of *CcEstA.*

Keywords: Esterase, β-Lactamase homologue, PBP, Nitrocefin

H032

Optimization of Bioconversion Condition for Amikacin Production

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Amikacin is one of the most popular aminoglycosides. It usually was produced chemically from Kanamycin A. By synthetic methods it is rather expensive and not cost-effective. However, an interesting 2-deoxystreptamine (DOS)-containing aminoglycoside antibiotic produced by *Bacillus circulans* is capable of converting Kanamycin A into amikacin by the addition of (S)-4-amino-2-hydroxybutyrate (AHBA) side chain. Therefore, it is fascinating to explore the synthetic of useful novel AHBA-bearing aminoglycosides by biosynthetic origin pathways. In this study, the 2-DOS-containing aminoglycosides have been selectively acylated at the N-1 position with clinically valuable AHBA side chain using the purified *Bacillus circulans* origin biosynthetic enzymes BtrH and BtrG in combination with a synthetic acyl-SNAC surrogate substrate. The process was optimized and performed in two steps in a sequential manner without purification of the intermediate product. The application of this *in vitro* enzymatic production is encouraging implications for the preparation of unnatural antibiotics AHBA-bearing aminoglycosides via directed biosynthesis.

[This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01316003)" Rural Development Administration, Republic of Korea.]

Keywords: Amikacin, Kanamycin A, (S)-4-amino-2-hydroxybutyrate

H033

Antibacterial Activity of the Plasma-activated Water Stored at Different Temperatures against *Staphylococcus aureus*

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Plasma activated water (PAW) is formed when plasma components disperse into the water via convection. Plasma consists of charged oxygen, electrons, and atoms of nitrogen species, forming reactive oxygen nitrogen species in PAW. PAW has been shown to be an effective medium to kill bacteria. To test the feasibility of the long term use of PAW with the bactericidal activity, the storage conditions should be further characterized. In this study, PAW (PAW-11) was produced by a dielectric barrier discharge device with the treatment of Milli-Q water for 11 min and antibacterial activities against a Gram-positive bacterium, *Staphylococcus aureus,* were tested during incubation at four different temperatures (25°C, 4°C, -20°C, -72°C) up to 10 days. In addition, changes of some physicochemical properties such as the acidity, conductivity, and oxidation-reduction potential (ORP), nitrate and nitrite concentrations were monitored during the incubation. Our results showed that during the incubation, acidity and nitrate concentrations were almost constant. The ORP values and antibacterial activities against *S. aureus* were maintained at high levels with the values initially obtained. Our study will help the extended use of PAW for disinfection.

Keywords: Plasma activated water, Antibacterial activity, *Staphylococcus aureus*

H034

Development of Mouthwashes to Suppress Periodontal Disease Pathogens and Reduce Bad Breath through Oral Microbiome Analysis

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For oral microbial community and population analysis, 22 saliva were tested for fungi, aerobic bacteria and anaerobic bacteria. 101 strains were isolated, identified, screened and collected. Identified physiological activity and antimicrobial activity for the strains. Also characterized oral microbial and data them. In addition, the oral microorganisms, oral disease, and bad breath problems from dietary changes were found in 18 objects. BigDB data was found through the analys through 20 objects NGS analys of oral microbiome. As a result of the analysis of bad breath for microorganisms having excellent antibacterial activity, physiological activity and bioconversion ability, two kinds of bad breath reducing microorganisms were established.

H035

Molecular Diagnostics for Oral Pathogenic Bacteria

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Molecular diagnosis of seven oral pathogenic bacteria (*Fusobacterium nucleatum* (Fn), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Aggregatibacter actinomycetemcomitans* (Aa), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Campylobacter rectus* (Cr) was performed in 24 objects. As a result of qPCR diagnosis on seven periodontal causative strains (Fn, Pg, Pi, Aa, Tf, Td, Cr), an average of 5.21% of periodontitis-causing strains could be diagnosed. Among oral microorganisms, periodontitis-inducing strains accounted for 5.21%, of which *Fusobacterium nucleatum*(**Fn**) strains accounted for 76.36%. (Others *Prevotella intermedaia*(**Pi**)7.49%, *Aggregatibacter actinomycetemcomitans*(**Aa**)7.29%, *Campylobacter rectus*(**Cr**)5.18%, *Porphyromonas gingivalis*(**Pg**)4.22%, *Tannerella Forsythia*(**Tf**)0.58%.)

H036

Analysis of Antibiotic Resistance Genes and Virulence Genes in Oral Microorganisms

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BigDB results were obtained through antibiotic resistance gene analysis and virulence gene analysis by real-time PCR. The presence and content of 15 antibiotic resistance genes in the human microbiome were analyzed by real-time PCR. As a result of the virulence gene analysis study, all 9 target genes were identified by qPCR in DNA sample 27, and virulence genes were not detected. As the Ct value was similar to the non-control, it was confirmed that there was no virulence gene in the sample. qPCR with the antibiotic resistance gene tetA Primer showed a lower ct value than non-control (NTC), indicating that the tetA antibiotic resistance gene was present in all samples except K-4. The antibiotic resistance gene tetA amplicon size was confirmed on agarose gel.

H037

Functional Analysis of *sngA* Related to Regulation of Production of Autoregulator Binding to SngR from *Streptomyces natalensis* ATCC27448 Producing Natamycin

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A *sngR* encoding a gamma-butyrolactone autoregulator receptor, which has a common activity as DNA-binding transcriptional repressors controlling secondary metabolism and/or morphological differentiation in *Streptomyces*, was cloned from *S. natalensis* ATCC27448 producing natamycin, and also SngA that acts as a pleiotropic regulator was also studied. Although PI factor as a novel type quorum-sensing inducer eliciting natamycin production was discovered in *S. natalensis*, a gamma-butyrolactone autoregulator regarded as *Streptomyces* hormone has never been studied. The effectiveness of these autoregulators, which are active at nanomolar concentrations, as well as the presence of the specific receptor proteins as mediators of autoregulator signaling. The presence of autoregulator in *S. natalensis* was confirmed with gel shift assay using ethyl acetate extracts of wild-type strain, *sngR*-disruptant, and *sngA*-disruptant of *S. natalensis*, and *S. avermitilis* producing avenolide, butenolide-type autoregulator, and recombinant SngR (rSngR). Corresponding rSngR-binding activities were only found in the ethyl acetate extracts of wild-type strain and sngR-disruptant of *S. natalensis,* which suggests that *sngA* may regulate production of autoregulator binding to SngR.

[This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B03034992)]

Keywords: *Streptomyces natalensis*, Autoregulator, Receptor, Pleiotropic regulator, Gel shift assay

H038

Inhibition of 3T3-L1 Adipocyte Differentiation by PPAR γ2 Aptamer

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Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor, which upon activation increase the growth and differentiation of adipocytes. Adipocytes are the major cellular component in fat tissue and excessive growth, differentiation and hypertrophy of adipocytes are fundamental processes of obesity. The differentiation of preadipocytes into adipocytes involves exposure of a confluent, quiescent population of cells to a variety of effectors that activate a cascade of transcription factors. Here, we prove that inhibiting the differentiation of mouse 3T3-L1 cells into adipocytes; regulate to inhibit PPARγ2. Aptamers are not only useful in their own right, but as escorts for therapeutic or diagnostic reagents. In the present study, aptamer aginst PPARγ2 was selected by SELEX. Aptamers obtained after 10 rounds of selection demonstrated high affinity and specific binding with PPAR γ2 using real-time PCR and SPR system.

[This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2016R1D1A1B03934258)]

Keywords: Aptamer, Adipocyte, Obesity, PPARg, Differentiation

H039

Selection of RNA Aptamer Specific Binding to CTHRC1 Protein for Pancreatic Cancer

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CTHRC1 (collagen triple-helix repeat-containing 1) protein which is secreted during the tissue-repair process is highly expressed pancreatic cancer. CTHRC1 is upregulated by promoter demethylation and TGF-β-mediated Smad signaling. Recent studies have proved that the expression of CTHRC1 protein is associated with cancer cell proliferation and angiogenesis. Here we developed an RNA aptamer against CTHRC1 protein for the prevention of pancreatic cancer. The RNA aptamer that have been treated with pancreatic cancer, will be downregulated the CTHRC1 function by blocking the Smad signal for cell growth. We selected the RNA aptamer using *in vitro* transcription SELEX. We obtained the 16 RNA aptamer against CTHRC1 protein through the 11 round of SELEX process. And we analyzed the binding structure of the protein-RNA aptamer complex, which is confirmed the RNA aptamer bind to the active site of the CTHRC1 protein using MOE program. We selected the RNA aptamer, which is binding to the CTHRC1 protein active site, has high affinity. The RNA aptamer efficacy experiments will be tested *in vitro* pancreatic cancer cell line.
[This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2019R1I1A1A01062365).]

Keywords: Aptamer, Pancreatic cancer, CTHRC1

H040

Characterization of PAT Protein-aptamer Complex Structure by X-ray Diffraction

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More than 80% of all GM crops grown worldwide have been engineered for herbicide tolerance. Phosphinothricin N-acetyltransferase (PAT) is herbicide-resistant protein in GM crops. The effects of GM crops on human health are not yet fully understood. An effective detection system is important for proper regulation of GM crops. Aptamers are ssDNA or RNA molecules that can bind to targets by recognizing the structure. Protein-aptamer complex structure analysis can help improve the binding specificity and sensitivity of protein and aptamer. In this study we have selected aptamers binding to PAT protein from a DNA library by SELEX (Systematic Evolution of Ligands of Exponential enrichment) method. PAT protein crystallization for structure analysis of PAT with and without aptamer was set up. The *Bar gene* was cloned in pET-21a and expressed by *E. coli* BL21 (DE3). The protein was purified by three step purification: Affinity chromatography, Ion-exchange chromatography and Gel filtration chromatography. Sitting drop vapor diffusion at 291K temperature was used for Crystallization. PAT protein-aptamer complex structure can be used to identify important protein-aptamer interactions.
[This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2017R1D1A1B03033366).]

Keywords: PAT, Aptamer, Protein-aptamer complex, Crystallization, X-ray

H041

Development of CD1d Binding Aptamer and Immobilization of Magnetic Beads

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There is a growing interest in the diagnostic markers for early disease detection and prognosis. Extracellular vesicles, such as microvesicles and exosomes, represent a fast-growing field of research with therapeutic and diagnostic applications. However, traditional methods for the isolation of exosomes are labor-intensive, time-consuming and heavily instrument-dependent. It is well known that a variety of different cells may produce different sizes of exosome, but all exosomes include the same surface markers. Various studies have revealed the presence of CD1d protein on the surface of exosomes extracted from dendritic cell. In this study, ssDNA aptamers were generated to specifically bind to CD1d through 10 rounds of SELEX. After *in vitro* selection, binding affinity of selected candidates were quantified by using surface plasmon resonance. To establish a direct method for fast, efficient, and selective isolation of exosomes, CD1d specific aptamers were covalently immobilized on magnetic beads.

[This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ012653)” Rural Development Administration Republic of Korea.]

Keywords: SELEX, CD1d, Aptamer, Exosome, Magnetic beads

H042

***In vitro* Selection of Aptamer Targeting NME2 Protein for HCC Detection**

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Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and the third leading cause of cancer mortality worldwide, with the highest incidence rates reported in East Asia. Increasing prevalence of Hepatitis C virus (HCV) infection, HCC has been observed in most industrialized countries. Recently, surgical resection and transplantation are the only curative methods for HCC, but only 10–20% of patients are suitable for these treatments. Nucleoside diphosphate kinase B (NME2) protein is HCC biomarker and it mediates the neoplastic transformation of epidermal cells in the early stages of carcinogenesis. Aptamers are ssDNA or RNA oligonucleotides that can bind to targets by recognizing the structure. In this study, *NME2* gene was cloned in pET-21a and expressed by *E. coli* BL21(DE3). The protein was purified by two step purification: Affinity and Ion-exchange chromatography. We selected aptamers binding to NME2 protein by using SELEX (Systematic Evolution of Ligands of Exponential enrichment) process. After SELEX rounds, we generated aptamers structures and the binding affinity was confirmed by SPR. The development of NME2 specific aptamers can be applied to the therapeutics of HCC by direct inhibitors or indirect vehicles for drug delivery.

[This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ012568)" Rural Development Administration, Republic of Korea.]

Keywords: HCC, NME2, Aptamer, SELEX, Detection

H043

Crystallization and X-ray Diffraction Analysis of Seleno-methionine Labeled SAM-dependent Methyltransferase

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S-adenosyl methionine, also known as SAM or AdoMet, is the universal methyl donor involved in different methylation reactions and present in all living organisms. S-Adenosyl-methionine-dependent methyltransferases (SMTs) are a large class of enzymes that catalyze the transfer of a methyl group from SAM to an acceptor substrate. Since SMTs catalyze most methylation reaction in many biological processes, it has become an important enzyme category of biotechnological interests, synthetic biology and chemical biology. In this study, we carry out crystallization of SMTs for structure analysis. Seleno-methionine (SeMet) labeled SMT and native SMT were purified and dispensed in the 96 well plates to ensure protein crystals. Based on the conditions under which protein crystals were formed, the pH and concentration of the buffer were varied to induce protein crystallization. Finally, X-Ray diffraction data were collected using ADSC Q270 detector on beamline 7ASB1 at the Pohang Accelerator Laboratory.

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Keywords: S-Adenosyl methionine (SAM), SAM-dependent methyltransferases (SMTs), Seleno-methionine (SeMet), Crystallization, X-ray diffraction

I001

Characterization of *Lactobacillus fermentum* PL9988 for a Probbiotic: Antibiotic Susceptibility and Attachment to Intestinal Epithelial Cells

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Several *Lactobacillus fermentum* strains were isolated from Korean people who are over 80 years old in Korean longevity villages. Among these *Lactobacillus fermentum* PL9988 showed ability to resistant to acid and bile acid, and enhance immune-enhancing activity. In the present study, its adhesiveness to the intestines was tested using a BALB/c mouse model and its susceptibility to various antimicrobials were assayed. Mice were administered with extremely high concentration of *Lactobacillus fermentum* PL 9988 daily for 28 days. There were no significant changes in feed intake, body weight, liver weight, or clinical signs. *Lactobacillus fermentum* PL9988 were found to be attached to the intestinal epithelial cells as well as detected found in feces showing its survival in intestines. Results from the previous study and this study showed beneficial characteristics of *Lactobacillus fermentum* PL9988 as a good probiotic.

I002

Survival and Cellular Responses of Food-poisoning Bacterium, *Escherichia coli* MK-7 Exposed to Allyl Isothiocyanate (AITC)

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The aim of this study was to characterize the food-poisoning bacterium, *Escherichia coli* MK-7 exposed to allyl isothiocyanate (AITC). Initially, MK-7 was enriched and isolated from stale food. The isolate was identified and assigned to *Escherichia coli* MK-7 using the 16S rRNA sequencing. Phylogenetic tree of MK-7 was plotted on 16S rRNA sequencing comparison. Bactericidal effects of MK-7 exposed to AITC ranging from 0 mg/ml to 2 mg/ml were monitored, and complete bactericidal effects were achieved within 24 h at 2 mg/ml and higher concentrations. SEM analysis demonstrated the presence of perforations and irregular rod forms with wrinkled surfaces in cells treated with sublethal concentrations of AITC. SDS-PAGE with silver staining revealed that the amount of LPS increased or decreased in the strain MK-7 treated to different concentrations and exposing periods of AITC in exponentially growing cultures. The stress shock proteins (70-kDa DnaK and 60-kDa GroEL), which might contribute to enhancing the cellular resistance to the cytotoxic effect of AITC, were induced at different concentrations of AITC exposed to cell culture of MK-7. In conclusion, this study provides important clues in understanding of the survival mechanism that affect the antibacterial effect of AITC exposure, and suggests its use as a phytochemical antibacterial.

Keywords: Allyl isothiocyanate, AITC, *Escherichia coli* MK-7, Cellular responses

I003

Influences of Salt and Capsaicin on Fibrinolytic Activities of *Bacillus subtilis* GK-5 Isolated from Green Onion Kimchi

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The purpose of this work was investigate the influences of salt and capsaicin on fibrinolytic activities of *Bacillus subtilis* GK-5 isolated from green onion kimchi. Initially, the physiological and biochemical characteristics of the strain were examined. 16S rRNA sequencing analysis was performed to identify the strain, and the strain could be designated as *B. subtilis* GK-5. The changes of bacterial growth, fibrinolytic activity, and pH were monitored, *B. subtilis* grew well at in the different ranges of NaCl (0–10%) and capsaicin (0–300 μg/ml) at 37°C. *B. subtilis* GK-5 showed the strongest fibrinolytic activity (2.97 U/ml) in absence of NaCl and capsaicin. Although when *B. subtilis* GK-5 was cultivated in harsh conditions tested in this work, such as in LB media containing 10% NaCl and 300 μg/ml capsaicin, the culture showed the highest fibrinolytic activity (0.62 U/ml) for 84 h. Considering its high fibrinolytic activity, significant salt- and capsaicin-resistance, and ability to grow, *B. subtilis* GK-5 can be used as a starter for salty as well as spicy fermented foods.

Keywords: Fibrinolytic activity, Salt, Capsaicin, Green onion, *Bacillus subtilis* GK-5

I004

Microbial Community Analysis of Korean Traditional Meju by Pyrosequencing Methods

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Traditional Korean Meju is consisted of a microbial ecosystem of bacteria and fungi and their enzymes play an important role to produce the flavor in soybean fermentation. In this study, against seven types of traditional Korean meju collected from nationwide, the bacterial and fungal communities were analyzed by pyrosequencing and their characteristics such as form, weight, water content, pH, and protease activity were compared. *Bacillus* species in bacteria and *Aspergillus, Penicillium*, and *Mucor* genera in fungi were distributed at a high ratio in all meju samples. Dominant microorganisms in each meju differed depending on the locality of sample collection and observable meju characteristics (weight, water content, and thickness). Among meju presenting high protease activity, *Aspergillus* dominated in the low water content and *Geotrichum, Mucor,* and *Clostridium* were prevalent in the high water content. *Cryptococcus, Debaryomyces*, and *Alternaria* increased pH of the meju. Two fungal genera, *Lichtheimia* and *Rhizomucor*, were first identified in meju. We conclude that it will be possible to promote specific enzymatic activity to produce target flavors either by using proper starters or by controlling meju production environment such as temperature, oxygen, and water content.
[The support of “Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01453902)” Rural Development Administration, Republic of Korea.]

Keywords: Meju, Pyrosequencing, Meju fungal community analysis, Meju microbial community analysis, Microbiota

I005

Complete Genome Sequence and Phylogenetic Analysis of Multidrug-resistant *Staphylococcus aureus* Isolated from Food Products of Animal Origin

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Staphylococcus aureus is a pathogen that causes food poisoning and community-associated infection with antibiotic resistance. The relatively small genome size and rapid evolution of antibiotic resistance genes in the species have been drawing an increasing attention in public health. To extend our understanding of the species, *S. aureus* stains were isolated from pork and beef in Korea. Among the isolates, KS101Sa isolated from pork was multidrug-resistant, with resistance to benzylpenicillin, oxacillin, gentamicin, ciprofloxacin, erythromycin, telithromycin, clindamycin, tetracycline, and trimethoprim/sulfamethoxazole. The genome sequence was determined using Illumina MiSeq platform, which assembled using HGAP3, producing 2 contigs with a total genome size of 2,989,207 bp (33% G + C content). Automatic annotation was performed using the Prokka, generating features potentially assigned to protein-coding genes. A comparison between the genome of KS101Sa and the genomes of *S. aureus* strains showed that the closest strain to KS101Sa is *S. aureus* subsp. *aureus* 71193, *S. aureus* subsp. *aureus* ST398, and *S. aureus* 08BA02176 with an average 99.9% (amino acid sequence) similarity. These strains have been known to colonize and infect humans and certain animal species such as dogs, horses, and pigs. Therefore, this report would be helpful for further studies of pathogenesis, rapid detection, and epidemiological investigation of *S. aureus*.

Keywords: *Staphylococcus aureus*, Complete Genome Sequence, Multidrug-resistant, Food Products of Animal Origin

I006

Seasonal Difference of Bacterial Community in the Chinese Chive (*Allium tuberosum Rottler*) Cultivated in the South Korea

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To understand seasonal features of bacterial community and potential pathogens on raw vegetables, The Chinese chive samples analyzed by 16S rRNA gene-based sequencing (V5-V6, Illumina Miseq). On March-April and June, Chinese chive samples collected at traditional market and grocery store in Seoul and Busan (n = 80). Total 11,122,877 reads were analyzed to compare seasonal difference. α-diversity indices of March-April samples were statistically higher than June samples ($p < 0.0001$), whereas total bacteria index of June samples more higher ($p < 0.0001$). Although the proportions of each component were different, similar components were found at each taxonomy level (phylum, class, order and genus). Potential pathogens such as *Pantoea*, *Pseudomonas*, *Escherichia* were detected at the genus level. The proportion of some potential pathogenic genera was different according to sampling time. These results suggest that seasonal factors can affect microbiota on Chinese chives. Further studies on identification of foodborne pathogens are needed.

[This research was supported by a grant (14162MFD972) from Korean Ministry of Food and Drug Safety.]

Keywords: Chinese chive, Microbiota, 16S rRNA gene sequencing

I007

Study on Sawdust Medium Composition to Improve Shiitake (*Lentinula edodes*) Productivity

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Shiitake (*Lentinula edodes*) sawdust cultivation is widely used, and in order to increase its productivity, researches on its cultivation have been done. Among them, the component of the sawdust medium is one of important parts in shiitake cultivation research. We investigated the effect of various medium components to improve the productivity of *L. edodes*. In this study, 10 materials (beet pulp meal, corn grain, corn meal, cotton seed meal, cotton seed hull, kapok meal, mixed medium, soybean meal, soy hull, and wheat bran) were used for mushroom cultivation. With the different mixing ratios of oak sawdust and additional materials, 2kg block type media were prepared and adjusted to 65% of water content. The strain (NIFoS 2778) was inoculated and cultivated for 80 days in dark condition, and 40 days in light condition. After the incubation period, mushroom fruiting was stimulated at 18°C and more than 85% humidity. The fruiting body was not produced on the sawdust media with soy bean meal and cotton seed meal. Mixed medium and soy hull media showed higher productivity and biological efficiency than wheat bran media. Other conditions had lower productivity than wheat bran media. Our results show there are better materials than wheat bran normally used in shiitake sawdust cultivation.

Keywords: Biological efficiency, Medium components, Productivity, Sawdust cultivation, Shiitake

I008

The Fate of Non- or Slightly Halophilic Bacteria in a Korean Traditional Fermented Fish Sauce, Jeotgal

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Jeotgal is a salty and fermented traditional Korean fish sauce. Unlike most other previous studies that investigated samples purchased from retail markets, this study focused on samples of jeotgal with traceable history to Yeonggwang, a time-honored fishing village in Korea. Jogi jeotgal, which was made from small yellow croakers, was selected based on information obtained from interviews with the local craftsmen and literature review. There are concerns on the contamination by undesired bacteria in the process of making jeotgal. The 16S rRNA gene-based metagenomic analysis revealed that halophilic bacterial species were predominant species while non- or slightly halophilic bacteria were infrequent. Since there is still a possibility that non- or slightly halophilic bacteria survive by forming endospores attempts were to isolate and classify bacteria from jogi jeotgal. 100 μl liquid samples were taken directly from 2-year-old jogi jeotgal, and plated out on Marine Broth agar plates. A total of eighty colonies were randomly picked isolated and subjected to 16S rDNA sequencing analysis. All of the strains tested were found to belong to the single family Bacillaceae, which includes *Bacillus* spp., *Virgibacillus* spp., *Pontibacillus* spp., *Halobacillus* spp., *Oceanobacillus* spp., *Piscibacillus* spp., and *Paraliobacillus* spp. Based on the known characteristics of each best match, it is apparent that none of the isolates can cause human health problems.

Keywords: Jeotgal, Fermentation, 16S rRNA sequencing, Bacillaceae

I009

Daily Consumption of Brewed Type Coffee Induces Changes on the Gut Microbiome, and These Changes are Dose-dependent

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Consumption of coffee has become the most natural part of daily life for the world population. Especially in Korea coffee consumption has been increased rapidly, and the annual consumption of coffee is three times the world average. Even though more than 1,500 chemical components in coffee are expected to affect our gut microbiota, its impacts and correlation with quantity have not been evaluated. In this study, we evaluated the effects of consuming small (2 shots, 85 ml per day) and large (4 shots, 170 ml per day) amount of brewed coffee on gut microbiota composition. Twenty-five Korean early adulthoods participated, and they consumed a small amount of coffee for a week, followed by a week of washout period they consumed a large amount of coffee for a week. Fecal samples were obtained before and after each period to verify the microbial changes using 16S rRNA gene-based sequencing. In *Bacteroides* type, coffee consumption induces moderate changes in the gut microbiome but *Bifidobacterium* decreased and this change is dose-dependent. In *Prevotella* type, coffee consumption induces dynamic change, and it more dynamically changes when they intake a large amount of coffee. These data suggest that consumption of coffee may induce changes on the gut microbiota and these changes vary by gut enterotype and amount of coffee.

Keywords: Coffee, Gut microbiome, Gut enterotype

I010

Screening of Makgeolli-derived Lactic Acid Bacteria from Human Feces after Makgeolli Consumption

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In order to isolate novel probiotics, it is required to evaluate acid resistance, bile resistance and mucus adhesion abilities of candidate strains. In this study, we identified *Lactobacillus*, derived from makgeolli that settles well in human gut environments. We collected human fecal samples before and after consuming makgeolli from 26 subjects and analyzed samples to identify gut microbiota of each subject using 16S rRNA gene-based sequencing technique (V4 region; Illumina MiSeq). Sequences were analyzed by QIIME Pipeline (v1.9.1) based on the open OTU (Operational Taxonomic Unit) picking method with EzBioCloud 1.5 database. One subject was selected from each enterotype (*Bacteroides* type or *Prevotella* type) that showed the largest increase in LAB after consuming makgeolli. For identification of LAB, the fecal samples were incubated in MRS medium and each colony was analyzed with BLASTN tool. 11 strains *of L. fermentum* and one strain of *L. plantarum* in *Prevetella* type and 8 strains of *L. paracasei* and 590 strains of *L. plantarum* in *Bacteroides* type were derived from makgeolli. Acid tolerance and bile salt tolerance tests were performed with the strains identified as makgeolli-derived LAB. Four strains showed better survival rate than LGG (control strain) in acid and bile salt tolerance test, suggesting the possibility of new probiotics candidates with high resistance in Korean gut microbiome environments.

Keywords: Probiotics, Lactic acid bacteria, Microbiota, Screening, Makgeolli

I011

Observation of Microbiota Changes of Home-made Yogurt while Fermentation and Storage

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With growing interest in healths, the fermented milk market continues to grow. There is a lot of information on how to make homemade yogurt, but there are few scientific-based results. In this study, we observed the microbiota changes of home-made yogurt while fermentation and storage hours. We selected four drinking yogurt products (Y1-Y4) from the market as starter culture and these products were chosen by purchase rank. The home-made yogurt was prepared with 42.5 ml of milk and 7.5 ml of drinking yogurt product with 8 h of fermentation at 42°C. The sampling points were before fermentation, during fermentation (4h, 8h), and during storage (after 1 d, 7 d in the refrigerator). To observe the microbiome changes of home-made yogurts, 16S rRNA gene-based sequencing technique was used based on the Earth Microbiome Project (EMP). Results showed that Fermented yogurts using Y1 and Y4 were dominated by *Streptococcus* with low relative abundance of *Lactobacillus*. Y2-fermented yogurt contained 80% of *Streptococcus*, 20% of *Lactobacillus*, and 2% of *Bifidobacterium*. In relation to other products, about 10% of *Lactococcus* was found in Y3-fermented yogurt. In Y3-fermented yogurt, the rate of *Lactococcus* decreased while that of *Lactobacillus* increased during fermentation. These results suggested that the home-made yogurt microbiota is stable at least 7 days during storage and composition of microbiota in yogurt varied by starter products.

Keywords: Yogurt, Fermentation, LAB, Probiotics, Microbiome

I012

Optimization of Culture Conditions for the Black *Aspergillus niger*

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Aspergillus niger is a group in the genus *Aspergillus* that is made up of 15 varieties, all with black conidia. At present, the filamentous fungus *Aspergillus niger* is widely used by the fermentation industry for the production of enzymes and organic acids, particularly citric acid. Spores of *Aspergillus niger* showed clear black color, which can be used to give black color in food industry. In this study, we selected the *Aspergillus niger* strain that produced the best clear black color and established optimal culture conditions. 12 strains of *A. niger* having ATCC numbers were tested in four culture media (Czapek's agar, Potato dextrose agar, Malt extract agar, Yeast malt agar), reveal that YM agar showed the best blackness spores in relation to other media. Further optimizations under various pH, dextrose content, amount of medium and temperature conditions were performed to find the optimum culture conditions. In conclusion, the culture condition of pH 7.0 and 37°C with 1% of dextrose content in 20 ml of solid agar per plate showed best result in showing black color.

Keywords: Fungi, Black pigment, Culture, *Aspergillus niger*

I013

Biodiversity and Characterization of Aerobically Cultured Bacteria from the Various Korean Hot Spring

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This research confirmed the diversity and characterization of microorganisms isolated from the various Korean hot springs by different temperature (33–60°C), collected on the Chung-Ju, Samcheonpo, Uljin, and Busan in South Korea. To isolate strains, Marine agar medium was basically used and cultivated at 37–60°C and pH7 for several days aerobically. After single colony isolation, total 91 pure single-colonies were isolated and phylogenetic analysis was carried out based on the result of 16S rRNA gene DNA sequencing, indicating that isolated strains were divided into 11 families, 14 genera, 34 species, and 91 strains. Bacillaceae family, the main group, comprised 65.9% with 4 genera and 16 species of Aeribacillus, Bacillus, Caldibacillus, and Geobacillus. To confirm whether isolated strain can produce industrially useful enzyme or not, amylase, lipase, and protease enzyme assays were performed individually, showing that 62 strains possessed at least one enzyme activity. Especially the thermophiles (*Bacillus thermoamylovorans* EF45103, *Geobacillus stearothermophilus* EF60053, and 60063 strains) showed all enzyme activity tested. This result indicated that isolated strains have shown the possibility of the industrial application. Therefore, this research has contributed for securing genetic resources and the expansion of scientific knowledge of the hot spring microbial community in South Korea.

Keywords: Diversity, Hot spring, Thermophiles

I014

Biodiversity and Profile for Extracellular Enzyme Production of Aerobically Cultured Halophiles from the Various Ethiopian and Korean Salts

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This research confirmed the diversity and characterization of microorganisms isolated from the various Ethiopian and Korean salts, collected on the saltern of Danakil Depression, Afdera lake, Shinan saltern, and Gomso saltern. To isolate strains, modified Marine agar medium (15% salt conc.) was basically used and cultivated at 37–45°C for several days aerobically. Total 230 pure single colonies were isolated and phylogenetic analysis was carried out based on the result of 16S rRNA gene DNA sequencing, indicating that isolated strains were divided into 4 phyla, 12 families, 25 genera, 64 species and 230 strains. Firmicutes phylum, the main group, comprised 89.6% with 3 families, 15 genera and 52 species of Bacillaceae, Staphylococcaceae, and Carnobacteriaceae. To confirm whether isolated strains can produce industrially useful enzyme or not, amylase, lipase, and protease enzyme assays were performed individually, showing that 177 strains possessed at least one enzyme activity. This result indicated that isolated strains have shown the possibility of the industrial application. Therefore, this study has contributed for securing domestic/abroad genetic resources and the expansion of scientific knowledge of the microbial community in salts.

Keywords: Biodiversity, Ethiopian and Korean salts, Halophiles

I015

Diversity and Profile for Extracellular Enzyme Production of Bacteria in the Gut of *Muraenesox cinereus*

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This research confirmed the diversity and characterization of gut microorganisms isolated from the intestinal organs of *Muraenesox cinereus*, collected on the Samcheonpo and Seocheon Coast in South Korea. To isolate strains, Marine agar medium was basically used and cultivated at 37°C and pH7 for several days aerobically. After single colony isolation, totally 49 pure single-colonies were isolated and phylogenetic analysis was carried out based on the result of 16S rRNA gene DNA sequencing, indicating that isolated strains were divided into 3 phyla, 13 families, 15 genera, 34 species and 49 strains. Proteobacteria phylum, the main phyletic group, comprised 83.7% with 8 genera and 26 species of Aeromonadaceae, Pseudoalteromonadaceae, Shewanellaceae, Enterobacteriaceae, Morganellaceae, Moraxellaceae, Pseudomonadaceae, and Vibrionaceae. To confirm whether isolated strain can produce industrially useful enzyme or not, amylase, lipase, and protease enzyme assays were performed individually, showing that 39 strains possessed at least one enzyme activity. Especially the *Aeromonas* sp. strains showed all enzyme activity tested. Therefore, this study has contributed for securing domestic genetic resources and the expansion of scientific knowledge of the gut microbial community in *Muraenesox cinereus*.

Keywords: Extracellular enzyme, Gut, *Muraenesox cinereus*

I016

Bacteriocin from *Lactococcus lactis* 3001 Can Inhibit the Growth of *Propionibacterium acnes*

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Propionibacterium acnes is a causing bacteria for acne. We have tried to isolate lactic acid bacteria which can inhibit the growth of *P. acnes*. From the screening, we found that *Lactococcus lactis* 3001 which was previously isolated and produces a bacteriocin inhibiting the growth of *Staphylococcus aureus,* showed antimicrobial activity against *P. acnes* KCTC 3314. Furthermore the bacteriocin presented mode of bactericidal action against the strain. Several bacteriophages which is specific to *P. acnes* were isolated and tested for their antimicrobial activities against the strain. Now we are investigating the optimal concentration of the bacteriocin and a selected bacteriophage for synergistic effect on the control of *P. acnes*.
[This was supported by Korea National University of Transportation in 2019.]

Keywords: *Propionibacterium acnes*, Bacteriocin, Bacteriophage, *Lactococcus lactis*, Synergistic effect

I017

Cultural Characteristics of *Sparassis latifolia* on Sawdust Medium of Conifer

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Special Forest Products Division, National Institute of Forest Science

Cultivation Characteritics of cauliflower mushroom (*Sparassis latifolia*) was investigated in mycelial growth on Potato dextrose agar (PDA) and mushroom production on sawdust medium with different nutrients. The maximum growth of each strain on PDA showed NIFoS 700 and NIFoS 923 at 20°C, NIFoS 1748 and NIFoS 1756 at 24°C, respectively. The fruiting bodies of four strains produced in 38 treatments of a total of 60 treatments with combination of three additional nutrients and three sawdust. NIFoS 1756 produced an average of 41 g fruiting bodies per 400 g medium and the color of the mushrooms showed 64.5 (L)-2.7 (a)-18.4 (b) and 64.9 (L)-1.4 (a)-15.0 (b) on *Pinus densiflora* and *Larix kaempferi* sawdust media, respectively. There were no fruiting bodies from sawdust media with bran nutrient. We don't think bran is suitable for cauliflower mushroom cultivation.

Keywords: Conifer, Cultural characteristics, Nutrient, Sawdust medium, *Sparassis latifolia*

I018

Basic Characteristics of Mycelial Growth of *Lentinula edodes* on Sawdust Medium

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Special Forest Products Division, National Institute of Forest Science

We investigated a total of 61 hybrid strains of *Lentinula edodes* to select fruiting body with good quantity. For the cultivation, rectangular shape polyethylene bags filled with 2 kg sawdust media containing 80% of *Querqus* spp. sawdust and 20% of wheat bran. The weight loss rate of sawdust media in 61 strains was 5.0–11.1% and the average weight loss was 7.6% after 60 days of incubation. Three six strains showed above average levels of weight loss rate. NIFoS 4504 had the highest weight loss rate (11.1%) among them, followed by NIFoS 4714 (10.4%), NIFoS 4473 (10.1%), NIFoS 4709 (9.7%), NIFoS 4549 (9.6%), NIFoS 4450 (9.5%), NIFoS 4496 (9.4%), and NIFoS 4508 (9.4%). NIFoS 4804 had the lowest weight loss rate (5.0%).
Cultural characteristics of newly bred *L. edode* (61 strains) were also investigated. After 60 days, 12 strains formed tunicate and 54 strains showed browning of the culture media. The medium elasticity was observed in 50 hybrid strains. Forty-nine strains formed mycelial lump and 42 strains of them excreted decomposition water.

Keywords: Cultural characteristics, Decomposition water, Hybrid strains, *Lentinula edodes*, Weight loss rate

I019

Introduction of Meju Fungal Study

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There is a saying that the taste of Jang (soy sauce, soybean paste, etc.) depends on molds of meju. It means that molds of meju are important to make Jang. However, only *Aspergillus oryzae* have been studied and used to make Jang in companies. *Aspergillus oryzae* is only one of many molds in Korean traditional meju. The aims of this study is to elucidate roles of the molds of meju and to develop them as starter for Jang manufacture. 1047 fungal strains have been isolated from meju and they were identified as 101 species. *Mucor circinelloides, M. racemosus, Lichtheimia ramosa, Aspergillus oryzae, Eurotium repens, Penicillium solitum, Scopulariopsis brevicaulis* etc. are predominant species on meju. We examined their extracellular enzyme production. We made Jang with each mold in small scale and examined quality properties of the soybean products. We selected 11 fungal species for more studies. We are going to examine roles of the 11 fungi in a lager scale and try to develop them as starter for Jang manufacture.

[Supported by grants from RDA, PJ014539]

Keywords: Fungus, Meju, Jang

J001

Identification of *Metarhizium majus* (Hypocreales, Cordycipitacae) Infecting *Protaetia brevitarsis* (Coleoptera, Scarabaeidae) from Industrial Insect Farms in the Republic of Korea

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Mycosis symptoms of *Protaetia brevitarsis* (Coleoptera: Scarabaeidae) from two industrial insect farms in the Republic of Korea were recorded in May 2019. The causal agent was morphologically identified. Using both morphological taxonomy and molecular phylogenetics, we identified 1 strain of entomopathogenic fungus classified as species of *Metarhizium majus*. Polymerase chain reaction (PCR) of 5' Transcription Elongation Factor (5TEF) was used for molecular identification and verification of the morphological determination. Both methods gave consistent results and we report for the first time the occurrence in insect farm of a fungal species belonging to the order Hypocreales (Phylum Asocomycota); *Metarhizium majus* (Hypocreales, Cordycipitacae).

Keywords: *Metarhizium majus*, Entomopathogenic fungi, Industrial insect farm, *Protaetia brevitarsis*

J002

Efficacy of Polymerase Chain Reaction Assays for the Prohibited Quarantine Pathogen, *Xylella fastidiosa* and Studies of Insect Vectors for Pierce's Disease

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Plant Quarantine Technology Center, Animal and Plant Quarantine Agency

Xylella fastidiosa, the causal agent of Pierce's disease (PD) in grapevines, is regarded as one of the most important quarantine bacterial diseases (EPPO A2 List) as well as the most destructive pathogen that causes yield losses in the world. Also, it is very difficult to determine the presence or absence of PD on the various host plants such as grapevines due to easily confused showing the similar symptom such fungal disease on grapevines caused by *Pseudopezicula tracheiphila*. In addition, 563 host plants and 22 Insect vectors were reported in the world. For this reason, we were conducted to developed the most specific and sensitive diagnosis method based on polymerase chain reaction (PCR) that is available for immediate detection of *X. fastidiosa* as prohibited pathogen from various importing plants. Thus, we also studied the 22 insect vectors of PD as well. According to results, we selected the RST31/RST33 primer set showing the most efficient on *X. fastidiosa* compare with XF1-F/XF6-R and CVC-1/272-1-int primer sets. In addition, we also proved that the limit of RST31/RST33 primer set on PCR assay to detect *X. fastidiosa* was very highly indicated from 1 pmol/µl to 10^{-4} pmol/µl of genomic DNA per reaction. Consequently, we proved the PCR assay was highly efficient to prevent the spread of *X. fastidiosa* and also studied the nine insect vectors' morphology for plant quarantine purpose.

Keywords: Prohibited quarantine pathogen, *Xylella fastidiosa*, Pierce's disease, PCR assay, Insect vectors

J003

The Imitative Cultivation of the Human Gut Microbiota in a Single Batch and Its Potential Possibility as a Microbial Agent for FMT

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Although FMT can be applied to treat gut diseases, the stool needs to be collected from the donor whenever needed and difficult storage. Moreover, the reproducibility of microbial composition from donor stool is challenging due to various environmental factors. The objectives of this study were to prove that the gut microbiota could be imitatively cultured in a single batch and that the cultured gut microbiota could have the same therapeutic effect when compared to the original stool as an FMT material.

For evaluating the cultured gut microbiota, the relative abundance, alpha and beta diversities of the cultured microbiota in several kinds of media were compared with selected original stool sample (SOS) and samples from healthy persons (HG). Alpha diversity showed that the cultured gut microbiota in BHI media from 72 hours shows recovery when compared with SOS. Also, the PCoA plot produced using the weighted unifrac (Beta diversity) revealed that the cultured microbiota in BHI from 72 h were found near the cluster of the HG.

The therapeutic effect of cultured microbiota was confirmed using the DSS induced IBD mouse model. The mice group introduced cultured microbiota transplantation after DSS treatment, was not different when it compared to the other FMT groups. Moreover, the cultured gut microbiota shows similar therapeutic effect when introduced to the IBD mice in not only the histological observation, but also the cytokine level compared to the other FMT groups.

Keywords: Gut microbiota, Imitative cultivation, Mouse model, FMT, IBD

J004

Genetic Analysis of Collected Dengue Virus Resources by National Culture Collection for Pathogen

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National Culture Collection for Pathogen (NCCP) has performed collection of virus and distribution of well characterization viral pathogen resource. Dengue fever is caused by dengue viruses (DENV) from the Flavivirus. DENV exists in four immunogenically distinct and genetically-related serotypes (DENV-1 to 4), each subdivided in genotypes. DENVs were not circulated by localization in Korea, however, the imported cases of all four DENV serotypes have continuously occurred in Korea. NCCP conducted this study to provide well identified Dengue virus and in-depth genetic analysis information. Twelves Korean DENVs were analyzed by year and genotypes that were compared with each genotype of reference strains. The viral E protein and full genome sequences were aligned by Muscle v 3.8 and variation and phylogenetic analysis performed with in-house Python scripts and RAxML v8. Estimates of evolution rates and time since the most recent common ancestor (tMRCA) were inferred via Bayesian phylogenetic analysis using the BEAST software package. The phylogenetic trees based on full genome and E gene sequences showed similar patterns. The Varied sites were observed in comparison to Dengue virus reference strains and phylogenetic relationship among full viral genome sequences was identified. In this study, we presented genetic characterize of collected DENVs by NCCP. These well characterized viral information and NGS data will be provided on distributed each DENV resources.

Keywords: Dengue virus, Flavivirus, NGS

J005

Functional Analysis of DNA Methyltransferases from *Cryphonectria parasitica*

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Two representative fungal DNA methyltransferases (DNMTase) genes from *Cryphonectria parasitica* had been identified as *CpDmt1* and *CpDmt2*, orthologous to repeat-induced point mutation (RIP) and RIP defective of *Neurospora crassa*, respectively. Although both mutants showed no significant differences from the wild type in responses to various stress, they showed significant changes in virulence but in opposite direction. These results indicated that each gene has a functional specificity. In addition, both mutants showed severe growth retardation after the hypovirus infection, suggesting epigenetic regulation in fungal responses. Moreover, spontaneous viral clearance was occurred from hypovirus-infected hyphae of both mutants, which resulted in robust colonial growth showing the virus-free colonial characteristics such as pigmentation and conidiation. Compared to the uninfected isogenic strain, drastically enhanced up-regulation of two key antiviral genes were observed in both mutants when the hypovirus infected. These results suggested the epigenetic regulation of the host responses to the hypovirus infection via antiviral RNA silencing pathway.

This study demonstrated that DNA methylation is important for the fungal virulence and hypovirus infection. Each fungal DNMTase affects fungal biology in a common as well as specific ways. These will help to understand epigenetic regulation of fungal virulence and responses to the virus infection.

[Supported by grants from NRF.]

Keywords: *Cryphonectria parasitica*, DNA methylation, Hypovirus, Virulence, RNA silensing

J006

Useful Multiplex PCR Assay for Rapid Identification of Pathogenic Staphylococci

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The *Staphylococcus* infection can cause a wide variety of diseases in humans and animals through either toxin production or penetration. These bacteria commonly inhabit the skin and nose where they are innocuous, but may enter the body through cuts or abrasions which may be nearly invisible. Bloodstream infections are a leading cause of morbidity and mortality in the United States and are associated with increased health care costs.

Animal experiments are essential to biological and medical research. High quality laboratory animal is most important in the experiment for get reliability and reproducibility data. However, most of the infections in laboratory animals are closely related to the hygiene of breeders and/or researcher. However, it is not easy to identify bacterial of similar colony morphology and morphology diagnosis is subjective.

In this study, we were using conventional PCR for objectively identification of *Staphylococcus* spp. Our PCR method will be used to improve quality control in laboratory animals and laboratory animal facilities.

Keywords: Staphylococci, PCR assay, Quality control

J007

Genetic Characterization of Murine Astrovirus on Laboratory Animal in South Korea

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Mice (*Mus musculus*) are the most commonly used laboratory animals. The murine astrovirus (MuAstV) was first isolated from a nude mouse with symptoms of diarrhea in 1985. The first complete genome sequence of a MuAstV was elucidated in 2011. Infection by MuAstV was reported in North America, Europe, Middle East and Asia from 2012 to 2017. Infections with MuAstV were recently reported at Australia, China and Japan in Asia but unknown at South Korea. The objective of this study was the evaluation of infection status of MuAstV on laboratory mice in South Korea.
Using PCR assay, we screened sentinel mice from 3 vendor, 6 research institutes and 5 universities in the South Korea. Mice in over half of the vendor (2/3), research institutes (3/6) and universities (3/5) revealed the presence of MuAstV. Two mice strains tested positive including ICR and C57BL/6. Comparison of the MuAstV RNA dependent RNA polymerase sequences (346 bases) showed numerous mutations (92% to 98% nucleotide homology) ongoing viral divergence. This study indicates that MuAstV has a wide geographical, institutional distribution in South Korea.

Keywords: Murine astrovirus, RNA dependent RNA polymerase

J008

Development of Duplex Real-Time RT-PCR Assay for Detecting of Rift Valley Fever Virus

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Rift Valley fever (RVF) is viral infectious disease caused by RVF virus belonging to genus Phlebovirus, family Phenuiviridae. The symptoms of RVF commonly include a bleeding and fever and can progress into severe condition such as high fever, low blood pressure, multiple organ failure and even death in a few patients. Since there is no effective vaccine or therapeutics for human, suspected or confirmed patients are depend on the supportive treatment. Therefore, accurate and rapid diagnosis is essential to prevent the spread of RVFV and reduce expected outcomes as like deaths.
This study aimed to develope genetic diagnostic method for simultaneously detecting M and S segment of RVFV. We designed two specific primer/probe sets corresponding to M or S segment, and conducted one-step duplex real-time RT-PCR assay. *In vitro* transcribed full length M or S segment RNA of 7 genotypes was employed for analytic evaluation of developed assay. LOD was calculated to be 10–10^2 copies/μl for S segment and 10 copies/μl for M segment, respectively, showing similar functional aspect on sensitivity to commercial RVFV RT-PCR assay. Additionally there was no cross-reactivity with the other RNA viruses, including CCHFV, LV, SFTSV, Hantavirus, etc. These results demonstrate that the developed real-time RT-PCR assay could be applicable for diagnosis and surveillance analysis of RVFV.
[Supported by intramural funds (2017-NI53004-02, 4837-301-210-13) from the National Institute of Health, KCDC]

Keywords: Rift Valley fever virus, Real-Time RT-PCR

J009

Study on Improvement of Shiitake (*Lentinula edodes*) Bottle Cultivation

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Various edible and medicinal mushrooms are produced using bottle cultivation method. On the other hand, Shiitake mushrooms (*Lentinula edodes*) are with the usage of plastic bags. They cause serious environmental problems, so it is important to reduce plastic bag usage while improving mushroom quality and productivity to improve cultivation methods. In this study, plastic bottle (1,000 ml capacity) was used to cultivate shiitake. Oak tree sawdust media were filled in plastic bottles and autoclaved at 100°C for 60 min and then 121°C for 90 min. Inoculated media were cultivated for 80 days, 100 days or 120 days. During the cultivation period in light condition, the sides of bottles were covered with black vinyl to block light transmission because minimizing lateral and bottom fruiting is important to succeed bottle cultivation method. After the incubation, the media showed shown the possibility of shortening incubation period. However, there was a disadvantage that the media were not suitable for multiple fruiting. Covering side was found to be more effective with shorter incubation periods. The strain suitable for column type media showed higher productivity than the other strain that suitable for block type media. In this study, we could observe inhibition of lateral fruiting and reduction of incubation period, so we should be select and develop strains suitable for bottle cultivation.

Keywords: Bottle cultivation, Lateral fruit, Light transmission, Shiitake

J010

The Value Evaluation of Actinobacterial Resource Based on Microbial Products

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Microbial natural products have continued to play an important role in the discovery of novel chemicals for the development of important therapeutic agents. Actinomycetes produce diverse secondary metabolites which have the primary importance in medicine, cosmetic, agriculture and food production. In this research, actinomycetes as the useful resource were isolated from multiple soil and were analysed 16S rRNA sequences, enzymes (protease, amylase, lipase, CMCase) and antimicrobial activities, culture conditions depend on temperature, pH and LC/MS profiles. Ultimately, we gather and supply not only actinomycetes secondary metabolites, but also their information of valuable characteristics and new functions, so that the researches can speed up the development of bio-R&D, strengthen the competitive power of bio-industry.
[This research was supported by a grant (NRF-2013M3A9A5076601) from A study on the strategies of improving the value of microbial resources funded by Ministry of Science, ICT and Future Planning of the Korea Government.]

Keywords: Microbial resources, Actinomycetes, Microbial products, Mass distribution

J011

Korea Mushroom Resource Bank (KMRB) with Great Value

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The Korea Mushroom Resource Bank (KMRB) was established as a national research resource bank in 2015, supported by the Ministry of Science, ICT and Future Planning. The main goals of the KMRB is to secure fungal biodiversity, to conserve an important fungal biological resource, especially mushroom-forming Basidiomycota, and to provide useful resources to research institute, company and university. KMRB has not only preserved 1631 isolates and 756 spore prints but also retained the dried specimen and genomic DNA. A various types of mushroom resources has been deposited in KMRB: a medicinal mushroom (e.g., *Fomitopsis* and *Ganoderma*), which is known to have antitumor and immune-stimulant properties, edible mushrooms (e.g., *Hypsizygus*, *Lentinula*, and *Lentinus*), which have nutritional and culinary values, and decaying mushrooms (e.g., *Irpex* and *Phanerochaete*), which present excellent enzyme activity. As the values of mushrooms increase, the role of KMRB has grown in importance. The crucial tasks of KMRB are as follows: 1) investigating the mushrooms in natural environments across South Korea to make a list of mushroom diversity, 2) establishing a resource management system for accurate identification of mushrooms, 3) assessing the availability of the discovered mushrooms, and 4) setting up a secure preservation and ordering system.
[This work was supported by Korea Basidiomycota Resources Center of the National Research Foundation (NRF-2015M3A9B8029237)].

Keywords: Basidiomycota, Fungal diversity, KMRB, Mushroom

J012

Fecal Microbiota by Enterotype and Gender in Korean Young Adolescents

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Enterotype is a classification based on the dominant group of bacteria present in the human intestine. In this study, the fecal microbiota of Korean young adults (n=62; ages 20–35 years) was analyzed. The standardized protocol of the Earth Microbiome Project was used based on 16S *rRNA* sequencing technology (Illumina MiSeq). Enterotypes of Korean young adults were clearly classified into two groups (*Prevotella*-dominant group or *Bacteroides*-dominant group). There were significant differences in microbial composition in accordance with enterotype and gender. The *Bacteroides*-dominant group showed more differences in microbiota composition according to gender, in relation to the *Prevotella* dominant group. This study will provide profiles of Korean young adults gut microbiota based on enterotype and gender. Additional studies are needed to determine functional differences by enterotypes and gender-based on functional metagenomics studies.

Keywords: Enterotype, Korean young adults, Prevotella-dominant group, Bacteroides-dominant group

J013

Preliminary Study on the Symptoms and Toxic Substances of Poisonous Mushrooms Based on Literatures in Korea

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Special Forest Products Division, National Institute of Forest Science

Mushrooms play an important decomposer role in the ecosystem, and currently 1,900 species are known in Korea. Among them, there are 517 kinds of edible mushrooms, 204 kinds of medicinal mushrooms, and 243 kinds of poisonous mushrooms. Mushrooms found in forests grow together with edible mushrooms and poisonous mushrooms, resulting in the ingestion of mushrooms and poisoning or death. In the past 10 years, about 30 cases of poisoning have occurred annually, so it is necessary to sort out the similarities and differences between poisonous mushrooms and edible mushrooms. The purpose of this study was to search the literature on the classification of poisonous mushroom poisoning accidents in Korea to help to understand the poisonous characteristics of poisonous mushrooms. Though this, we want to understand the toxic substrate of poisonous mushroom and use it as a basic data of the possibility of using it as a functional substance.

Keywords: Poisonous mushroom, Mushroom poisoning accidents, Toxic substrate, Functional substance

J014

Activation of Endophytic Bacteria Useful for Plants by Atmospheric Plasma Treatment

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National Fusion Research Institute

The application of Plant growth promoting bacteria (PGPB) to the field as a microbial fertilizer requires consistent efficiency. To overcome this limitation of PGPB, we investigated the potential of micro Dielectric Barrier Discharge (DBD) plasma to increase the bacterial activity of a PGPB, *Klebsiella pneumonia,* KW7-S06. Bacterial proliferation and vitality increased after plasma treatment. The infrared band of the observed FTIR spectrum showed no significant difference in plasma treated bacteria and untreated bacterial cell membranes. These results demonstrate that cells are not damaged by oxidative stress caused by plasma treatment. Germination rates of rice and barley seeds inoculated were significantly increased, the growth of rice plants was also improved. The level of SA (Salicylic Acid) hormone was higher in rice plants infected with plasma treated than with untreated bacteria. Our results demonstrated that plasma can accelerate bacterial growth and vitality, and the increased bacteria improved the adhesion of plant seed surfaces and elevated the level of phytohormones, leading to the enhancement of plant growth and tolerance to disease.
[Supported by grants from ‘Plasma Advanced Technology for Agriculture and Food (Plasma Farming)’ program through the National Fusion Research Institute of Korea (NFRI).]

Keywords: Plant growth-promoting bacteria (PGPB), Micro Dielectric Barrier Discharge (DBD) plasma, KW7-S06, FTIR, Rice, Barley, Germination rate

J015

Development of Direct PCR Polymerase and Molecular Diagnostic System for the Detection of Pathogen

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Direct PCR is rising as one of the most useful molecular techniques applied in many fields of biological research and diagnostic. We investigated the PCR amplification directly from minute amount of samples without undergoing DNA isolation process. Environmental samples such as soil or water may be contaminated with microorganisms, the direct PCR allows the identification of potential pathogens and provides a rapid tool to evaluate the risk assessment. The direct PCR analysis was detection of five pathogenic bacteria (*E. coli*, *Salmonella* sp., *Staphylococcus aureus*, *Bacillus cereus*, *Listeria* sp.) using specific primers of functional genes. The direct PCR was carried out using universal primers and standard cycling conditions. Our data resulted in a high-quality yield with clear band profile of PCR products observed after agarose gel electrophoresis. Each sample was feasible to successfully amplify constitutive genes by direct PCR independently from target templates. Implementation of molecular diagnostic system was developed and validated on a wide range of templates including bacterial, plant species, animal tissues and different kinds of environment samples. its implementation will offer new insights for several investigations biomedical diagnosis, plant biotechnology, as well as in applied environmental and food microbiology.

Keywords: Direct PCR, Molecular diagnosis, Molecular detection

J016

Screening and Selection of Endophytic Bacteria against Phytophthora Root Rot in *Ligularia fischeri*

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Cultivation of a wild vegetable, *Ligularia fischeri,* called *gom-chwi*, is usually hampered by *Phytophthora drechsleri* causing the Phytophthora root rot. For the biological control strategies of Phytophthora root rot, a total of 122 bacterial strains were isolated from root of gom-chwi. Out of the 122 strains, 21 strains effectively inhibited the mycelial growth of *P. drechsleri* by dual culture assay. Subsequently, 8 potentially antagonistic strains were selected based on gom-chwi seedling assay with significant difference compared to the water treated control. Among these 8 strains, seven bacterial strains belonging to the genus *Bacillus* and one strain belonging to the genus *Lysinibacillus* were identified by 16S ribosomal RNA gene sequence analysis. The endophytic bacteria might be candidates as potential biocontrol agents against Phytophthora root rot.
[Supported by grants from Rural Development Administration (Project No. PJ01259502)]

Keywords: Biological control, Endophytic bacteria, *Ligularia fischeri*, *Phytophthora drechsleri*

J017

Expression, Purification, Crystallization of Cystathionine β-Lyase from Multidrug-resistant *Acinetobacter baumannii*

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Today super bacteria seriously threaten humanity. If new antibiotics are not developed for super bacteria until 2050, more people will die from super bacteria than those who die from cancer. *Acinetobacter baumannii* (Ab) is a nosocomial pathogen and one of the multidrug-resistant bacteria. The biosynthesis of methionine is an attractive antibiotic target, based on its importance in protein and DNA metabolism and its absence in mammals. Cystathionine β-lyase (CBL) is involved in the methionine biosynthesis and considered as a new drug target for the development of antimicrobial agents. We performed the gene cloning and the expression, purification, and crystallization of CBL from Ab. The structure will provide the valuable information for the catalytic mechanism and inhibitor study.

Keywords: Cystathionine β-lyase (CBL), Drug target, Antibiotics, *Acinetobacter baumannii*, X-ray crystallography

J018

Structure and Mechanism of Inteins for Protein Splicing

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CRISPR/Cas9 system established a new way to make precise, targeted changes to the genome of living cells without leaving no footprint. Inteins do the same work in proteins, which perform the cleavage and formation of peptide bonds in protein such as protein splicing. The gifted function of inteins can be used in myriad ways from basic science to biomedical and industrial applications. The scaffold of inteins belong to the Hedgehog/intein (Hint) domains, of which core structure contains several two-or-three-strand β-sheets and loops with short α-helices. The conserved structure and mechanism of inteins are systematically studied. The catalytic function of inteins can be used for any protein chemistry and engineering such as protein purification and protein labeling.

Keywords: Protein splicing, Inteins, Crystal structure, Catalytic mechanism

J019

Cell Penetration Assay of Antibiotics with Gram-negative Bacteria

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Gram-negative bacteria have two cell membranes, inner-membrane and outer-membrane with lipopolysaccharide. Small molecules are difficult to cross the outer-membrane, making barrier of developing new antibiotics for these Gram-negative bacteria. Thus, the accumulated concentration of new drug, how efficiently is it penetrated, inside the cell membrane is a critical issue. Gram-negative bacteria, as *Escherichia coli* k-12, were used in this study. The cell culture were grown to an optical density (OD_{600}) is 0.55 roughly and CFUs were determined by spreading culture at that point. There are two kind of control compound. The positive control is ciprofloxacin and negative control is kanamycin. Only compounds that were penetrated in the cell membrane were analyzed with LC-MS/MS. Finally, the accumulated concentration of new drug against target protein were compared with concentration of control compounds.

Keywords: Gram-negative bacteria, Penetration, Antibiotics, Accumulation

J020

Expression, Purification, and Crystallization of Fibroblast Growth Factor 10 from Human and Whale

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The family of Fibroblast growth factors (FGFs) is known for playing an essential role in various cellular signal transductions such as proliferation, differentiation, and migration. FGF10, one of the FGF family members, is a multifunctional signaling growth factor, which regulates mammalian embryonic development and adult tissue homeostasis by binding and activating its receptor (FGFR2b). Binding specificity between FGFs and their receptors (FGFRs) is critical for the proper regulation of FGF signaling. Binding specificity between FGF10 and FGFR2b is essential for performing its functions. In this study, we tried to FGF10 make stable. The FGF10 gene from *Human* and *Whale* was cloned and its protein was overexpressed in *E. coli*, and purified. To further understand the structure of FGF10, the crystallization of FGF10 is ongoing to solve the structure.

Keywords: Fibroblast growth factor 10 (FGF10), X-ray crystallography

J021

Expression and Purification of FGF10 from Human and Whale

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FGF family members possess broad mitogenic and cell survival activities and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. FGF10 is also one of the major markers of the early cardiac progenitor cells and a crucial regulator of differentiated cardiomyocyte proliferation in the developing embryo, and is also implicated to be a primary factor in the process of wound healing. We expressed and purified FGF10 proteins of human, mink whale, and killer whale. Crystallization of purified FGF10 proteins is being carried out.

J022

Structure and Function of UDP-Glucose Pyrophosphorylase (UGPase) from *Pseudomonas aeruginosa*

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Antibiotic resistant super-bacteria and lack of effective antibiotics are becoming a global concern. *Pseudomonas aeruginosa* also resist multi-drug, antibiotics, so new bacterial target is essential in a broad range of bacteria and a tight and conserved inhibitor binding site.
UDP-glucose, synthesized from bacteria by UDP-glucose pyrophosphorylase (UGPase), is an essential metabolite in the various processes of cells in all organisms and there is no homology between eukaryotic and prokaryotic UGPases. UGpase gene from *Pseudomonas aeruginosa* used pET system for over expression of PaUGPase in *E. coli*, and purified. It was purified with a Ni-NTA column 1st, and anion column for 2nd purification. We try to make PaUGPase crystal for structure study. The study of PaUGPase structure will be useful to develop antibacterial agents and define mechanism.

Keywords: UDP-glucose pyrophosphorylase (UGPase), Drug target, Antibiotics, *Pseudomonas aeruginosa*

J023

Development of Rapid and Highly Sensitive Loop-mediated Isothermal Amplification Primer Set for the Detection of Human Astrovirus

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Human *Astrovirus* (HuAstV) identified in 1975 and classified into the genera *Mamastrovirus* and *Avastrovirus*. Genus *Mamastrovirus* species can infect mammals, including humans. HuAstV are mainly causes of diarrhea, followed by nausea, vomiting and fever. Transmission of HuAstV is fecal-oral route and has been associated with foodborne, waterborne outbreaks. In this study, we designed three loop-mediated isothermal amplification (LAMP) primer sets, one LAMP primer set was selected based on specificity and sensitivity test. To validation of positive LAMP reaction, restriction fragment length polymorphism (RFLP) assay were developed. Amplification of 223 base pair using the LAMP outer primer set with reverse-transcription polymerase chain reaction from HuAstV RNA, and restriction enzyme Hae III used to generate two bands. Developed LAMP primer set will be useful for detection of HuAstV from various sample.
[This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT).]

Keywords: Human astrovirus, Virus, Loop-mediated isothermal amplification, Waterborne virus

J024

Inhibition of Influenza Virus Infection by *Poncirus trifoliate* Seed Targeting Virus-mediated Cellular Pathway

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The influenza virus is a major infectious disease that causes public health threats. Despite currently being treated with commercially available antiviral drugs, the emergence of resistance variants to antiviral agents requires the development of novel and effective antiviral agents.
Natural products such as traditional herbal medicines have been used as a major source of antiviral development, and numerous researchers have focused on developing new antiviral agents using natural products. *Poncirus trifoliata* (PT) is widely used in oriental medicine as a treatment for gastritis dysentery, inflammation, and digestive ulcers. Also, many studies have been reported various pharmacological effects of PT.
In this study, we investigated the potential antiviral activity of PT ethanol extract (PTex) against influenza virus. PTex inhibited the replication of influenza viruses, in particular, oseltamivir-resistant strains. Unlike oseltamivir, PTex exerted a significant inhibitory activity on the cellular penetration pathway of the virus rather than HA-receptor binding. Antiviral effect of PTex has been shown to greatly inhibit the influenza endocytosis by interfering the cellular EGFR/PI3K signaling pathway required for influenza entry.
The potent antiviral effect and novel antiviral mechanism of PTex support the development of effective natural antiviral agents with broad-spectrum activity against influenza and oseltamivir-resistant viruses.

Keywords: Influenza, *Poncirus trifoliata*, Antiviral agent, Natural product

J025

Immunogenicity of Middle East Respiratory Syndrome Virus Subunit Vaccine

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Middle East respiratory syndrome coronavirus is a pathogen that can transmit between humans as well as animals and humans. While high MERS-induced mortality rates in humans and pandemic cases are reported worldwide, no licensed vaccines are available at present. The MERS antigen, MERS-CoV spike protein (eS770), was expressed in the baculovirus/insect cell system. Subunit vaccine formulations with or without the adjuvants were intramuscularly administered into mice at an eS770 dose of 1 μg. The ability to protect against MERS-CoV was confirmed through infection. Compared to the Alum-containing eS770 formulation, the MF59-containing formulation gave higher serum antibody titers against the spike protein of MERS. Induction of neutralizing antibody was additionally affected by the vaccine formulation. Subunit vaccine formulations with MF59 conferred 1.5-fold higher levels of neutralizing antibody against MERS-CoV pseudovirus relative to Alum-containing formulations. Moreover, the MF59 adjuvant induced higher levels of spike protein-specific IgG1 and IgG2a antibodies than Alum. Following challenge with MERS-CoV, the group vaccinated with the eS770 formulation containing MF59 was observed the lowest viral titer in the lung. Histological analysis of lung further revealed the most intact features in the group administered the eS770 vaccine with MF59 adjuvant. eS770 subunit vaccine formulation containing the MF59 adjuvant can be used for a potential vaccine candidate against MERS-CoV.

Keywords: MERS-CoV, Adjuvant, Subunit vaccine, Alum, MF59

J026

PNGM-1; Structure Based Analysis of PNGM-1 and Related Proteins

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Nosocomial infection is one of the most potent threats in the modern health system. Many superbugs were reported to nestle in the hospital due to the over-usage of antibiotics. Carbapenem resistance deserves special note as carbapenem is one of the most successful antibiotics. Over the globe, about 50% of the antibiotics market is occupied by carbapenem and its derivatives[1]. The estimated number of casualties caused by antibiotics exceeds that of cancer by 2050[2]. Until now reported carbapenemases can be sorted into 2 big branches, serine β-lactamase (SBL) and metallo β-lactamase (MBL). The mechanism and origin of SBLs are well understood and documented. Whereas there is little known about the origin and catalytic mechanism of MBLs. Newly discovered from metagenomic research of Papua new guinean deep-sea sediments, PNGM-1 has both RNAse Z activity as well as carbapenemase activity. Its thought to bridge the evolutionary gap between MBLs and RNAse Z.

Keywords: X-ray crystallography, PNGM-1, Metallo β-lactamase, Carbapenemase, Rational drug design

J027

Immunogenicity of Recombinant Baculoviral DNA Vaccine against Middle East Respiratory Syndrome Coronavirus in Mice

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Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel beta coronavirus that has been emerging infectious disease in human. In 2015, the MERS-CoV outbreak occurred with 186 cases in the Republic of Korea. To control the MERS-CoV outbreak, we developed a MERS-CoV DNA Vaccine using the baculoviral delivery system, recombinant baculovirus coated with human endogenous retrovirus envelope (AcHERV). First, we constructed a recombinant baculovirus encoding each of Spike full gene, S1, and RBD genes under the control of CMV promoter, and confirmed expression by western blot in Huh7 cell. To investigate the efficacy of the vaccine, recombinant baculoviruses were immunized in Balb/c mice. All three AcHERV delivering each of MERS-CoV S, S1 and RBD genes elicited a high level of IgG, neutralizing antibody, and IFN-γ secretion. Especially AcHERV-MERS-S1 showed the highest humoral and cellular immune response. Finally, the challenge with infectious MERS-CoV verified the protection results correlated with the neutralized antibody titers. In conclusions, AcHERV baculovirus could be a potential prophylactic vaccine against MERS-CoV.

Keywords: MERS-Cov, Baculovirus, Vaccine, MERS

J028

Prevalence of Colistin Heteroresistance in *Acinetobacter baumannii* Isolated from Humans, Animals and Environment

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Colistin is one of the last treatment options against MDR *A. baumannii* infections, but colistin heteroresistance have been reported increasingly. We investigated the prevalence of colistin heteroresistance in *A. baumannii* isolated from humans, companion animals and environment in South Korea.
Antimicrobial susceptibility test of 13 antimicrobial agents was performed using the broth microdilution or disk diffusion method. Heteroresistance to colistin was determined using population analysis profiles (PAPs).
Among 158 *A. baumannii* isolates tested, only one isolate (0.6%) was resistant to colistin. Sixty-two (39.5%) of the 157 colistin-susceptible *A. baumannii* isolates were revealed as colistin-heteroresistant strains. By specimen origin of human-animal-environment, the prevalence of colistin heteroresistance in *A. baumannii* isolated from humans was 44.1% and 54.2% in pathogens and non-pathogens, respectively. The heteroresistance rates in the isolates from animals and environment were 37.5% and 25.6%, respectively, relatively lower than that from specimens of human origin.
Our data suggests that the presence of colistin-resistant subpopulations seems to be common in colistin-susceptible *A. baumannii* isolates. Colistin-heteroresistant *A. baumannii* would be a significant threat to the treatment of *A. baumannii* infection because these isolates may lead to increase of resistance in *A. baumannii* by suboptimal usage of colistin.

Keywords: Colistinn heteroresistance, *Acinetobacter baumannii*, One Health

J029

Short to Medium-term Insecticidal Evaluation of *Isaria javanica* pf185 against Green Peach Aphid (*Myzus persicae*)

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The short to medium-term insecticidal activity of *Isaria javanica* pf185 was evaluated for four successive weeks against the green peach aphid (*Myzus persicae*) in pepper seedlings. Under two different tents, seedlings were sprayed with a suspension of the entomopathogenic fungi 10^7 conidia/ ml, and 0.05% Tween 80 in distilled water; respectively. Each week, six leaf discs (2 cm diameter) sampled from top, middle, and bottom of three seedlings per tent were placed on moistened filter paper in a Petri dish. The insecticide test consisted of placing 5 neonate aphids on each leaf disc (0.5 mm diameter), and incubated (25 ± 1°C, and 100% RH). Mortality of the aphids was recorded from day 3 to day 6 during each week. Insecticidal efficacy was 20.43, 39.82, 72.32, 66.43, and 70.04%; during weeks 0, 1, 2, 3, and 4; respectively. This work shows a gradual action of the entomapathogen and suggests a preventive use by treating seedlings as soon as the first aphids appear in fields.

Keywords: Aphid, Entomapathogen, *Isaria javanica, Myzus persicae*, Mortality

J030

Characterization of Entomopathogenic Fungi, *Metarhizium anisopliae* FT284 for Microbial Control against Phytophthora Blight and *Thrips palmi*

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Crops suffer damage from diseases and pests during their development. These different plant enemies reduce the yield and quality of the products. Facing these biotic constraints, producers often depend on chemicals that are expensive with adverse effects on the environment, the operator, and beneficial insects. In addition, resistance is developed because of the repeated use of chemicals. In recent decades, the use of microorganisms in crop protection has become a credible alternative because it is environmental eco-friendly. In this study, we investigated characterization of *Metarhizium anisopliae* FT284 for control. *M. anisopliae* FT284 showed > 90% germination rate at 30–35°C and mycelial growth of it was most excellent at 25°C. Dose-response experiment indicated critical concentration of fungal spore required for control. *M. anisopliae* FT284 at 1×10^5, 1×10^6 and 1×10^7 conidia/ml results in 68.9%, 66.1% and 73.9% mortality in adult thrips at 25°C. *M. anisopliae* FT284 is more effective at 30–35°C as 95.6–97.8% than 25°C as 87.8%. When we treated *M. anisopliae* FT284 (1×10^7 conidia/ml) to 3 leafy cucumber pot in field, population of thrips reduced 78.3% compare with control. Average temperature, humidity and light intensity at field were 24.3°C, 56.5% and 1803.9 lum/ft^2.

Keywords: Entomopathogenic fungi, *Metarhizium anisopliae*, Thrips

J031

Insecticidal Activity of Entomopathogenic Fungi, *Isaria fumosorosea* FG340 at Field

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Insecticidal activity of entomopathogenic fungi, *Isaria fumosorosea* FG340 developed at our previous project was evaluated against beet armyworm (*Spodoptera exigua*) and thrips in greenhouse and farm. 1/100, 1/200, 1/500 diluted suspension of granular formulation of *I. fumosorosea* FG340 (1×10^9 conidia/g) was treated at Chinese cabbage inoculated with 3rd instar larvae of beet armyworm in greenhouse and survival larvae were counted every other day for 8 days in June and October 2018. The corrected mortality by *I. fumosorosea* FG340 was 90.3–93.8% in June and 70.1–86.2% in October. Control efficacy of *I. fumosorosea* FG340 against thrips was evaluated in the non-chemical pesticide cultivated pepper greenhouse and field of farm in August to September 2019. We treated *I. fumosorosea* FG340 every 7 days and counted population of thrips in pepper flowers before treatment. *I. fumosorosea* FG340 reduced population of thrips effectively compare with conventional part of farm which managed with natural extracts and other organic materials.

Keywords: Beet armyworm, Entomopathogenic fungi, *Isaria fumosorosea*, Thrips

J032

Characterization of a Novel Agarose-degrading Bacterium Isolated from Freshwater

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Agar is a polysaccharide that is difficult to be decomposed by digestive enzymes, and has been used in food industry such as jelly and other raw materials for a long time. Agar is composed of agarose and agarosepectin. A few organisms that produce enzymes that break down agarose have been reported such as ginseng, invertebrate etc, but most of them are microorganism. These bacteria are called agarolytic bacteria. Most agarolytic bacteria in the literature have been known to be of marine origin and fresh water-derived agarolytic bacteria have been rarely reported. Recently. We isolated a bacterial strain which has a high agrolytic activity from a stream in IKSAN, SOUTH KOREA. For the taxonomic identification of the isolate, the 16S rDNA sequence was determined. When the nucleotide sequence of the 16S rDNA was aligned with sequence available in the gene bank database by using the BLAST W search program, it exhibited the maximum homology (99%) with those of *Cellvibrio fulvus* strain NCIMB 8634, *Cellvibrio fibrivorans* strain R-42 1491 and *Cellvibrio ostraviensis* strain LMG 19434. At present we are conducting experiment about enzymatic characteristics of the purified agarase