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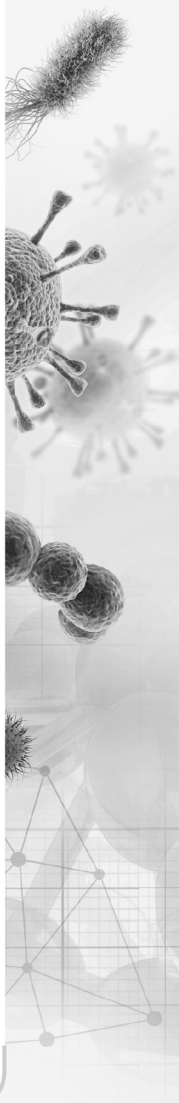

A001

Taxonomic Characterization of *Micromonospora* spp. Isolated from Riverside Soil

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During a survey for the isolation and characterization of rare actinobacteria, 25 strains belonging to the genus *Micromonospora* were isolated from riverside soil samples using humic acid vitamin agar medium. According to phylogenetic analysis of 16S rRNA gene sequences, the isolates formed 10 distinct phylogenetic clusters within *Micromonospora* clade, which could be affiliated to 9 different known species. The most abundant strains were those affiliated to *Micromonospora wenchangensis* (6 strains) and *Micromonospora haikouensis* (5 strains). The isolates developed characteristic deep orange colored colonies, which later turned black with sporulation and with various morphology. All strains grew well on ISP3, ISP7 and starch casein agar medium, and grew optimally at 30-37°C, at pH 8-9 and in 0-1% NaCl. A PCR based analysis was employed to check the presence of biosynthetic gene clusters for secondary metabolites, and the resultant data indicated that over 50% of the isolates contained the biosynthetic gene clusters for non-ribosomal polypeptides and polyketide synthase type I, while those of polyketide synthase type II were less abundant. *Micromonospora* spp. are known to have a high potential as the producers of antibiotics compounds, and thus the ongoing studies include taxonomic characterization and examination of antimicrobial potential.

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

A002

Three New Species of *Nocardioides*, *Nocardioides euryhalodurans* sp. nov., *Nocardioides seonyuensis* sp. nov. and *Nocardioides eburneoflavus* sp. nov., Isolated from Soil

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Aerobic, rod-shaped actinobacterial strains designated MMS17-SY117^T, MMS17-SY207-3^T and MMS17-SY213^T were isolated from soil, and their taxonomic positions were analyzed with a polyphasic approach. The isolates showed best growth at 30℃, pH 7, and 0–1% (w/v) NaCl. On the basis of 16S rDNA sequence similarity, the isolates were affiliated to the genus *Nocardioides*, and the closest species to MMS17-SY117^T, MMS17-SY207-3^T and MMS17-SY213^T were *N. aestuarii* JC2056^T (97.76%), *N. terrigena* DS-17^T (96.83%) and *N. exalbidus* RC825^T (98.71%), respectively. Each isolate formed a distinct cluster within the *Nocardioides* clade in the phylogenetic tree. The major polar lipids for the strains were PI, PG and DPG, and predominant fatty acids were iso-$C_{16:0}$ and $C_{17:1}$ $\omega 8c$. MK-8(H_4) was the major isoprenoid quinone, and LL-DAP the major diamino acid. Galactose, glucose and rhamnose were present in the whole-cell hydrolyzate. The polyphasic data supported the classification of each strain to a new species of *Nocardioides*, and *Nocardioides euryhalodurans* sp. nov. (type strain = MMS17-SY117^T = JCM 32831^T = KCTC 49175^T), *Nocardioides seonyuensis* sp. nov. (type strain = MMS17-SY207-3^T = JCM 32832^T = KCTC 49176^T) and *Nocardioides eburneoflavus* sp. nov. (type strain = MMS17-SY213^T = JCM 32833^T = KCTC 49177^T) are proposed accordingly.

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

Keywords: Actinobacteria, *Nocardioides*, Polyphasic taxonomy, Seonyu island

A003

Two Unrecorded Fungi in Korea: *Mortierella amoeboidea* and *Plectosphaerella pauciseptata*

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During a survey of fungal diversity of order Mortierellales and Glomerellales in Korea, two unrecorded strains, EML-Ge1-3-1 and EML-GW2S7-1 were isolated from a soil sample collected at Geumsan Park located in Jeju and water sample collected at Gwangju stream, Gwangju, Korea, respectively. Based on the morphological characteristics and sequence analysis of the internal transcribed (ITS) regions, the isolates EML-Ge1-3-1 and EML-GW2S7-1 were confirmed as *Mortierella amoeboidea* and *Plectosphaerella pauciseptata*, respectively. BLASTn search of the rDNA ITS sequence via NCBI database indicated that the isolates, EML-Ge1-3-1 and EML-GW2S7-1 matched *M. amoeboidea* (GenBank accession No. KC009083) and *P. pauciseptata* (GenBank accession No. KU751875) with similarity values of 99.3% (555/559 bp) and 100% (490/490 bp), respectively. The colony of EML-Ge1-3-1 was initially white and then changed to pale beige, reaching 39 mm in diameter after 7 days culture at 25℃ on PDA. On the other hand, the colony of EML-GW2S7-1 was initially pale beige, later changed to pale buff. To our knowledge, *Mortierella amoeboidea* and *Plectosphaerella pauciseptata* are unrecorded species in Korea.

[This work was supported by a research grant from the National Institute of Biological Resources (NIBR) and Nakdonggang National Institute of Biological Resources (NNIBR) funded by the Ministry of Environment (MOE) of the Republic of Korea.]

Keywords: *Mortierella amoeboidea*, *Plectosphaerella pauciseptata*, Unrecorded fungi

A004

Description of *Agromyces seonyuensis* sp. nov., Isolated from Island Soil

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A Gram-positive, non-motile, pale yellow colored actinobacterial strain designated MMS17-SY077^T was isolated from an island soil, and its taxonomic position was investigated by using a polyphasic approach. Strain MMS17-SY077^T grew optimally at 30℃, pH 7, and 0% NaCl on R2A agar. Based on the 16S rRNA gene sequence, the isolate was assigned to the genus *Agromyces*, and the closest species were *Agromyces italicus* DSM 16388^T (sequence similarity = 98.76%), *Agromyces allii* UMS-62^T (98.07%) and *Agromyces terreus* DS-10^T (97.79%). The isolate formed a distinct cluster within the *Agromyces* clade in the phylogenetic tree. The main polar lipids were diphosphatidylglycerol, an unidentified phospholipid and and unidentified glycolipid, and an unidentified phosphaminolipid and an unidentified glycolipid were present in minor amounts. The cell-wall amino acids were diaminobutyric acid (DAB), glycine, glutamic acid and alanine. Based on API ZYM, the isolate differed from other related species, for example by the presence of alkaline phosphatase activity and absence of cystine arylamidase activity. The phylogenetic, phenotypic and chemotaxonomic data supported the recognition of the strain as a new species of *Agromyces*, for which the name *Agromyces seonyuensis* sp. nov. (type strain = MMS17-SY077^T) is proposed.

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

Keywords: Actinobacteria, *Agromyces*, Polyphasic taxonomy, Seonyu island

A005

Three New Records Isolated from Freshwater Sample of Wonhyo Valley in Korea

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In this study, six isolates including EML-MSW11-6-2, -MSW11-6-2-1, -MSW242-6, -MSW242-8, -MSW24-4 and -MSW24-4-1 were isolated from freshwater samples collected at Wonhyo valley located in Mudeung Mt, Gwangu, Korea. To accurately identify them at the species level, detailed molecular phylogenetic analysis and morphological study were performed. Phylogenetic analysis of the internal transcribed spacer (ITS) region by BLASTn search indicated that MSW11-6-2, MSW242-6 and MSW24-4 isolates were closest to *Cadophora novi-eboraci* (GenBank accession no. KM497036), *Mycoarthris corallina* (GenBank accession no. AH009124) and *Paraconiothyrium fungicola* (GenBank accession no. KU212362) with identity values of 99.6% (584/586 bp), 99.8% (416/417 bp) and 100% (416/416 bp), respectively. These isolates were well matched with previous descriptions and sequences analyses of ex-type strain. Our study showed that the EML-MSW11-6-2, -MSW11-6-2-1, MSW242-6, MSW242-8, -MSW24-4 and -MSW24-4-1 isolates were unrecorded species, *Cadophora novi-eboraci, Mycoarthris corallina* and *Paraconiothyrium fungicola* in Korea.
[Supported by grants from NNIBR]

Keywords: *Cadophora novi-eboraci*, *Mycoarthris corallina*, *Paraconiothyrium fungicola*, Taxonomy, Undescribed species

A006

Gordonia insulae sp. nov., Isolated from an Island Soil

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A Gram-positive, aerobic, non-motile, mycolic acid containing, pinkish-white actinobacterium designated MMS17-SY073 was isolated from soil of an island. R2A agar was the optimal medium for MMS17-SY073, but the strain could also grow TSA and MA. MMS17-SY073 optimally grew R2A and grew at pH 6.0 and 25℃. The temperature range for growth was 10–37℃ (optimum=25℃), the pH range was 6–7 (optimum=6.0), and NaCl range was 0–8% (w/v, optimum=0%). Based on API ZYM test, MMS17-SY073 was positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, α-glucosidase and β-glucosidase. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showed that MMS17-SY073 belongs to the clade comprising *Gordonia* species. It was most closely related to the type strains of *Gordonia soli* (98.5% similarity), *Gordonia polyisoprenivorans* (98.07% similarity), and *Gordonia hankookensis* (97.79% similarity). The major isoprenoid quinone was MK-9(H_2), and the major polar lipids were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI) and phosphatidyl inositol mannoside (PIM). The whole cell hydrolysates contained galactose, and arabinose. Based on the phenotypic, chemotaxonomic and phylogenetic analysis, MMS17-SY073 should be classified as a new species of the genus *Gordonia*, for which the name *Gordonia insulae* sp. nov. is proposed (type strain=MMS17-SY073).

Keywords: Bacteria, Gordonia, New species

A007

One Undescribed *Aspergillus* Species, *Aspergillus europaeus* Discovered in Korea

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During a survey of fungal species belonging to order Eurotiales, one strain designated as CNUFC-WJC9 was isolated from a corn grain commercially produced from Wanju, in Korea. Based on the results of phylogenetic analysis using beta-tubulin (BenA) and calmodulin (CaM) gene regions and morphological characteristics, the strain CNUFC-WJC9 was found to be new record in Korea, *Aspergillus europaeus* belonging to family Aspergillaceae. The regions of beta-tubulin (BenA) CNUFC-WJC9 and calmodulin (CaM) were matched *A. europaeus* (GenBank accession no. LN909018 and GenBank accession no. LN909019) with identity values of 100% (405/ 405 bp) and 99.6% (507/ 509 bp), respectively. The colony of CNUFC-WJC9 was initially pale yellow and then changed to dark brown. Conidia were globose to subglobose, and measured 3.1–4.6 × 3.2–4.2 µm. Vesicle was pyriform or globose, and measured 11–32 µm. Phialides were ampulliform, and measured 6.4–9.2 × 2.4–3.9 µm. To our knowledge, the species of *A. europaeus* is unrecorded species in Korea.

Keywords: *Aspergillus europaeus*, Corn, Eurotiales, Aspergillaceae

A008

Isolation and Characterization of Two Unrecorded Fungal Species in Korea: *Arcopilus aureus* and *Tetracladium globosum*

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In this study, two unrecorded strains, CNUFC-KMHY6-1 and CNUFC-CPWS-1 were isolated from a soil sample collected at Geumgol Mountain located in Jindo, and water sample collected at a pond in Chonnam National University arboretum, Korea, respectively. Based on their morphological characteristics and sequence analysis of the internal transcribed (ITS) regions, the CNUFC-KMHY6-1 and CNUFC-CPWS-1 strains were identified as *Arcopilus aureus* and *Tetracladium globosum*, respectively. BLASTn search of the rDNA ITS sequences via NCBI database indicated that the isolates matched *A. aureus* (GenBank accession no. KX976582) and *T. globosum* (GenBank accession no. JX029133) with similarity values of 100% (500/500 bp), respectively. Perithecia of CNUFC-KMHY6-1 strain were superficial, ostiolate, globose or oval. Ascospores became more brown when they mature, reniform, 9.2–11.5 x 4.6–6.5 µm, with one or two apical germ pores. Conidia of CNUFC-CPWS-1 strain were globose, hyaline, attaching to the hyphae with short conidiophores, and measured 4.7–6.5 µm in diam. To the best of our knowledge, the species *A. aureus* and *T. globosum* have not been previously described in Korea.

Keywords: *Arcopilus aureus*, *Tetracladium globosum*, Undescribed, Taxonomy

A009

Pseudolabrys koreensis sp. nov., Isolated from Artificial Wetland

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The strain GY_H^T was isolated from artificial wetland in Okcheon, Chungcheongbuk-do Province in Republic of Korea. The strain GY_H^T was closely related to *Pseudolabrys taiwanensis* CC-BB4^T based on 16S rRNA gene sequence (94.9% similarity). The isolate was Gram-negative, catalase-negative, oxidase-positive and its color of colony was white or pale transparent. It could respire both aerobically and anaerobically. It could grow within pH 5~11 and optimum pH was 7. Growth of GY_ H^T in range from 10°C to 45°C was identified. Optimal temperature for growth was 25°C. The strains grew between 0 and 4% NaCl. Also optimal NaCl concentration was 0.5%. Of the substrates tested, GY_H^T was able to utilize D-turanose, D-fructose-6-phosphate, pectin, D-glucuronic acid, glucuronamide, β-hydroxy-D,L butyric acid, acetoacetic acid and formic acid. The major fatty acids were $C_{19:0}$ cyclo ω8*c* (35.75%) and summed features 8 ($C_{18:1}$ ω7*c*/$C_{18:1}$ ω6*c*, 27.36%). The major quinone was Q-10. Diphosphatidylglycerol (DPG), Phosphatidylethanolamine (PE), Phosphatidylglycerol (PG), Phosphatidylcholine (PC) comprised its polar lipids. The G+C content of genome of GY_H^T was 63.28%. On the basis of phylogenetic and phenotypic attributes, we suggest *Pseudolabrys koreensis* as the name of novel species of the genus *Pseudolabrys*.

Keywords: Bacteria

A010

Winogradskyella aestuarii sp. nov., Isolated from a Seawater Sample Collected from the South Sea

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A Gram-stain-negative, oval-to rod shaped, non-motile, aerobic and orange pigmented bacterium, designated strain KYW1333^T, was isolated from seawater of Gwangyang Bay (34°54'41.55"N, 127°41'41.02"E), Republic of Korea. *Winogradskyella aquimaris* DPG-24^T was the nearest neighbor of strain KYW1333^T with 95.99% 16S rRNA gene sequence similarity. Growth occurs at 4–30°C (optimum, 25°C), at pH 6–9 (optimum, pH 7–8) and with 2–5% (w/v) sea salts (optimum, 3%). Flexirubin-type pigments are absent. Catalase-positive and oxidase-negative. The major quinone was menaquinone-6 (MK-6). On the basis of phenotypic-, chemotaxonomic data and phylogenetic inference, strain KYW1333^T should be classified into the genus *Winogradskyella*, as a member of a novel species, for which the name *Winogradskyella aestuarii* sp. nov. is proposed. The type strain is KYW1333^T (=KCTC 62354^T = JCM 32500^T).
[This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea. It was also supported by the CK (university for Creative Korea)-I]

Keywords: *Winogradskyella*, Seawater

A011

A Marine Bacterium Representing a Novel Species in the Genus *Polaribacter*

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A Gram-stain-negative, non-motile, aerobic and yellow pigmented bacterium, designated strain WD7^T, was isolated from seawater collected in the South Sea, Republic of Korea. Strain WD7^T grow at 10–35°C (optimally at 30°C) and 2–6% (w/v) sea salts (optimally at 4%). Catalase- and oxidase-positive. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain WD7^T formed a distinct lineage within the genus *Polaribacter* and was closely related to *Polaribacter reichenbachii* 6Alg 8^T (96.88% 16S rRNA sequence similarity). The predominant fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$. The only respiratory quinone is menaquinone 6 (MK-6). On the basis of phenotypic, chemotaxonomic data and phylogenetic inference, strain WD7^T should be classified into the genus *Polaribacter*, as a member of a novel species, for which the name *Polaribacter lutea* 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. It was also supported by the CK (university for Creative Korea)-I]

Keywords: Polaribacter, Marine bacterium

A012

Korean Novel Indigenous Bacterial Species belonging to the Phylum *Bacteroidetes*

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This study aimed to assay the taxonomic hierarchy of the phylum *Bacteroidetes* and to understand the isolation and classification state of the phylum *Bacteroidetes* with valid names for Korean indigenous isolates. At the time of this writing, the phylum *Bacteroidetes* consists of 5 classes, 7 orders, and 41 families. Until Mar. 2018, 450 species assigned within the phylum *Bacteroidetes* which originated from Korean territory were approved. All species were affiliated with four classes (*Bacteroidia*, *Cytophagia*, *flavobacteriia,* and *Sphingobacteria*), four orders (*Bacteroidales*, *Cytophagales*, *Flavobacteriales*, and *Sphingobacteriales*), 10 families, and 52 genera. Families *Flavobacteriaceae* (230 species) and *Sphingobacteriaceae* (76 species) were abundant. Twelve novel genera were created first with Korean indigenous isolates. A large number of Korean indigenous *Bacteroidetes* species were isolated from natural environments such as air, soil, seawater, tidal flat sediment and fresh-water. In addition, a considerable number of species were isolated from sea animals, marine algae and solar saltern.
[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.]

A013

Novel *Proteobacteria* Species Originated from Various Environments in Korea

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This study focused on the isolation and classification state of the phylum *Proteobacteria* with valid names for Korean indigenous isolates. *Proteobacteria* species isolated from various environments in Korea have been reported from 1999, and 644 species have been approved until Feb. 2018. All *Proteobacteria* species were affiliated with four classes (*Alpha-, Beta-, Epsilon-* and *Gamma-proteobacteria*), 27 orders (including two unclassified orders), 69 families (including four unclassified families), and 258 genera. Three hundred and four species belonged to the class *Alphaproteobacteria*, 257 species to the class *Gammaproteobacteria*, 82 species to the class *Betaproteobacteria*, and only one species to the class *Epsilonproteobacteria*. Eighty-six novel genera were created first with Korean indigenous isolates. Most of the Korean indigenous *Proteobacteria* species were isolated from natural environments such as soil, seawater, tidal flat sediment, marine organism, seashore sand, fresh-water and solar saltern and salt lake, and a number of species were also isolated from artificial resources such as fermented foods, wastewater, compost and water-cooling systems. Several species were associated with animals and plants.

[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.]

A014

Two New Records of Undescribed Species from Freshwater and Saline Water in Korea

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During a survey of fungal species belonging to the class Sordariomycetes, two strains designated as CNUFC-YJS7 and CNUFC-BCSM3 were isolated from freshwater and saline water samples collected at Yeosu, Jeonnam province, and Dadepo beach located in Busan, Korea. Based on their morphological characteristics and phylogenetic analysis of the internal transcribed spacer (ITS-rDNA), 28S rDNA (large subunit) sequences and beta-tubulin, the isolates, CNUFC-YJS7 and CNUFC- BCSM3 were identified as *Mariannaea fusiformis* and *Collariella robusta*. Colony of CNUFC-BCSM3 is white on PDA (Potato dextrose agar), turn from white to black like mycelium when grown on OA (Oatmeal agar) media and shows ascospores broadly limoniform. The other isolate, CNUFC-YJS7 shows purple color on PDA and fusiform to subglobose conidia. The designated strains, CNUFC-YJS7 and CNUFC- BCSM3 were placed in order Hypocreales and order Sordariales in the Sordariomycetes class, respectively. Our study shows that the two strains are unrecorded fungal species in Korea.

Keywords: *Mariannaea fusiformis*, *Collariella robusta*, Freshwater, Saline water, Sordariomycetes

A015

Genetic Distance of RNA Dependent RNA Polymerase May Affect Coupling of ORF2 in Norovirus Genogroup II P16

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In this study, we collected partial RdRp nucleotide sequence dataset (the 3'-end of ORF1; 674 nt length; n=779) of norovirus GII.P16 from web database; GenBank. Maximum clade credibility tree analysis showed several sub-lineages (alpha, beta, gamma etc.). For analytical feasibility, we focused on alpha (70 sequences) and beta (71 sequences) sub-lineages isolated in 2010–2014. GII.2 was the most ORF2 genotype combining with alpha sub-lineage sequences (68 sequences); ORF2 genotypes in beta sub-lineages had more diverse composition (GII.2, 20 sequences; GII.3, 22 sequences; GII.13, 26 sequences; GII.16, 2 sequences; GII.17, 1 sequence). Group mean nucleotide distances showed that beta sub-lineage was 11.7 nucleotides/group; alpha (GII.2 ORF1 strains) sub-lineage had less 9.4 nucleotides/group. To confirm whether the difference of genetic distances between the alpha (GII.2 ORF1 strains) and the beta had been occurred in on yearly basis also, we plotted distance graphs showing nucleotide distances between an "GII.P16 origin sequence (isolation date; 1975)" and each sequence of the two sub-lineages according to the isolation years. The sequences in the beta sub-lineage showed wider nucleotide distance ranges in each year than those in the alpha sub-lineage. According to these data, our findings suggested that genetic distance of a sub-lineage in GII.P16 may related with diversification of VP1 genotype through ORF1/2 intergenotypic recombination.

Keywords: Norovirus, RdRp, Intergenotypic recombination, Genetic distance

A016

Characteristics of Norovirus Genogroup II Epidemic Variants in Generation of ORF1/2 Intergenotypic Recombinants

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In this study, we amplified ORF1/2 junction (1.0 kb) of norovirus GII in water samples (2014–2018; n=262) harvested from environmental areas neighboring human communities in South Korea. By using both RDP4 and Simplot programs, two ORF1/2 intergenotypic recombinants were identified; GII.P16/GII.4 and GII.P7/GII.4. We found that parental sequences of these recombinants were GII.Pe/GII.4, GII.P16/GII.13, GII.P16/GII.2, and GII.P7/GII.6, which were believed to had coexisted in human community for multiple years. Maximum clade credibility analysis revealed that GII.P16 and GII.P7 lineages have had several sub-lineages classified by following criteria; "prone/not-prone to ORF1/2 intergenotypic recombination". Additionally, we identified two intra-capsid recombinants; GII.17 and GII.8. These recombinants had replaced shell domain partially with those same- or different genotype. Our findings reveal that 1) large-scale epidemic variants have been "capsid gene (ORF2; VP1) donor" to create intergenotypic ORF1/2 recombinant; 2) small-scale epidemic variants could be "polymerase (ORF1; RdRp) gene donor"; 3) viral lineages maintained for multiple years in human community seem to be essential for occurrence of new intergenotypic ORF1/2 recombinant; 4) there were "ORF1/2 intergenotypic recombination prone sub-lineages" in GII.P16 and GII.P7 RdRps.

[This research was supported by National Research Foundation of Korea (NRF); (Project No. 2017R1D1A1B03029973)]

Keywords: Norovirus, Intergenotypic recombinant, Environmental water

A017

Candidate of New *Pogonoloma* Species (*Pseudoclitocybaceae*, Agaricales) in Korea

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A new candidate species of mushroom genus *Pogonoloma* was discovered from Hallasan Mountain in Jeju province during a recent floristic survey. Based on morphological characteristics and molecular identification, our specimen was not matched with previously reported *Pogonoloma* species. The main characters of collected specimens are Tricholomatoid basidiomata, whitish pileus with in rolled margin and covered with soft appressed tomentum, stipe whitish, staining yellowish when injured. Here, we taxonomically described this species as a candidate of new to science. In addition, we provided a morphological comparison between our species and closely related species.
[This research was supported by the Korean National Arboretum (Project No. KNA 1-1-14, 14-2).]

Keywords: *Pogonoloma*, ITS, Taxonomy, Phylogeny

A018

Ottowia oryzae sp. nov., Isolated from Andong Sikhye, a Korean Traditional Rice Beverage

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A Gram-stain-negative, non-spore-forming, non-motile, short rod-shaped bacterial strain designated KADR8-3T, isolated from an Andong sikhye from Andong-si, Gyeongsangbuk-do, Republic of Korea, was characterized using a polyphasic approach. On the basis of morphological, genetic and chemotaxonomic characteristics, it was determined to belong to the genus Ottowia. The phylogenetic similarity based on the 16S rRNA gene sequences indicated the strain formed a clade with of *O. beijingensis* GCS-AN-3T, *O. thiooxydans* DSM 14619T, *O. pentelensis* RB3-7T and "*O. shaoguanensis*" J5-66T, showing the highest with *O. beijingensis* GCS-AN-3T (96.3%). The major fatty acids were $C_{16:0}$, summed feature 3 ($C_{16:1}$ w6c and/or $C_{16:1}$ w7c) and summed feature 8 ($C_{18:1}$ w6c and/or $C_{18:1}$ w7c). The predominant respiratory quinone was Q-8. All polar lipids present were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, two unknown aminolipids and two unknown lipids. The genomic DNA G+C content was 66.80 mol%. These results supported that strain KADR8-3T was clearly distinguished from its closely related species and represents a novel species of the genus Ottowia for which the name *Ottowia oryzae* is proposed. The type strain is KADR8-3T (=KACC 19325T =NBRC 113109T).
[This study was carried out with the support (PJ0135409) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea]

Keywords: *Ottowia oryzae*, New taxa, Andong sikhye

A019

Phreatobacter cathodiphilus sp. nov., Isolated from Cathode of Microbial Fuel Cell

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A novel bacterial strain, S-12T, of the genus Phreatobacter was isolated from a cathode of microbial fuel cell, Suwon city, South Korea. The cells were Gram-stain negative, aerobic, non-sporulating rods with a flagellum, and formed white round colonies. It grew at the range of 10–40°C (optimum, 28–30°C), pH 6.0–10.0 (optimum 7.0–8.0) and 0–1% NaCl (not growing at 2% NaCl). The 16S rRNA gene sequence analysis showed relatedness of strain S-12T to *Phreatobacter stygius* YC6-17T (98.2%) and *Phreatobacter oligotrophus* PI_21T (98.1%). The major respiratory lipoquinone was Q-10. Polar lipids consist of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine and an unknown lipid. The major fatty acids were summed feature 8 ($C_{18:1}$ w7c and/or $C_{18:1}$ w6c). The DNA G+C content was 69.25 mol%. Based on its differences from validly published Phreatobacter species, strain S-12T is identified as a new species, for which the proposed name is *Phreatobacter cathodiphilus* sp. nov., with S-12T as the type strain (=KACC 18497^T =JCM 31612^T).
[This study was carried out with the support (PJ0135409) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea]

Keywords: *Phreatobacter cathodiphilus*, New taxa, Cathode

A020

Simplicispira suum sp. nov., Isolated from Dust Collector of Pig Farm

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A novel Gram-stain-negative, strictly aerobic, polar-flagellated and rod-shaped bacterium, designated SC1-8T, was isolated from a dust collector of a pig farm located in Wanju-gun, Jeollabuk-do, South Korea. The strain grew at the range of 4–37°C (optimum 28–30°C), pH 7.0–9.0 (optimum pH 7.0–8.0) and with 0–2% (w/v) NaCl (optimum 0%). Colonies were white-beige, circular and convex after 4 days of incubation on R2A. Based on the 16S rRNA gene sequence analysis, strain SC1-8T was a member of the genus Simplicispira, revealing the highest sequence similarities with *Simplicispira limi* EMB325T (97.9%), *Simplicispira psychrophila* DSM 11588T (97.4%), *Acidovorax defluvii* BSB411T (97.3%), *Simplicispira piscis* RSG39T (97.1%) and *Simplicispira metamorpha* DSM 1837T (97.0%). The predominant respiratory quinone was Q-8. The polar lipids were phosphatidylethanolamone, diphosphatidylglycerol and phosphatidylglycerol. The major fatty acids (>10% of the total fatty acids) were composed of C16:0 and summed feature 3 (C16:1 w6c and/or C16:1 w7c). The DNA G+C content was 63.3 mol%. On the basis of phenotypic, genotypic and phylogenetic evidence, strain SC1-8T is presented as a novel species, for which the name *Simplicispira suum* sp. nov. is proposed. The type strain is SC1-8T (=KACC 19329T =NBRC 113111T).
[This study was carried out with the support (PJ0135409) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea]

Keywords: *Simplicispira suum*, New taxa, Pig farm

A021

Genomic and Phenotypic Characteristics of *Hydromonas* sp. F02 Isolated from Gut of the Aquatic Insect

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Gram-stain-negative, non-motile and catalase- and oxidase-positive bacteria, designated F02^T, were isolated from of gut of *Cincticostella levanidovae* (Tshernova). Growth occurred at a temperature range of 4–30℃, at pH 6–9 and in the presence of 0–0.5% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain F02^T showed highest 16S rRNA gene sequence similarity with the type strain of *Hydromonas duriensis* (96.82%). The major isoprenoid quinone was Q-8. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The major cellular fatty acids were summed feature 3 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c) and iso-$C_{13:0}$ 3-OH. The polyamines were cadaverine and putrescine. The draft genome sequence of F02 is 2,723,407 bp, with a G+C content of 48.3%. Combined data of phylogenetic, phenotypic and chemotaxonomic analyses demonstrated that strain F02^T represent a novel species.

Keywords: Proteobacteria, Burkholderiaceae

A022

First Report of Two *Filosporella* Species Isolated from Freshwater Ecosystem in Korea

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NNIBR

For investigation of aquatic fungi diversity, we collected deposit of soil and plant from pond. NNIBRFG1552 was isolated from soil and NNIBRFG3013 was from plant in Namsaengi-mot in Jeju, Korea on 2016. Based on morphological characteristics and phylogenetical analysis of internal transcribed spacer (ITS), NNIBRFG1552 and NNIBRFG3013 were confirmed as *Filoporella exilis* (100%, KC834046) and *F. fistucella* (99.8%, KC834047), respectively. Neither *Filosporella* genera has previously been reported in Korea.

Keywords: *Filosporella*, Aquatic fungi, Freshwater

A023

Two New Species of the Genus *Candelariella* from China and Korea

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Candelariella is a widespread lineage of lichenized ascomycetes with ambiguous relationships among species that have not solved completely. In this study, several specimens belonging to *Candelariella* were collected from China and South Korea, and the ITS gene was generated to confirm the system position of the newly collected specimens. Combined with a morphological examination and phylogenetic analysis, two sorediate areolate species, *Candelariella rubrisoli*, and *C. subsquamulosa*, are new to science. Detailed descriptions of each new species are presented. In addition, *C. canadensis* is first reported from mainland China.
[Supported by grants from Korea National Arboretum and National Research Foundation of Korea]

Keywords: Lichen, New species, New record, Taxonomy

A024

A Catalogue of Lichen-forming and Lichenicolous Fungi from Dokdo Islands (South Korea) Including New for Science Taxa

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First data on lichen diversity of Dokdo Islands, Republic of Korea based on identification of more than 230 lichen specimens collected in September of 2017 are presented. A catalogue of 45 species of lichen-forming and lichenicolous fungi is reported for the first time for the Dokdo Islands. One new for science lichenicolous fungus of the genus *Bryostigma*, as well as one crustose lichen of the genus *Rufoplaca* are described, illustrated and compared with closely related taxa. The position of new taxa in the phylogenetic tree of the Arthoniaceae based on 12S mtSSU and RPB2 gene sequences is illustrated, while the position of the newly described *Rufoplaca* (Caloplacoideae of the Teloschistaceae) is confirmed by phylogenetic tree based on ITS nrDNA data. Three species, i.e.: *Diplotomma alboatrum*, *Intralichen lichenum*, and *Stigmidium marinum* are reported for the first time for Korea.
[Supported by grants of Korea National Arboretum and National Research Foundation of Korea]

Keywords: Lichen, New species, New record, Taxonomy

A025

New Genera of the Teloschistaceae (Teloschistales, Lichen-forming Ascomucota) Proved by Three Gene Phylogeny

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Five new genera in the family Teloschistaceae (Teloschistales, Lichen-Forming Ascomycota) are described in 2018. New genus *Upretia* S. Y. Kondr., A. Thell & J. S. Hur of the Caloplacoideae including the '*Caloplaca' amarkantakana* clade is characterized by partly pruinose, lobate to subsquamulose, olivaceous grey to brown thallus, small ascospores and narrowly bacilliform conidia. The new genus is closely related to *Ioplaca* Poelt according to phylogeny analysis based on ITS1/ITS2 nrDNA, 28S nrLSU and 12S mtSSU sequences. Two new genera, i.e.: *Hosseusiella* S. Y. Kondr., L. Lőkös et A. Thell for the *Caloplaca chilensis* group including three South American species and *Rehmanniella* S. Y. Kondr. et J.-S. Hur for the new species, *R. wirthii* S. Y. Kondr. from South Africa are described in the subfamily Teloschistoideae. Two more new genera are proposed for species groups of the Xanthorioideae. All new taxa will be illustrated.
[Supported bya grant from National Research Foundation of Korea]

Keywords: Lichen, New Genera, ITS1/ITS2 nrDNA, 28S nrLSU, 12S mtSSU

A026

A Revision of the Lichen Genus *Collema* (Collemataceae, Lichenized Ascomycota) in South Korea

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Collema F.H. Wigg, is rarely scattered throughout in many countries in the East Asian region including South Korea, and nearly from two decades no detailed taxonomic or revisionary study has been done. This study was based on the specimens deposited in the lichen herbarium at Korean Lichen Research Institute, and samples were identified following the recent literature. During the current revisionary study, a total of sixteen species of *Collema* including five new records such as *Collema furfuraceum* var. *luzonense* (Räsänen) Degel., *Collema pulchellum* var. *subnigrescens* (Müll. Arg.) Degel., *Collema rugosum* Kremp., *Collema subconveniens* Nyl., *Collema tenax* (Sw.) Ach., and one variety *Collema leptaleum* var. *leptaleum* Tuck. were documented. Detailed descriptions of each species with their morphological, anatomical and chemical characteristics are given. A key to the all known *Collema* species of South Korea is also presented.

Keywords: Lichen, New record, Variety, Taxonomy

A027

Three New Species of Foliicolous Lichens in the Genus *Strigula* in South Korea

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Foliicolous lichens inhabit the surface of living leaves mainly in the tropics. South Korea has a subtropical climate along its southern coast and on Jeju Island, featuring evergreen broadleaved forest particularly on the latter and thus providing good conditions for the growth of these organisms. Here we report on more detailed collections made recently from Gotjawal Forest. Using molecular data from the nuclear LSU and ITS markers, we identified three species, which remarkably are not identical with any of the previously reported taxa but represent three species new to science. The most common species is morphologically similar to *Strigula smaragdula* and corresponds to material previously identified as *S. concreta*, *S. macrocarpa*, *S. melanobapha*, and *S. smaragdula*. *Strigula smaragdula* s.str. is only distantly related, and so the four names *S. concreta*, *S. macrocarpa*, *S. melanobapha*, and *S. smaragdula* have to be removed from the Korean lichen biota. A second new species is also somewhat similar to *S. smaragdula*, but differs in the numerous, concentrically arranged, black perithecia with depressed top. A third new species corresponds to what had previously been identified with the name *S. subelegans*, which requires to remove that name from the Koran lichen biota as well. Together with studies in China, these results identify Korea and adjacent parts of (sub-)tropical eastern Asia as an evolutionary hotspot for the genus *Strigula*.

Keywords: South Korea, Foliicolous lichens, Strigula, New species, Phylogenetic tree

A028

Cryptococcus cellulosum sp. nov. a Cellulose Degrading Yeast Isolated from the Gut of Grasshopper

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During our study on the novel microbial community, two yeast strains ON2 and ON17 were isolated from the gut of grasshopper collected in Onam-ri, Gyeonggi Province, South Korea. Phylogenetic analysis based on the D1/D2 domains of the large subunit rRNA gene and the internal transcribed spacer (ITS) regions revealed that the strains are most closely related to *Cryptococcus laurentii* (FN689393). In YM broth, the colonies were ovoid to elongate, smooth and cream colored after grown for 2 days at 25°C. Growth was observed at 15°C, 25°C and 37°C but not at 40°C. Growth on YM agar with 10% sodium chloride was negative. The major ubiquinone was Q10. The growth was observed with 50% glucose and 1% cycloheximide. The ability to produce starch and cellulose degradation made the strains differ from their closely related species. Based on sequence analysis and physiological characteristics, strains ON2 (= KCTC 27805) and ON17 (= KCTC 27806) represents a novel species in the genus *Cryptococcus*, for which the name *Cryptococcus cellulosum* sp. nov. is proposed.
[This work was supported by a grant from the 'National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201839201). 2018 Graduate Program of Undiscovered Taxa.]

Keywords: *Cryptococcus* species, Yeast, Grasshopper, rRNA

A029

A Novel *Alsobacter* Species, Strain D20^T, Isolated from a Shallow Stream Sediment

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A novel Gram-stain-negative, non-motile, and aerobic rod-shaped bacterium, designated strain D20^T, was isolated from a shallow stream sediment in Shinan-gun, South Korea. Growth occurred at 15–40°C (optimum 30°C), at pH 6.0–8.0 (optimum pH 7.0), and in 0–1% NaCl (optimum growth occurred in the absence of NaCl). The phylogenetic analysis based on the 16S rRNA gene sequences showed that strain D20^T belonged to the genus *Alsobacter*, a member of the order *Rhizobiales*, with the highest sequence similarity to *Alsobacter metallidurans* SK200a-9^T (96.2%), followed by *Methylosinus sporium* NCIMB 11126^T (94.9%), and *Methylosinus trichosporium* OB3b^T (94.6%). The major cellular fatty acids (>5% of the total) of strain D20^T were summed feature 8 ($C_{18:1}\ w7c$ and/or $C_{18:1}\ w6c$), $C_{18:1}\ w7c$ 11-methyl, $C_{16:0}$ and summed feature 3 ($C_{16:1}\ w7c$ and/or $C_{16:1}\ w6c$). The major respiratory quinone was Q-10. Based on the phylogenetic analysis and physiological and biochemical characterization, strain D20^T represents a novel species of the genus *Alsobacter*. The type strain is D20^T (=KACC 19718^T).

Keywords: *Alsobacter*, Sp. Nov., Dankook University

A030

Rhodotoralla cellolyticus sp. nov. a Cellulose Degrading *Basidiomycetes* Yeast Isolated from the Gut of Grasshopper

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[1]Kyungpook National University, [2]Seoul Women's University, [3]Korea University

Strains ON15 representing a species of *Rhodotoralla* were isolated from the gut of grasshopper collected in Onam-ri, Gyeonggi Province, South Korea. Phylogenetic analysis based on the D1/D2 domains of the large subunit rRNA gene and the internal transcribed spacer (ITS) regions revealed that the novel species was most closely related to *Rhodotorula taiwanensis* (KY109163) and *Rhodotorula sphaerocarpa* (KY109151). In YM medium, the colonies were ovoid, elongated and cream colored, after 2 days growth at 25°C. The growth was observed at 25, 30 and 42°C. The ability to produce starch like substance, positive to diazonium reaction, cellulose degradation and growth at 0.1% cycloheximide made the strain differ from other species in the genus *Rhodotoralla*. The sequence analysis and physiological characteristics revealed that the strain ON15 is a member of the genus *Rhodotoralla* and the name *Rhodotorula cellolyticus* sp. nov. is proposed.
[This work was supported by a grant from the 'National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201839201). 2018 Graduate Program of Undiscovered Taxa.]

Keywords: *Rhodotoralla* species, Yeast, Grasshopper, rRNA.

A031

Marinomonas DY-2 sp. nov Isolated from Marine Sea Water Collected from the Yeongilman, Pohang, South Korea

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Life Science Major, Division of Bio-convergence, Kyonggi University

A Gram-stanining-negative, motile, aerobic, catalase positive, oxidase negative. Cream color separately in marine agar but together, it has a pale orange color. Rod-shaped bacterial strain, designated DY-2T, isolated from sea-water samples collected off the Yeongilman, Korea and subjected to a polyphasic taxonomic study. On the basis of 16S rRNA gene sequence data, the strains were 93–97% similarity with the seven recognized species of Marinomonas. The most closely related species were *Marinomonas rhizomae* and *Marinomonas polaris*, with 98.6 and 98.2% similarity at the 16S rRNA gene sequence level, respectively. Major fatty acid C16:0, C16:1v7c and C18:1v7c Phosphatidylethanolamine and phosphatidylglycerol were identified as the predominant phospholipids. Growth range condition of temperature was 10°C to 35°C and pH 7 was the best PH condition in marine agar. All the above characteristics support the affiliation of strain DY-2T to the genus Marinomonas. Differential phenotypic properties showed that strain DY-2T can be differentiated from other Marinomonas species. On the basis of the data presented, strain DY-2T is considered to represent a novel species of the genus Marinomonas, for which the name *Marinomonas* DY-2 sp. nov. is proposed. The type strain is DY-2T.

Keywords: Marinomonas, Gammaproteobacteria, Oceanospirillales, Marine bacteria

A032

Candidate of New *Melanophyllum* Species (*Agaricaceae*, Agaricales) in Korea

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Division of Forest Biodiversity, Korea National Arboretum

A new candidate species of Macrofungi genus *Melanophyllum* was discovered from Korea. Based on morphological characteristics and molecular identification, our specimens were not matched with previously reported *Melanophyllum* species. Morphological features of our specimens are Lepiotoid, pileus and stipe covered with an epithelium. Lamellae free from stipe, strong purplish red when fresh, with lamellulae. Here, we taxonomically described this species as a candidate of new to science. In addition, we provided a morphological comparison between our species and closely related species.
[This research was supported by the Korean National Arboretum (Project No. KNA 1-1-22, 17-2)]

Keywords: *Melanophyllum*, New species

A033

Algoriphagus F21 sp. nov. Isolated from Spoil of the Yeongilman, Pohang, Korea

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Gram-negative, non-motile, non-spore-forming, rod-shaped strain. Microorganism grew optimally at 37°C, pH 7–8 and in the presence of 2–5% (w/v) NaCl. The stain F21 did not grow without NaCl or in the presence of >8% (w/v) NaCl. Strain F21 was characterized chemotaxonomically as having MK-7 as the predominant isoprenoid quinone and iso-C15:0 as the major fatty acid. The strain F21 showed a 16S rRNA gene sequence similarity value of 98.13% with *Algoriphagus marincola* (KCTC = 12181). On the basis of phenotypic and chemotaxonomic properties and phylogenetic distinctiveness, strain F21 should be placed in the genus Algoriphagus as members of a novel species.

Keywords: *Algoriphagus, Sphingobacteria, Sphingobacteriales*, Marine bacteria

A034

Discovery of Four Rare Coprophilous Zygomycetes from Korea

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In this study, four rare zygomycete fungi were isolated from specific niches including dung samples of praying mantis and amphibians in Korea. Four rare fungal strains, CNUFC-MID1-1, CNUFC-FF1-1, CNUFC-FF2-3, and CNUFC-FF3-3, were isolated using direct plating method. Sequence analysis by BLASTn search indicated that the isolates, CNUFC-MID1-1, CNUFC-FF1-1, CNUFC-FF2-3, and CNUFC-FF3-3 were closest to *Mucor* sp. INBio2958 (GenBank accession no. GU827502), *Choanephora cucurbitarum* (GenBank accession no. MF942131), *Blakeslea trispora* (GenBank accession no. LN609582), and *Mucor ellipsoideus* (GenBank accession no. NR_111683) with identity values of 94.1% (554/589 bp), 99.7% (590/592 bp), 99.6% (517/519 bp), and 99.5% (568/571 bp), respectively. On the basis of their morphological characteristics and phylogenetic analysis of their internal transcribed spacer regions and 28S rDNA sequences, the CNUFC-MID1-1 isolate was identified as a new *Mucor* species, named *M. orantes-mantis* sp. nov. The CNUFC-FF3-3 isolate was identified as an unrecorded species, *M. ellipsoideus* in Korea. The CNUFC-FF1-1, and CNUFC-FF2-3 isolates were identified as *C. cucurbitarum*, and *B. trispora*, respectively. To our knowledge, this is the first report of *C. cucurbitarum*, *B. trispora,* and *M. ellipsoideus* from a specific habitat of fecal sample in the world.

Keywords: Morphology, Mucorales, Phylogeny, Rare fungi

A035

Gymnosporangium in China

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Gymnosporangium species are mainly distributed in the northern hemisphere. Approximately 61 species of *Gymnosporangium* have been reported worldwide, and 16 species have been recorded from China. Most *Gymnosporangium* species are heteroecious and demicyclic. They usually produce telia on the needles, stems, and branches of Cupressaceae and aecia on the leaves and fruits of plants belonging to Maloideae, causing significant economic losses. Because there is no correlation of the occurrence time of aeciospores and teliospores as well as their hosts, so many taxonomists regard the characteristics of aeciospores and teliospores as independent taxonomic basis for the classification of species. Only a few taxonomists have determined the life cycle of some *Gymnosporangium* species by artificial inoculation. On the basis of the correlation of aeciospores and teliospores, they combined with the morphological characteristics of aeciospores and teliospores to classify the species. Thus, species and their interspecific relationships identified by morphological observation are still very confusing and the life cycle of most *Gymnosporangium* species is not clear, which leads to a lot of problems to taxonomy on the species level. Therefore, it is necessary to use phylogeny to determine the life cycle of *Gymnosporangium* and to correctly define the species of *Gymnoporangium* combining morphology and phylogeny. Totally 747 specimens from China are included in the present studies. Morphological characteristics of aecial and telial stages on different hosts are observed under the optical microscope and scanning electron microscope. At the same time, phylogenetic studies based on ITS2, LSU and TEF-1α sequences of *Gymnosporangium* have been conducted.

Keywords: *Gymnosporangium*, Rust fungi, Taxonomy, Phylogeny

A036

Phylogenetic Characterization of Novel *Vitreoscilla* Strain Isolated from Fish Intestine

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A bacterial strain designated FH7-10^T was isolated from intestine of ice-fish (*Hypomesus nipponensis*) collected from Sagimak reservoir in Gangneung-si, Gangwon-do and was examined in detail applying by a polyphasic taxonomic approach. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain FH7-10^T belong to the genus *Vitreoscilla* of the family Neisseriaceae. Strain FH7-10^T is closely related to *Vitreoscilla stercoraria* DSM513^T with a sequence similarity value of 94.5%. Cells are aerobic, ivory-colored and Gram-stain-negative. Growth occurred at 4–37°C (optimum 20–30°C), pH 6–8 (optimum pH 7), within salt range of 0–1.0% (optimum 0%). It contained summed feature 3 (comprising $C_{16:1}$ w7c and/or $C_{16:1}$ w6c), $C_{16:0}$ and summed feature 8 ($C_{18:1}$ w6c and/or w7c) as the major fatty acids. Differential phenotypic properties and phylogenetic distinctiveness suggested that strain FH7-10^T represents a novel species of the genus *Vitreoscilla*.

Keywords: Fish microbe

A037

Identification and Characterization of *Colletotrichum* Species Associated with Bitter Rot Disease of Apple in Korea

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During the years 2015 and 2016, distinct symptoms of bitter rot disease were observed on apples in different regions of Korea. In the present study, infected apples from these regions were utilized to obtain eighteen isolates of *Colletotrichum* spp. These isolates were identified and characterized according to their morphological characteristics and nucleotide sequence data of internal transcribed spacer regions and glyceraldehyde-3-phosphate-dehydrogenase. The result of molecular analyses suggested that the isolates of *Colletotrichum* causing the bitter rot disease in Korea belong to four species: *C. siamense*; *C. fructicola*; *C. fioriniae* and *C. nymphaeae*. *C. siamense* and *C. fructicola* belonged to Musae Clade of *C. gloeosporioides* complex species, and *C. fioriniae* and *C. nymphaeae* belonged to the Clade 3 and Clade 2 of *C. acutatum* complex species. *C. gloeosporioides* species-complex isolates were found to be more aggressive than the isolates in the *C. acutatum* species complex via pathogenicity tests. Our results suggest that morphological characters coupled with molecular phylogenetic analysis and pathogenicity tests can be useful for accurate identification of *Colletotrichum* species.

Keywords: Apple, Bitter rot, *Colletotrichum*, Morphological characters, Nucleotide sequences, Pathogenicity

A038

Multigene Phylogeny and Morphology Revealed *Hyaloperonospora brassicae* Complex Consists of Several Cryptic Species

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Hyaloperonospora (Peronosporaceae; Oomycota) is an oomycete genus that causes downy mildew diseases on Brassicaceae plants, including many economically important crops, such as cabbage, radish, broccoli, oilseed rape. Members of this genus are well known to be obligate biotrophic pathogens, and thus display high levels of species diversity and host-specialization. Several species of three brassicaceous genera, *Brassica*, *Raphanus*, and *Sinapis*, are the host plants of the *Hyaloperonospora*, but the causal agents have been attributed to only a species *H. brassicae*. In the present phylogenetic analyses on ten multigene sequences, including ITS rDNA, LSU rDNA, *cox*1 mtDNA, *cox*2 mtDNA, β-tubulin, *Hsp*, NADH, *rps*, RPB2, and *ypt* gene, however, *H. brassicae* complex parasitic to the specific species of *Brassica*, *Raphanus*, and *Sinapis* has been divided into several distinct clades. In addition, morphological distinction, mainly in size and shape of conidia, supports the phylogenetic inference.
[This study was supported by a grant of the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT & Future Planning (2016R1C1B2008013)]

Keywords: Oomycete, Downy mildew, Brassicaceae, Host-specialization, ITS rDNA

A039

New Report of Six Oomycete Species Isolated from Freshwater Environments of Korea

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Oomycetes are widely distributed in varying environments with different lifestyles. They have evolved with both, saprophytic and pathogenic lifestyles, and execute various strategies depending on their hosts and environment. Of the diverse environments, freshwater ecosystem is one of the most important habitats for members of oomycetes. However, most studies on oomycetes diversity tended to be biased towards pathogenic species, rather than aquatic species, although their role as saprophyte is essential for freshwater ecosystems. In this study, we isolated oomycete strains from soil sediment, plant litter, and algae in freshwater stream of Korea. The strains were identified based on morphological characteristics and molecular phylogenetic analyses of ITS rDNA, *cox*1, and *cox*2 mtDNA sequences. In addition, cultural features including the mycelial growth rate and colony growth pattern were investigated on three different media. As a result, we discovered six oomycete species that have not been reported so far in Korea, namely *Phytophthora lagoariana*, *Phytopythium litorale, Pythium heterothallicum*, *Pythium oopapillum*, *Pythium diclinum*, and *Pythium coloratum*. Diversity and ecology of freshwater oomycetes in Korea are poorly understood. This study could contribute to understand their distribution and ecological functions in freshwater ecosystem.
[Supported by grants from NRF and NNIBR]

Keywords: Oomycete, Freshwater, Taxonomy, Peronosporales, Pythiales

A040

Isolation and Identification of a β-Glucosidase Producing Bacterium, *Chitinophaga ginsengi* sp. nov., from Rhizoplane of Ginseng

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The distribution of β-glucosidase producing bacterial species have been investigated on the rhizoplane of ginseng. A novel β-glucosidase producing bacterium, designated 2MR8^T was shown to belong to the genus *Chitinophaga* in *Chitinophagaceae* according to 16S rRNA gene sequence analysis. Cells of strain 2MR8^T was found to be a Gram-stain-negative, yellow-pigmented, non-motile and rod-shaped bacterium. Strain 2MR8^T grow optimally at 28°C and pH 8.0, and produces β-glucosidase and degraded esculin. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain 2MR8^T belongs to the genus *Chitinophaga*, showing highest sequence similarity to *Chitinophaga arvensicola* DSM 3695^T (97.5%), *C. taiwanensis* CC-ALB-1^T (97.5%) and *C. niastensis* JS16-4^T (97.1%). Levels of DNA-DNA relatedness between strain 2MR8 and the type strains of other species of the genus *Chitinophaga* ranged from 7.7 to 16.9%. The predominant isoprenoid quinone was identified as MK-7. The major fatty acids were identified as $C_{16:0}$ and $C_{16:1}$ $\omega 7c$ and/or $C_{15:0}$ iso 2-OH. The major polar lipids were identified as phosphatidylethanolamine, unidentified phospholipids and unidentified aminophospholipids. On the basis of the phylogenetic, phenotypic and chemotaxonomic analyses in this study, strain 2MR8^T represents a novel species of the genus *Chitinophaga*, for which the name *Chitinophaga ginsengi* sp. nov. (=KACC 19545 =NBRC 113194) is proposed.
[Supported by grants (Project No. PJ01287601) from RDA.]

Keywords: β-Glucosidase, Chitinophaga, Ginseng

A041

Isolation and Characterization of a Novel Aerobic and Extremely Thermophilic Bacterium from Food Sludge Compost

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A novel extremely thermophilic bacterium, strain FW80, was isolated from food waste compost in Jeongeup-si, Republic of Korea. Cells were Gram positive, spore-forming, strictly-aerobic and motile rods with peritrichous flagella. Strain FW80 grew at 50–85℃ (optimum 77℃), pH 6.0–10.0 (optimum pH 7.5) and 0.0–3.0% NaCl (optimum 1.0%). The optimal growth temperature of strain FW80 was higher than that of *Thermaerobacter composti* JCM 15650T (70℃) and showed the highest growth temperature in Genus *Thermaerobacter*. The isolate was heterotrophic and grew on yeast extract and peptone as a nutrient substrate. The G+C content of the genomic DNA was 71.2 mol%, as measured by the thermal denaturation method. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain FW80 was closely related to *T. composti* JCM 15650T (99.7%). However, DNA-DNA hybridization showed that DNA relatedness value for strain FW80 and *T. composti* JCM 15650T was below 19.5%. On the basis of physiological and molecular properties, strain FW80 is considered to represent a novel species of the genus *Thermaerobacter*.

Keywords: *Thermaerobacter*, Thermophilic bacterium, Food waste compost

A042

Bacillus salinus sp. nov., Isolated from Solar Salt

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A novel aerobic, yellowish colored, Gram-stain-positive bacterium, designated strain HMF5848^T was isolated from solar salt developed in Shin-an, Republic of Korea. Phylogenetic determination based on 16S rRNA gene sequences indicated that the isolate was classified in the genus *Bacillus*, exhibiting the highest level of sequence similarity with the type strain of *Bacillus humi* LMG 22167^T (96.1%). Subsequently followed by *B. luteolus* YIM 93174^T (96.0%) *and B. pervagus* 8-4-E12^T (95.6%). The major fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$ The predominant quinone was MK-7. Based on the whole-genome sequences, the G+C content of the strain HMF5848 was 37.4 mol%. On the basis of its phylogenetic, phenotypic, and chemotaxonomic properties, strain HMF5848^T represents novel species of the genus *Bacillus*, for which the name *Bacillus salinus* sp. nov. (=KCTC 43010^T =CECT-ing^T) is proposed.

Keywords: Solar salt, *Bacillus*, 16S rRNA gene

A043

Cellulomonas citreus sp. nov., Isolated from Paddy Soil

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A Gram-stain-positive, facultative anaerobic bacterium, designated AO-9^T, was isolated from paddy soil. Strain AO-9^T was found to be motile rods which can grow at salinities of 0–2% (w/v) NaCl, at pH 3.0-9.0 and at 20–45°C in TSB medium. Phylogenetic analyses based on 16S rRNA gene sequence revealed that strain AO-9^T belongs to the genus *Cellulomonas*, showing the highest sequence similarity to *Cellulomonas marina* FXJ8.089^T (96.5 %), *Cellulomonas gelida* DSM 20111^T (96.2%), *Cellulomonas uda* DSM 20107^T (96.1%) and sequence similarity of the other closely related taxa was below 96%. Level of DNA-DNA relatedness between strain AO-9^T and closely related species in the genus *Cellulomonas* was below 70%. The major cellular fatty acids were identified as anteiso-$C_{15:0}$ (49.9%), $C_{14:0}$ (12.9%) and iso-$C_{14:0}$ (12.1%). The predominant isoprenoid quinone was identified as MK-9 (H_4). The polar lipids were identified as diphosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylinositol dimannosides and one unidentified phospholipid. The cell-wall sugar was found to contain ribose. The DNA G+C content was determined to be 67.3 mol%. Based on its distinct phenotypic, phylogenetic, and chemotaxonomic characteristics, AO-9^T represents a novel species of the genus *Cellulomonas*, for which the name *Cellulomonas citreus* sp. nov. (=KACC 19069^T =NBRC 112523^T) is proposed.
[Supported by grants (Project No. PJ01287601) from RDA.]

Keywords: *Cellulomonas citreus* sp. nov, Paddy soil

A044

Elioraea reseus sp. nov., a Plant Growth Promoting Bacterium Isolated from Paddy Soil

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A Gram-stain-negative, facultative chemolithoorganotrophic bacterium, designated strain PF-30^T, was isolated from paddy soil in south Korea. Strain PF-30^T was found to be a strictly aerobic, motile and pink-pigmented rods which can grow at 25–40°C (optimum, 28°C), at pH 5.0–9.0 (optimum pH 7.0) and at salinities of 0.5–3 % NaCl (optimum 0.5% NaCl). Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain PF-30^T belongs to the genus *Elioraea*, showing highest sequence similarity to *Elioraea tepidiphila* TU-7^T (97.1%) and sequence similarity of the other closely related taxa was below 91.3%. Level of DNA-DNA relatedness between strain PF-30^T and closely related species in the genus *Elioraea* was below 70%. The major fatty acids were identified as $C_{18:0}$ and $C_{18:1}$ ω7*c*. The polar lipids were identified as phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and one unidentified phospholipid. The predominant respiratory quinone was identified as Q-10. The DNA G+C content was determined to be 68.3 mol%. The strain PF-30^T was observed to produce plant-growth-promoting materials such as indole-3-acetic acid (IAA), siderophore and phytase. On the basis of phylogenetic, chemotaxonomic and phenotypic data, we concluded that strain PF-30^T represents a novel species of the genus *Elioraea*, for which the name *Elioraea reseus* sp. nov. is proposed.
[Supported by grants from iMAF (Project No. 918016-4).]

Keywords: *Elioraea reseus* sp. nov, Paddy soil, Plant growth promoting bacterium

A045

A Novel Bacterium of the Family *Rhodospirillaceae* Isolated from Coastal Seawater

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A Gram-negative, catalase- and oxidase-positive, motile and rod shaped bacterium, designated strain HT1-32, was isolated from coastal seawater, Tongyeong of Korea. Colonies were whitish, circular with entire margin, slightly convex and had size of 0.3-0.6 mm on Marine broth 2216E agar plate at 25°C for 7 days. Sodium ion was not essential requirement for growth and optimum concentration was 2.5-3.5%. On API 20NE kit test, reduction of nitrates to nitrites and urease were positive. Phylogenetic analyses based on 16S rRNA gene sequence revealed that strain HT1-32 belonged to the family Rhodospirillaceae, and closely related to *Aliidongia dinghuensis* 7M-Z19^T with 89.7% sequence similarity. The low 16S rRNA gene sequence similarity support the assignment of the strain HT1-32 as a novel species into the family *Rhodospirillaceae*.
[Supported by NRF-2017R1D1A3B04033871]

Keywords: Novel bacterium, *Rhodospirillaceae*

A046

Novel Species Candidate *Cohnella* sp. nov., KS 22, Isolated from a Clinical Specimen in Korea

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Korea National Institute of Health

One of the main businesses of NCCP is the discovery and exploration of novel species pathogen. The aim of this study is resource identification and discovery of a novel species pathogen, among 143 non-identification strains collected from bank branches of NCCP.
Initial identification was performed using matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) with the MALDI Biotyper software (Bruker Daltonik, Germany). The 16S rRNA gene sequence similarity was calculated by comparing with sequences on the EzTaxon server (http://www.eztaxon.org/). A phylogenetic tree was constructed by the neighbor-joining and maximum-likelihood methods with MEGA 6.0. For analysis of biological activity test was perfomed in comparison to reference strains (*Cohnella luojiensis*).
Using 16S rRNA sequencing and MALDI-TOF MS, 62 strains of 46 species were identified. KS 22 was considered as a novel species according to its initial identification by MALDI-TOF MS was inaccurate (not in database). Phylogenetic analysis based on 16S rRNA gene sequences revealed that KS 22 belonged to the genus Cohnella and was closely related to *Cohnella luojiensis* DSM 24270T (97.87%). Biological activity test showed the difference between the reference strains. KS 22 was the possibility of a novel species.
NCCP will continued to have perform discovery of novel species such as pathogen resource and distributes to health and medical treatment researchers.

Keywords: Novel species, *Cohnella* sp.

A047

Sphingomonas ginkgonis sp. nov., Isolated from Phyllosphere of *Ginkgo biloba*

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Strain HMF7854^T, isolated from a ginkgo tree of the Yongin, Republic of Korea, was an orange-pigmented, Gram-staining-negative, motile, strictly aerobic, rod-shaped bacterium. The isolate grew optimally on R2A agar at 30°C, pH 7.0–8.0 and 0.5% NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain HMF7854^T belonged to the genus *Sphingomonas* and was most closely related to *Sphingomonas daechungensis* CH15-11^T (96.5% sequence similarity). The major fatty acids were $C_{17:1}\omega 6c$, summed feature 8 ($C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$), summed feature 3 ($C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$) and $C_{16:0}$. The predominant isoprenoid quinone was ubiquinone-10. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, sphingoglycolipid and one unidentified glycolipids. The genomic DNA G+C content was 68.4 mol%. Thus, based on the phylogenetic, phenotypic and chemotaxonomic data, strain HMF7854^T represents a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas ginkgonis* HMF7854^T sp. nov. is proposed. The type strain of the species is strain HMF7854^T (=NBRC 113337^T=KCTC 62461^T).

Keywords: *Sphingomonas*, Ginkgo biloba, 16S rRNA gene

A048

Agromyces soli sp. nov., Isolated from Soil of a Framing Field

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A Gram-stain-positive, designated strain ANK073, was isolated from a farming field at Daejeon in Korea. Strain ANK073 was found to be an aerobic, non-motile actinomycete which forms branching hyphae and can grow at 15–40°C (optimum, 28–30°C), at pH 6.0–10.0 (optimum, pH 6.5–7.5) and at salinities of 0–1% (w/v) NaCl (optimum, 0% NaCl). Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain BH258^T belongs to the genus *Agromyces*, showing highest sequence similarity to *Agromyces humatus* DC5^T (98.8%), *Agromyces iriomotensis* IY07-20^T (98.4%), *Agromyces neolithicus* 23-23^T (98.3%) and *Agromyces subbeticus* WDSM 16689^T (98.3%). The predominant isoprenoid quinone was identified as menaquinone-7 (MK-11), and the major fatty acids were identified as anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. The DNA G+C content of this novel isolate was determined to be 70.2 mol%. On the basis of the phylogenetic, phenotypic and chemotaxonomic analyses in this study, strain ANK073 is considered to represent a novel species of the genus *Agromyces*, for which the name *Agromyces soli* sp. nov. is proposed. The type strain is ANK073 (= KACC 18681 = NBRC 111825).
[Supported by grants (Project No. PJ01287601) from RDA]

Keywords: Actinobacteria, *Agromyces*, Taxonomy

A049

Genetic Characteristics of *Panellus edulis* Strains and Their Artificial Cultivation on Sawdust Medium

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Molecular analysis using the internal transcribed spacer region sequences revealed that the strains used in this study which are formerly identified as *Panellus serotinus*, are *Panellus edulis*. As a result of Universal Fungal PCR Fingerprinting (UFPF) analysis eight strains of *P. edulis* divided into two groups. We conducted fundamental research on mycelial growth and sawdust cultivation to understand cultural characteristics of eight wild strains of *P. edulis* collected from Korean forests. Mycelial growth of *P. edulis* on sawdust media was faster on *Quercus acutissima* and *Q. mongolica* than the others, and mycelial density was higher on Quercus spp. Sawdust cultivation of *P. edulis* was successful. The conditions were 80 to 85 days for cultivation period after spawn inoculation, 10 to 11 days for primordial formation at 17–18℃ and 15 to 20 days for fruiting growth. NIFoS 2804 and 3993 were selected as a good strain in terms of cultivation period and mushroom production. These results could be useful for the artificial cultivation of *P. edulis*.
[Supported by grants from NIFoS]

Keywords: *Panellus edulis*, Universal Fungal PCR Fingerprinting, Mycelial growth

A050

Collinsella canisensis sp. nov., Isolated from a Dog Feces

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A novel, Gram-staining-positive, strictly anaerobic, non-motile and singly or in long chains of short rod-shaped bacterium, strain BA40 was isolated from a dog feces (Beagle; Canis lupus familiaris). The colonies of the new isolate were circular, opaque and 0.4–0.65 μm diameter after 2 days incubation on DSMZ 104 medium at 37℃. Based on the 16S rRNA gene sequence similarity, the new isolate was most closely related to *Collinsella tanakaei* KCTC 15132^T (96.19%, sequence similarity). Strain BA40 grew optimally at 37°C, at pH 7.0 and in the presence of 0.5% (w/v) NaCl. Catalase-negative and oxidase-negative. The G+C content of the genomic DNA was 64.61 mol%. On the basis of polyphasic evidence from this study, the isolate is considered to represent a novel species of the genus *Collinsella*, for which the name *Collinsella canisensis* sp. nov. is proposed.
[This work was supported by a grant from of the Korea Health Technology R&D Project (HI14C0368) and was partially supported by Grants from the National Research Foundation of Korea (NRF) (2015M3C9A4053394).]

Keywords: *Collinsella*, Canis lupus familiaris, Feces

A051

Cultural Characteristics and Enzyme Activities of *Hypholoma lateritium*

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Hypholoma lateritium is an edible mushroom which occurs in oak trees in autumn. Six strains of *H. lateritium* were investigated in the optimal mycelial growth for artificial cultivation. The mushroom culture media were potato dextrose agar (PDA), malt extract agar (MEA), sabouraud dextrose agar (SDA) media. Optimum mycelial growth of *H. lateritium* was potato dextrose agar (PDA), temperature 25℃, respectively. The minimum and maximum temperatures for mycelial growth were 10℃ and 30℃, respectively. The optimal pH was observed on the potato dextrose broth (PDB) medium incubated at pH 5.0, respectively. We reported the efficient production of cellulose and laccase enzyme by *H. lateritium*. Enzymatic activities were highest NIFoS 1097 in cellulose and NIFoS 1098 in laccase, while cellulose activity of NIFoS 2411 and laccase activity of NIFoS 2398, 2411 were the lowest. In seven kinds of each carbon and nitrogen source, mycelial growth and density were good at pectin in carbon source and yeast extract in nitrogen source, respectively.
(Supported by grants from NIFoS]

Keywords: *Hypholoma lateritium*, Mycelial growth, Cellulose and laccase enzyme

A052

Curvibacter sp. NFH^T nov., a Novel Bacterium of Isolated from a Nakdong Liver

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A creamy yellow-colored, catalase and oxidase positive short-rod-shaped, Gram-negative bacterium, was isolated from a fresh water sample collected at Nakdong Liver, South Korea. The isolate aerobically grew at 15–25°C (optimum 20°C), pH 5.0–9.0 (optimum pH 7.0) and in the presence of 0–0.5% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequence of strain AQ9(T) revealed that it belongs to the genes *Curvibacter* in the family Comamonadaceae. The highest degree of sequence similarities of 98.2% with *Curvibacter fontanus*. By distinct phenotypic, genotypic, physiological and chemotaxonic properties, strain AQ9 should be placed in the genus *Curvibacter* as members of a novel species.

Keywords: *Curvibacter*, Proteobacteria, Burkholderiales

A053

Complete Genome Sequence of *Pedobacter ginsengisoli* T01R-27, a Plant Growth-promoting Bacterium, Isolated from Tomato (*Solanum lycopersicum* L.) Rhizosphere

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Pedobacter ginsengisoli strain T01R-27 was isolated from the rhizosphere of tomato plants in Korea. The bacteria showed plant growth promoting activity under abiotic stress conditions, including low and high temperature and salinity. Here, we report for the first time the complete genome sequence of *P. ginsengisoli* strain T01R-27. The genome of the strain consists of a circular chromosome with 5,373,360 base pairs with a G+C content of 37.82%. The genome includes 4,399 coding genes, 16 rRNAs, and 53 tRNAs. Genes related to root colonization, antioxidant activity, proline and exopolysaccharide biosynthesis, phosphate solubilization, and phytohormone modulations, which may contribute to the promotion of plant growth under environmental stresses, were also found in the genome. Our results will help provide a better understanding of the molecular mechanisms of plant-growth-promoting rhizobacteria and advance the research for the development of a powerful bio-stimulant to enhance crop productivity.

Keywords: Pedobacter, Genome sequence, Plant growth-promoting bacteria, Abiotic stress

A054

Isolation and Identification of *Duganella* YL-20^T sp. nov. Isolated from Soil

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Strain YL-20^T was isolated from soil at Kyonggi University, Republic of Korea. Morphological characteristics of the isolated strain was aerobic, gram-negative and rod shape bacteria. Its colony is pale yellow, flat, smooth and round with scalloped margin. Biochemical and physiological characterization of the isolated strain was some different aspect from its type strains. Based on full-length 16S rRNA sequence, phylogenetic analysis showed that strain YL-20^T was shown to belong to the Betaproteobacteria and showed the highest levels of sequence similarity to *Duganella sacchari* Sac-22^T (98.6%), *Pseudoduganella danionis* E3/2^T (98.3%), *Duganella phyllosphaerae* T54^T (98.3%), *Massilia buxea* A9^T (98.3%), *Duganella zoogloeoides* IAM 12670^T (97.9%) and *Duganella radicis* Sac-41^T (97.7%). Growth occurred at 10–30℃ (optimum 20℃), at pH 5–9 (optimum pH 6.0) and with ≤3% NaCl. The main cellular fatty acids of the strain were summed feature 3 fatty acids (16:1 w7c/16:1 w6c), C16:0, C12:0 and feature 8 fatty acids (18:1 w7c). Based on phylogenetic, chemotaxanomic, genomic and phenotypic analyses, we propose a novel species of the genus *Duganella*, named *Duganella* YL-20^T sp. nov.

Keywords: *Duganella*, Oxalobacteraceae, Betaproteobacteria, Soil

A055

Atopobacter sp. AH10, Isolated from Monkey Vagina

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A novel atopobacterial strain, AH-10, was isolated from monkey vagina. The strain was a facultatively anaerobic, Gram-stain-positive, non-spore-forming, non-motile, catalase-negative and oxidase-negative, coccus-shaped. The most closely related strains were *Atopobacter phocae* ATCC® BAA-285™ (95.86% 16S rRNA gene sequence similarity, respectively). The isolate grew optimally at 37℃ and pH 7 in the presence of 0% (w/v) NaCl. The The DNA G+C content was 38.8 mol%. The major cellular fatty acids (>10 %) were C16:0, C12:0 and C14:0.
On the basis of its phenotypic and genotypic properties, and phylogenetic distinctiveness strain AH-10 should be classified in a novel species in the genus Atopobacter, for which the name *Atopobacter* sp. nov. is proposed.
[This work was supported by Metabolic Regulation Research Center (MRRC), Korea Research Institute of Bioscience and Biotechnology (KRIBB).]

Keywords: Bacteria

A056

Phylogenetic Characterization of *Aureobasidium* spp. Isolated from flowers in Korea

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For yeast isolation, 57 wildflowers were collected in seven areas in Jeolla-do and yeast strains were isolated using different mediums from wildflowers. *Aureobasidium* spp., which is dominant in 441 strains, was obtained in various yeast strains. *Aureobasidium* spp. has been reported in variety environments and *A. pullulans* is industrially important yeast such as producing the polysaccharide pullulan etc. To classify the *Aureobasidium* spp. in wildflowers, the ITS and LSU-rRNA gene were sequenced and the strains were aligned for phylogenetic analyses using MEGA7.0. Alignments included 540 base pairs for ITS, 580 for LSU and 1,120 for ITS+LSU. The strains are compared with ITS and LSU-rRNA type strains and 192 strains were classified as *A. pullulans* var. *pullulans*, 10 strains as *A. pullulans* var. *melanogenum* and 1 strain as *A. leucospermi*. The three species (*A. pullulans* var. *pullulans*, *A. pullulan* var. *melanogenum* and *A. leucospermi*) can be identified using ITS or LSU analysis, but comparisons of ITS, LSU and ITS+LSU of other 3 species (*A. pullulans* var. *subglaciale, A. pullulans* var. *namibiae* and *A. leucospermi*) showed different phylogenetic results from each other. Several *Aureobasidium* spp. in Korea are presumed to be different or variable species than the species already reported. For accurate classification of *Aureobasidium* spp., further studies of selection genes should be considered.
[Supported by the National Institute of Agricultural Sciences, RDA, Korea (PJ013549)]

A057

Analysis of Culturable Bacterial Diversity in Soil and Water of Wind Hole

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Wind holes or air holes sites, in which cool air blows out and ice forming may happen even in the summer and can be categorized into three types: Talus, Cave, and Sink type. This study investigated the diversity of bacterial communities in the soil and water of wind hole in Uiseong, Cheongsong and Jeongseon. They were divided broadly into 4 phyla and bacterial communities were composed of 6 classes 7 orders 7 families 9 genera in the Uiseong, 6 classes 8 orders 12 families 20 genera in the Cheongsong and 8 classes 15 orders 23 families 36 genera in the Jeongseon. Bacteroidetes and Proteobacteria were the dominant bacteria in Uiseong, Cheongsong and Jeongseon, respectively.
[This work was supported by a grant from the Nakdonggang National Institute of Biological Resource (NNIBR) funded by the Ministry of Environment (MOE) of the Republic of Korea.]

Keywords: Bacteria, Wind Hole, 16S rRNA gene

A058

Genomic Characterization of *Nocardia seriolae* Strains Isolated from Diseased Fish

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Members of the genus *Nocardia* are widespread in diverse environments; a wide range of *Nocardia* species are known to cause nocardiosis in several animals, including cat, dog, fish and humans. Of the pathogenic *Nocardia* species, *N. seriolae* is known to cause disease in cultured fish, resulting in major economic loss. We isolated two *N. seriolae* strains, CK-14008 and EM15050, from diseased fish and sequenced their genomes using the PacBio sequencing platform. To identify their genomic features, we compared their genomes with those of other *Nocardia* species. Phylogenetic analysis showed that *N. seriolae* shares a common ancestor with a putative human pathogenic *Nocardia* species. Moreover, *N. seriolae* strains were phylogenetically divided into four clusters according to host fish families. Through genome comparison, we observed that the putative pathogenic *Nocardia* strains had additional genes for iron acquisition. Dozens of antibiotic resistance genes were detected in the genomes of *N. seriolae* strains; most of the antibiotics were involved in the inhibition of the biosynthesis of proteins or cell walls. Our results demonstrated the virulence features and antibiotic resistance of fish pathogenic *N. seriolae* strains at the genomic level. These results may be useful to develop strategies for the prevention of fish nocardiosis.

Keywords: *Norcardia seriolae*, Comparative genomics, Fish pathogen, *Channa argus*, *Anguilla japonica*

A059

Genome Based-identification of a Novel *Lactobacillus* Species Using ChunLab's TrueBacTM ID System

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The strain EH110, which is a lactobacilli isolated from fecal sample of Korean baby, showed high level of 16S rRNA gene similarity (99.9%) with *Lactobacillus gasseri* ATCC 33323T. To analyze the taxonomic relationship between the strains EH100 and ATCC 33323T, we determined genome sequence of the strain EH110 and bacterial identification was conducted with TrueBac™ ID platform in ChunLab, Inc. Results from TrueBac™ID platform showed that an average nucleotide identity (ANI) value between the strains EH110 and ATCC 33323T was 93.5%, which is clearly higher than the cutoff proposed for bacterial species boundary. Therefore, we suggest that the strain EH110 should be classified as a novel species in the genus *Lactobacillus*.

Keywords: *Lactobacillus*, *Lactobacillus gasseri*, Average nucleotide identity, TrueBac™, EzBioCloud

A060

Genome-based Reclassification of Bacterial Species

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Currently, similarity value of the 16S rRNA gene sequence and relatedness value of DNA-DNA hybridization between two species are major criteria for bacterial species identification. However, the 16S rRNA gene analysis is not enough for tens of thousands of bacteria identification due to the short length of gene size, about 1,500 bp, and DNA-DNA hybridization experiment is highly labor intensive and has a lot of errors. In this study, we determined genome sequence of several bacteria species, which have more than 99% of 16S rRNA similarity with closely related species, and analyzed the phylogenetic relationship using orthoANI, UBCG pipeline, and TrueBac reference database of ChunLab Inc. The results indicated that some species have more than 95% of average nucleotide identities (ANI), which is higher than the cutoff proposed for bacterial species boundary, with phylogenetically close other species. Therefore, we suggest that bacterial taxonomic identification should be conducted based on genome sequence level analysis.

Keywords: OrthoANI, UBCG, TrueBac, EzBioCloud, Average nucleotide identity

A061

Comparative Genomic Analysis of *Pseudoalteromonas* Strain EH1, Isolated from Arctic Sea

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Pseudoalteromonas strain EH1 was isolated form Chukchi Sea, one of the Arctic oceans located between Siberia and Alaska, for the cold-active amylase activity. The temperature, pH and NaCl ranges for growth were determined to be 10-30℃, pH 6-8, and 0.5-3% with optima of 20℃, pH 7, and 2%. Analysis using the pathway/genome databases (PGDBs) revealed that EH1 is most closely related to *Pseudoalteromonas haloplantkis* TAC125. Interestingly, EH1 has one chromosome (4,594,697 bp) while TAC125 has two with a total size of 3,850,272 (3,214,944 bp + 635,328) bp. Also, EH1 contains a total of 3,984 putative genes and 138 RNA genes including 104 tRNAs, while TAC125 has a total of 3521 putative genes and 137 RNA genes including 107 tRNAs. It is noteworthy that, despite the significant size difference between chromosomes, the number of RNA genes is almost identical. The COG distribution pattern showed involvement of more core genes in translation, ribosomal structure and biogenesis(J) and amino acid transport and metabolism(E), while accessory and unique genes appear to be enriched in signal transduction metabolism(T), Cell wall/membrane/envelope biogenesis(M) related functions. Furthermore, this study also identified the bioactive compound in *Pseudoalteromonas* strain EH1. There are four genes related to bacteriocins, especially, 962 gene is similar to *Pseudoalteromonas tunicata* strain D2's which is known to L-Lysine oxidase. Also, depending on resistance on vancomycin, the shape of EH1 is described by TEM.

Keywords: *Pseudoalteromonas*, Genomics, COG, Bioactive compound, Vancomycin

A062

Genome Sequences of Phenol-degrading Bacteria Isolated from Activated Sludge of Industrial Wastewater

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Acinetobacter beijerinckii belonging to the phylum Proteobacteria is known that a Gram-negative and strictly aerobic bacterium. *Pseudomonas nitroreducens* (the phylum Proteobacteria) is Gram-negative and aerobic bacterium. In this study, it was confirmed that *A. bereziniae* and *P. nitroreducens* isolated from activated sludge of industrial wastewater decompose phenol at high concentration (500 ppm). To identify genes related to degradation of phenol, the genome sequencing was performed. The genome of *A. bereziniae* is a circular DNA molecule of 3.5 Mbp, with a GC content of 37.5%. This strain has 3,300 protein-coding genes, 21 rRNA genes, and 80 tRNA genes. In the de novo genome sequencing of *P. nitroreducens*, two contigs (5.4 and 0.5 Mbp respectively) were generated. The large contig is a circular DNA molecule, with a GC content of 64.3% and contains 5,123 protein-coding genes, 12 rRNA genes, and 62 tRNA genes. Functional annotation was performed using NCBI nr and SwissProt databases. The resulting data were assigned to Gene Ontology functional categories. COG analysis was performed by the BLASTP method using the COG database. These genome sequences provide information that will help better understand genetic characteristics of the phenol-degrading bacteria. [This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201828102).]

Keywords: *Acinetobacter bereziniae*, Bioremediation, Phenol degradation, *Pseudomonas nitroreducens*

B001

Detecting Environmental Fungi with the Loop-mediated Isothermal Amplification Method

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The loop-mediated isothermal amplification (LAMP) is a useful DNA amplification and detection method that involves an enzymatic reaction for DNA elongation at a constant temperature of 60–65°C for 1–2 h with high specificity and sensitivity. Recently, LAMP has been used for detecting microbes, not only for medical purpose but also for environmental control. Detection of pathogenic microbes, bacteria, viruses, and parasites has been reported based on the LAMP method, and some fungal species-specific methods are available. We developed a LAMP primer set for detecting a wide range of fungi by aligning the sequences of the large subunit ribosomal RNA gene of *Candida albicans* (Ascomycota), *Cryptococcus neoformans* (Basidiomycota), and *Mucor racemosus* (Mucorales). The threshold of *C. albicans* rDNA as template with our LAMP primer set was in the range of 10–100 copies per a reaction. In this study, we evaluated the correlation between colony forming units (CFU) and LAMP detection rate using the LAMP method for environmental fungi. The LAMP method should be a useful means of detecting fungi not only in indoor environments, but also disaster areas, or even in confined manned spacecraft to prevent allergies or infections caused by fungi.

Keywords: Environment, Fungi, LAMP, Rapid DNA extraction, Rapid fungal detection

B002

Microbiological Activities and Stable Isotope Ratio Estimated in a Stream Environment with Frequent Harmful Algal Bloom

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Although the water quality of Chungju Lake is relatively good, there are frequent occurrences of harmful algal blooming in Jecheon Stream, a tributary of Namhan River. We selected several sampling sites upstream of Jecheon Stream to compare the pollution characteristics among the tributaries. Heterotrophic plate counts (HPC), extracellular enzyme activities (EEA), and community-level physiological profiles (CLPP) of water sample were analyzed during Feb–Oct, 2017. We also measured stable isotope ratio (δ^{15}N, δ^{18}O) to trace the origin of pollutant (nitrate) of each sample. As a result, HPC was the highest during summer at 1.7×10^6 – 1.0×10^7 CFU/ml just downstream of the Jecheon Sewage Treatment Plant (STP). EEA were as high as 674.7 – 841.1 RFU at the same sampling point. However, there was no significant seasonal variation of Metabolic Potential Index (MPI), an indicator of potential physiological activity, in Jecheon STP site compared to other sampling sites. Stable isotope ratio of δ^{15}N and δ^{18}O was 8.8‰ and 2.9‰ at Jupo Stream and 11.4‰ and 1.4‰ at Jangpyeong Stream, respectively. The origin of nitrate, therefore, presumed to be from soil organic-N and from discharged sewage in Jupo and Jangpyeong Stream, respectively. Therefore, it is necessary to strictly control the effluent discharged from the STP as a point source especially during summer. Also prevention of organic-N input from watershed is necessary in Jupo Stream as a non-point source control.

Keywords: Bacteria, Stable isotope, Algal bloom, Heterotrophic, Trace

B003

A Putative ABC Transporter Contributes Radicicol Resistance of *Cylindrocarpon estructans* and *Fusarium solani*

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Ascomycete fungi *Cylindrocarpon destructans* (Cd) and *Fusarium solani* (Fs) cause ginseng root rot and significantly reduce the quality and yield of ginseng. Cd produces radicicol which targets the molecular chaperone Hsp90 and suppresses NO synthase. Fs is resistant to radicicol while other fungal genera associated with ginseng disease are sensitive to it. To understand the resistant mechanism of Cd and Fs to radicicol, we checked transcriptome profiles in mycelia of Fs and Cd treated without or with radicicol through RNA-seq. We functionally annotated all expressed genes to *Fusarium graminearum* transcript database and found out the differentially expressed genes. Changes of transcripts encoding ABC transporter, aflatoxin efflux pump, ammonium permease 1 (*mep1*) and nitrilase were commonly found in both strain. Among the four genes, only ABC transporter was up-regulated in both species. Aflatoxin efflux pump and *mep1* were up-regulated in Cd but down-regulated in Fs while nitrilase was down-regulated in both species. The transcriptome analyses provided the pathways for radicicol resistance and additionally genetic manipulation showed that the transporter genes contribute radicicol resistance. Further studies on signal transmission of radicicol to induce expression of genes will help to elucidate the resistance mechanism of Fs and Cd more explicit and help to provide information for control ginseng disease in field.
[Supported by Rural Development Administration (PJ010119).]

Keywords: *Cylindrocapon destructans*, *Fusarium solani*, Ginseng root rot, Radicicol, Transcriptomes

B004

The Microbiome Characteristics of Kimchi Cabbage (*Brassica campestris* ssp. *pekinensis*) Cultivated Soil Depending on Cropping System

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Kimchi cabbage (*Brassica campestris* ssp. *pekinensis*) is one of the most important crops in Korea. It is cool-season crop which could be growth only in highland area in summer season of Korea with a few problems due to cropping system. To solve this problem, the research of cropping system was required. We tried to applying soybean (*Glycine max L.*) and potato (*Solanum tuberosum L.*), in cropping system. Kimchi cabbage cultivated continuously was also collected as comparison group. Rotation treatment showed higher growth value than continuous treatment. To explain this phenomenon in terms of soil microorganism, we investigated soil microbiome characteristics. Kimchi cabbage cultivated soil samples were collected on 3 major growth. Soil chemical properties were analyzed on before seeding to get basic soil information. To analyze soil microbiome characteristics, Dehydrogenase activity (DHA) analysis, Ecoplate made Biolog™ which is constructed with 31 different carbon sources indicated diversity and availability of soil microbiome and 16S rRNA and ITS gene-targeted next generation sequencing were conducted. DHA values were highest in leaf growth stages on both and rotation soil were higher in almost growth period than continuous soil showing coincidence result of average well color development of ecoplate. Community level physiological profiles of ecoplate and diversity profiles of NGS also demonstrated distinct profiles in two treatments.
[Supported by grants from RDA]
Keywords: Kimchi cabbage, Cropping system, Soil microbiome

B005

Antimicrobial Activity of Some Bacteria and Synergistic Effects between Antimicrobial Substances against Several Human Pathogens and Mycotoxigenic Fungi

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The aim of this study is to evaluate the activity of antimicrobial substances produced by isolated bacteria DS381, DS518, DS620 and DS1515 against diverse microorganisms (human pathogens and mycotoxigenic fungi) and synergistic effects between antimicrobial substances. All isolated bacteria showed 15.3 to 35.3 mm inhibition zone diameter against most bacteria and yeast, and inhibited mycelial growth (~66.7%) and sporulation (~85.7%) of target fungi. The purified antimicrobial substances (lipopeptide, chitinase, siderophore, protease and anthracyclic antibiotics, etc.) from isolated bacteria exhibited low minimum inhibitory concentrations (0.0078–10,000 mg/ml) on target organisms. When the synergistic effect of antimicrobial substances was investigated, combinations of antimicrobial substance displayed synergistic effects against each different target organism (0 < fractional inhibitory concentration index < 0.75). In time-kill assays, most combinations reduced more than 10^5 in colony count of bacteria and yeast during 24 h. Also, combination of antimicrobial substances showed spore degradation (28.2–91.6%) and spore germination inhibition (97.2–100%). These results suggest that isolated strains and antimicrobial substances may be utilized as an environment-friendly preservative and biocontrol agent against human pathogens and mycotoxigenic fungi.

Keywords: Antimicrobial activity, Antimicrobial substance, Synergistic effect, Human pathogens, Mycotoxigenic fungi

B006

Mycotoxins Biodegradation and Growth Inhibition against Mycotoxigenic Fungi by *Streptomyces* spp.

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This study aims to explore mycotoxins (AFB_1 and FB_1) degradation and antifungal activity against mycotoxigenic fungi by isolated strains, *Streptomyces sporoverrucosus* JS383 and *S. lavendulae* JS669. Mycotoxins are secondary toxic metabolites produced by various filamentous fungi including *Aspergillus* and *Fusarium* spp. JS383 and JS669 degraded AFB_1 (0.1 mg/L) by 93.7 and 96.8%, respectively in nutrient broth. They also degraded FB_1 (0.1 mg/L) by 91.2 and 95.9%, respectively on same conditions. JS383 and JS669 showed excellent thermostability in AFB_1 degradation (up to 121℃), and JS383 also displayed broad temperature range (4–75℃) for FB_1 degradation. The antifungal activity of JS383 and JS669 was evaluated by co-culture with 3 strains of aflatoxigenic *A. flavus* (KACC44986, 45068 and 45146) and 4 strains of fumonisigenic *Fusarium* spp. (*F. fugikuroi* KACC46888 and 48352, *F. verticillioides* KACC48354 and *F. proliferatum* KACC48356). JS383 and JS669 effectively inhibited mycelial growth of target mycotoxigenic fungi (68.4–90.2%) and suppressed sporulation of target organisms up to 97.3 and 97.2%, respectively. They also displayed inhibition of spore germination by 99.0 and 97.3%, respectively. Ethyl acetate extracts of bacterial cultures showed low minimum inhibitory concentrations (1.25–5 mg/ml) on target mycotoxigenic fungi. Conclusionally, JS383 and JS669 can be used for mycotoxin (AFB_1 and FB_1) biodegradation and control of mycotoxigenic fungi in food and feed industry.

Keywords: Aflatoxin, Fumonisin, Biodegradation, Growth inhibition, Mycotoxigenic fungi

B008

Characterization of Mycelial Growth of *Tricholoma* spp. under Diverse Culture Conditions

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Some species in *Tricholoma* are economically interesting mushrooms as edible resources. So far, their production depends on natural development. To find for the way we could apply them, we examined the mycelial growth properties of four *Tricholoma* species (*T. bakamatsutake*, *T. fulvocastaneum*, *T. matsutake*, *T. retterum*) at different physical and chemical environmental condition. In the tests of physical culture conditions, all the four species grew well at 20–25℃, pH 4–7 and dark condition. Among them, *T. matsutake* did not grow at 30℃. In the tests of growth with different NaCl concentration, the four *Tricholoma* spp. could grow with 1% concentration. In case of *T. matsutake,* it could grow with 2% concentration. In the test with various heavy metals and pesticides, the growth of all the species was the most inhibited by cadmium and emamectin benzoate, respectively. But, they were able to grow with Cu^+ ion supplemented media. The growth of *T. matsutake* was not inhibited with other three kinds of insecticides. In the test of extracellular enzyme activities on chromogenic media, *T. bakamatsutake* and *T. fulvocastaneum* showed amylase and *ß*-glucosidase. Our results will be applied to do better culture of these *Tricholoma* spp. *in vitro* system.

B009

Isolation and Characterization of Endophyic Bacteria from *Oenothea adorata* Seeds and Their Potential for Seed Germination

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Endophytic bacteria are considered important because of their rapid growth, metabolites production and active colonization potential. However, the seed-borne bacterial endophytes are not fully explored. Therefore, the current study was undertaken to isolate and identify bacterial endophytes associated with *Oenothea adorata* seeds, their potential to produce IAA and ABA and their role in host-plant seeds germination. Among isolated strains, YNA11 and YNA12 were selected based on salkowski test and were identified as *Pantoea dispersa* and *Klebsiella pneumoniae* by sequencing their 16sRNA and phylogenetic analysis. Seed germination assay using *Echinochloa crus-galli* and *Oenothera odorata* showed that germination rate, seedling length and fresh weight of host weeds *Oenothera odorata* seeds were significantly inhibited by YNA12 while YNA11 showed non-significant difference. The pure cultures of YNA11 and YNA12, supplied with deuterated internal standards, were subjected to gas chromatography and mass spectrometric selected ion monitoring (GC-MS/SIM) for quantification of IAA and ABA. As a result, *K. pneumoniae* YNA12 produced more IAA and ABA than *P. dispersa* YNA11. In conclusion, *K. pneumoniae* YNA12 could be a suitable bio-herbicide for seed germination and growth inhibition of *Oenothera odorata*, an exotic weed.

Keywords: Endophytic bacteria, *Oenothea adorata*, Seed germination, Plant hormone

B010

Bacterial Diversity of Green-house Soil

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Green-house soils are not affected by precipitation since they are blocked by the outside natural environment. Due to intensive cultivation, material circulation is limited and many ions such as nitrate dissolved in water are accumulated in the soil. The objective of this study is to investigate the bacterial diversity of green-house soil with the unique characteristics described above. Soil samples were collected from green-house which cabbage is cultivated at Sunchon National University, Republic of Korea. A total of 277 bacterial strains were isolated using the standard plating technique. Based on the 16S rRNA gene sequences comparison analysis, the isolates belonged to four phyla: *Proteobacteria* (46.6%), *Actinobacteria* (24.5%), *Bacteroidetes* (18.4%), and *Firmicutes* (10.5%). Isolates were affiliated with 31 families, 78 genera and 168 species. The most dominant genus was *Pseudomonas* (11.9%). Two isolates BO139 and BO170 were proposed as new species of the genus *Luteimonas*.
[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: Bacterial diversity, 16S rRNA gene, Green-house soil

B011

Flora of Yeonchoen Wind Hole

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Wind hole is called ice hall, ice valley, etc, and has low temperature than ordinary forests or valley. It has been thought of as a fantastic place because of freezing ice in summer. Korea National Arboretum focuses on the species specificity of wind hole and surveys lichen diversity. The Yeonchoen wind hole, the area of the talus is 432 m^2 and is located at 83 m above sea level. We collected 22 lichen species, among them fruticose lichen, *Cladonia rangiferina* var. *rangiferina*, is unusual to be found at low elevation. This species was mainly collected at over 900 m above sea level, but the only one seen in 100 m low altitude except for Haui-island. Also, we collected *Maronea* sp., which is first found genus in Korea. Wind hole is thought to be a highly biodiversity-rich region of lichen, and further study is needed.

Keywords: Lichen, Diversity, Flora

B012

Cultivated Iron-reducing Bacteria in the Tidal Mudflat and Aquaculture Sediments

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Diverse anaerobic bacteria using various terminal electron acceptors (MnO_2, NO_3^-, FeOOH, and SO_4^{2-}) are responsible for most organic carbon oxidation in marine sediment. Iron-reducing bacteria (FeRB) are considered an important microbial group because FeRB are major carbon oxidizer and inhibit the accumulation of H_2S by limiting the activity of sulfate-reducing bacteria. Despite the significance of FeRB, little is known about FeRB communities inhabiting marine sediment. To elucidate FeRB communities in the marine sediment, we inoculated the coastal sediments in anoxic basal medium with amorphous iron oxide to selectively culture the FeRB. 16S rRNA genes amplifying from extracted DNA in the sediments and culture media were sequenced on an Illumina MiSeq. Sediment samples were collected at four geochemically distinct sites-*Spartina anglica* (SA) and *Suaeda japonica* (SJ) habitats and unvegetated mudflat (UMF) in Ganghwa, and abalone farm (AF) in Wando. Most cultivated FeRB belonged to *Clostridiales*, *Alteromonadales* and *Desulfuromonadales*. Interestingly, the bacteria in genus *Geobacter* were cultivated a major FeRB in SA and UMF sediments (16–18% of the total reads). In contrast, the bacteria in genus *Desulfuromonadaceae* grew as a major FeRB in SJ and AF sediments (34% of the total reads). Thus, unique geochemical characteristics might control the community composition and growth of FeRB in the culture media.
[Supported by grants from NRF, L-TMER and BrainKorea21 Plus]

Keywords: Fe-reducing bacteria, Marine sediment, 16S rRNA gene

B013

Roles and Diversity of Bacteria in the Phycosphere of *Microcystis aeruginosa*

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Roles of epiphytic bacteria on freshwater microcystin-producing *M. aeruginosa* strains are largely unknown. Fluorescent dye-based microscopic and scanning electron microscopic observation confirmed strong bacterial attachment on the surface of *M. aeruginosa* cells. Culture-independent community analysis showed that *M. aeruginosa* KW and *M. aeruginosa* FBC2 have relatively simple phyla including *Bacteroidetes*, and *Proteobacteria*. At the genus level, uncultured bacteria are dominant and *Sediminibacterium, Porphyrobacter, Hydrogenophaga* species are commonly found in both *Microcystis* species. Most of cultured bacteria belong to *Rhizobium* species. Bioinformatics using cyanobacterial genomes suggested the absence of catalase gene in *Microcystis aeruginosa* strains, but not in other nitrogen fixing cyanobacteria including *Anabaena* and *Synechococcus* species. Catalase activities of axenic *M. aeruginosa* NIES-298 cells could be ignored compared to xenic KW strain. Low concentration of H_2O_2 could be toxic to NIES-298 cells, but not to *Rhizobium*-added NIES-298 cells and xenic KW strains. Growth of *M. aeruginosa* NIES-298 cells measured by flow cytometry were enhanced in the presence of *Rhizobium* species. Our data suggested that many uncharacterized bacteria are associated with *M. aeruginosa* strains and epiphytic bacteria such as *Rhizobium* species could be beneficial to the growth of *M. aeruginosa* probably due to bacterial stress protection and nutrient supply.
[Supporyed by NIBR]

B014

Isolation of Microbial Strains for Microbial Degradation of Livestock Carcass to be Slaughtered Owing to Epidemics Such as Foot and Mouse Disease and Avian Influenza

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When a livestock epidemic occurs, not only the infected livestock but also the nearby livestock must be slaughtered. Leakage of leachate due to poor management of the landfill of livestock carcasses has caused secondary environmental pollution problems such as soil pollution and underground water pollution, outflow of pathogens, and landfill collapse. Therefore, the livestock carcasses are treated as a rendering method in which a livestock carcass is buried in a glass fiber reinforced plastic tank, and then the microorganism additive is added and degraded microbiologically. However, it has been found that the livestock carcass treated with the rendering method of degradation in the FRP tank does not decay in the tank even after a long period of time. Consequently, it is thought that the microbial additives distributed in the existing market are not effective. In this study, four microbial strains are isolated finally from environments which are elucidated to be very effective in degradation of chicken carcass. Final four candidates were tested the protease activity, the growth characters, the biochemical characters, and identification through 16S rRNA phylogenetics. It was studied that microbial agents degraded chicken carcass in pilot scale similar to the landfill of livestock carcasses.
[This work was supported by research grant from Korea Technology and Information Promotion Agency (TIPA), funded by Ministry fo SMEs and Startups of the Republic of Korea (C0563438)]

Keywords: Isolation, Microorganism, Degradation, Livestock, Carcass

B015

Metagenomic Analysis of the Effects of Tetracycline Residue on the Human Gut Microbiota Composition and the Emergence of Antibiotic Resistance in an *in vitro* Continuous Culture System

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The human gut microbiota, which is a stable ecosystem and plays important roles on host health or physiology, could be altered by the ingestion of antibiotic residues in foods derived from animals. In this study, we determined using metagenomic approachs if tetracycline at residue-level can influence on the human gut microbial community and functions and the antibiotic resistance gene. The effects of 0.015, 0.15, 1.5, 15, and 150 μg/ml tetracycline in 3% (w/v) human fecal suspensions collected from one individual were investigated using single-stage chemostat model. Metagenomic microbial community profiling revealed that *Firmicutes* and *Bacteroidetes* were the predominant phyla in the fecal samples. The evaluation of bacterial community changes from control to tetracycline-treated fecal samples suggested that tetracycline could lead to differences in the composition of intestinal microbiota. In particular, genus *Bacteroides* was increased 3.80 to 17.27% at tetracycline concentrations of 1.5 μg/ml or above after 2 days of tetracycline exposure and 3.39 to 24.42% at 0.15 μg/ml or above after 3 days, respectively. Blastx against ARDB shows that antibiotics resistome has 14 ARG classes and eight genes (*tet*40, B, 32, M, O, Q, W, and X) were major TRGs in control and tetracycline-dosed fecal samples. This in-depth metagenomic study provides a comprehensive understanding of the impact of residue-level of tetracycline on the microbiota in the human gastrointestinal tract.

Keywords: Tetracycline, Human intestinal microbiota, Antibiotic resistance, Metagenomics, *In vitro* gut model

B016

Relevance of Alpha- and Gammaproteobacterial Methanotrophs to N_2O Emissions from Denitrifying Microbial Consortia Enriched from Soils

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KAIST

In our recent study, we have observed that methanobactin (Mb) inhibits N_2O reduction in axenic denitrifier cultures, leading to N_2O emissions. To investigate whether this interplay between methanotrophs and denitrifiers is relevant to soil environments with established complex microbiomes, denitrification and N_2O production in methanotroph-enriched soil and sediment suspensions were monitored in laboratory settings. 0.1 g of rice paddy soils and varying amounts of *Methylosinus trichosporium* OB3b culture was added to 50 ml minimal medium. Initial enrichment with 20% of CH_4 was followed with addition of one mmole NO_3^- and N_2O production was monitored. In another set of cultures originating from stream sediment, indigenous methanotrophic population was enriched with CH_4 and denitrification progression was monitored. The amounts of added OB3b cells were directly correlated to the amount of N_2O produced from subsequent denitrification, and permanent accumulation of N_2O-N observed upon addition of 3 ml OB3b. Unexpectedly, >1000-fold increase was observed also in the sediment enrichment where *Methylococcaceae* group was the vast majority and Alphaproteobacterial methanotrophs were unseen minority. These findings suggest that methanobactin-mediated N_2O emission enhancement may be an environmentally-relevant phenomenon in copper-depleted soils and also that Gammproteobacterial methanotrophs may also utilize a yet unidentified copper-sequestering mechanism.
[Supported by grants from NRF]

Keywords: Methanotroph, Soil denitrifier, Methanobactin, Copper, Soil enrichment

B017

Bacterial Community Dynamics in Artificially Warmed Lakes, Konin Lake in Poland

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Microbial colonization on the zooplankton has been proposed to provide numerous ecosystem services including nutrient cycling, stress protection, detoxification and habitat provision. To understand the microbial community composition and diversity as well as their role in the functioning of freshwater food webs and lake biogeochemistry in artificially warmed lakes, here, we assessed bacterial composition of zooplankton (copepods and cladocerans), phytoplankton and bacterioplankton. Microbial community composition varied significantly across different microbiomes (e.g., bacterioplankton, phytoplankton, copepods, and cladocerans). The water parameters temperature revealed a significant effect on microbial community composition. Two phyla (e.g. *Proteobacteria* and *Bacteroidetes*) are predominant in zooplankton microbiome whereas *Actinobacteria* showed high abundance in bacterioplankton. The microbial richness and Shannon diversity were lower in zooplankton compared to bacterioplankton and phytoplankton. The indicator species analysis showed that total 266 OTUs were significantly associated with microbiomes, and only 23 and 22 unique OTUs were significantly associated with copepods and cladocerans, respectively. This study demonstrates that only selective bacterial community harbour to a certain type of zooplankton due to the effect of ecological niche, and in addition, microbial community structure is affected to all microbiomes due to the influence of temperature.

B018

Inhibition of Quorum Sensing by Inhibitor Substance from AMF-249

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Several types of bacterial cell-cell communication signals have been identified in the last two decades, such as quorum sensing that it's to take the upper hand in a competitive environment. Single-celled bacteria have adopted a community genetic regulatory mechanism, known as quorum sensing (QS). It is looked like phenomenon that collective activity system by network. But it's controlled by depending on bacterial population density. There are many phenomenon that in first, bioluminescence of *Vibiro fischeri*, many bacteria come out to induce activated autoinducer(AI) like switch that transfer gene by performing the biofilm that has virulence food poison and bacterial soft rot like a chili pepper, tomato, potato, radish and virulence by bacterial infection in human body. For this study, we isolated the microorganisms from the soil to inhibit quorum sensing. The present time, those are compared for optimization at the growth temperature 28℃ to 37 in cultures grown in aeration effects-LB (Luria-Bertani broth), based on bioassay of *Agurobaterium tumefaciens* NT1. At last, we refer to significance and impact of this study: The results suggest the bio-active constituent from edible and these could be interfered with bacterial quorum-sensing system, regulate its associate function and prevent bacterial pathogenesis from bacteria using quorum sensing, evaluate them as quorum sensing inhibiotor and analyze the exact mechanism of action.

Keywords: Quorum sensing, Bacterial cell-cell communication, Autoinducer

B019

Fungal Diversity of Konara Oak (*Quercus serrata*) Pure Forest in Gwangneung Forest

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For the investigation of fungal diversity in Konara oak pure forest of Gwangneung forest. We collected the soil samples and fruit bodies of macrofungi (mushroom) from 2013 to 2014. As a result, a total of 126 collected fruit bodies were classified into 2 phyla, 5 classes, 13 orders, 29 families, 49 genera and 88 species. Among them, the dominant species was *Clitocybe fragrans* (3.97%). The soil-fungal communities (belong to Ascomycota and Basidiomycota) were classified into 2 phyla, 14 classes, 35 orders, and 58 families 88 genera and 130 OTUs (19,744 reads). Totally, 132 genera were detected in this site. We found that only two genera (*Auricularia* and *Daedalea*) were commonly detected between macrofungi and soil-fungal communities.
[This research was supported by the Korean National Arboretum (Project No. KNA 1-1-14, 14-2)]

Keywords: Fungal diversity, Macrofungi, Soil-fungal communities, *Quercus serrata*

B020

Improved PCR assay for the Subspecies-specific Identification and Quantification of *Bacillus subtilis* subsp. *subtilis*

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Bacillus subtilis is frequently isolated from various niches, including fermented foods, water, and soil. Within the Bacillus subtilis group, *B. subtilis* subsp. *subtilis* have received significant attention as biological resources for biotechnology-associated industries. Nevertheless, radical solutions are urgently needed to identify microbes during their ecological succession to accurately confirm their action at the species or subspecies level in diverse environments, such as fermented materials. Thus, in this study, previously published genome data of the *B. subtilis* group were compared to exploit subspecies-specific genes for use as improved qPCR targets to detect *B. subtilis* subsp. *subtilis* in kimchi samples. *In silico* analyses of the selected genes and designed primer sequences, in conjunction with SYBR Green real-time PCR, confirmed the robustness of this newly developed assay. Consequently, this study will allow for new insights into the ontogeny and succession of *B. subtilis* subsp. *subtilis* in various niches.
[This study was conducted with support from the Next-Generation BioGreen 21 Program (SSAC, Grant nos. PJ013195 and PJ011844) of the Rural Development Administration of the Republic of Korea.]

Keywords: Bacillus, *Bacillus subtilis* subsp. *subtilis*, Succession, Quantitative assay

B021

A New Member of *Fusarium solani* Species Complex (FSSC) is the Causal Agent of Grafted Twig Rot on Citrus in China

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Fusarium solani is a common agent causing dry root rot on citrus trees. The disease can be destructive incorporated with stressed citrus, such as trunk girdling or grafting. In June 2017, grafted twigs were rotten starting from both ends on *Citrus sinensis* trees in more than 10 orchards in Zigui, China and only 5% grafted twigs were survived. Severe infection resulted in a whole twig rotten covered with white mycelia. One *Fusarium* like fungus was frequently isolated from the twigs. It was determined based on cultural and conidial characteristics and multi-gene phylogenetic analysis including partial gene sequences of the internal spacer rDNA region (ITS), translation-elongation factor 1 alpha (EF-1α), and the second largest subunit (RPB2). The results indicated that it is a new member of the *Fusarium solani* species complex (FSSC) and closely related to FSSC22 isolated from *Xanthoxylum piperitum* in Japan. Pathogenicity tests confirmed that the new member of FSSC is a causal agent of grafted twig rot on *Citrus sinensis*.

Keywords: *Citrus sinensis*, Fusarium, Morphology, Phylogeny, Pathogenicity

B022

Diversity of Arbuscular Mycorrhizal Fungi in Orm, Jeju Island

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Arbuscular mycorrhizal fungi (AMF) are one of the most important symbiont in ecosystem. AMF provide better nutrient absorptivity to host plant, enhance tolerance against plant root pathogen, and improve resistance against the stress by heavy metal or salinity. Due to their benefits for plants, AMF have importance in plant and soil ecosystem. There are many satellite cones in Jeju island, and we call them 'Orm'. Soils in Orm have higher permeability than general forest soils, so they form soil ecosystem with harsh environment. In this study, we sampled rhizospheres in Moolme-Orm and Nokome-Orm, Jeju island. We extracted AMF spores from field soils and identified AMF spores using morphological and molecular analysis. As a result, 10 species from 6 genera were identified. *Rhizophagus clarus* was the most dominant species, and we confirmed that community structures of AMF spores have difference between two rhizospheres.

Keywords: Arbuscular mycorrhizal fungi, Symbiosis, Jeju-island

B023

Symbiotic Fungi Isolated from *Calanthe discolor* in Jeju Island

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The Orchidaceae is the world's largest family in the plant kingdom, with estimates of more than 25,000 species. Most members of Orchidaceae have interactions with their mycorrhizal and endophytic fungi. As the seeds of orchids are very minute and contain few stored food reserves, orchids are dependent for germination and early development on symbiotic fungi for nutrient. Despite many researches have showed that the importance of using fungi to restore and preserve orchids, there were little studies about symbiotic fungal comunities in endangered native orchids in Korea. This study aimed to isolate and identify the endophytic fungi, including mycorrhizal fungi, from roots of *Calanthe discolor* (terrestrial orchid) in the Jeju island, Korea. The taxa of strains were classified based on morphological characteristics and molecular analysis. Currently, 17 species of endophytic fungi, including species of orchid mycorrhizal fungi belonging to the genus *Tulasnella* were indentified from 21 morphotypes. The result of this study showed community structure of symbiotic fungi isolated from in *C. discolor*. There are needed more studies about this diverse and valuable association to restoration of endangered species belonging to Orchidaceae.

Keywords: Orchid mycorrhiza, Jeju-island, *Calanthe discolor*, Orchidaceae

B024

Lignin Degradation by Fungi Isolated from Fresh Water in Korea

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Lignin is non-biodegradable organic polymers and present predominantly in woody plants. Biological degradation of lignin is very difficult due to the strong and not-hydrolysable carbon-carbon linkages and aryl ether bonds. Pulp-and-paper mill wastewater contains large amount of pollutants characterized by high biochemical oxygen demand (BOD), chemical oxygen demand (COD), mainly due to alkali–lignin. Several studies have been carried out on biological delignification of pulp-and-paper mill wastewater using bacteria and fungi. However, most studies used microorganisms which isolated from soil or decaying plant. In this study, biological degradation of lignin by fungal strains isolated from fresh water was investigated.

Fresh water samples were collected from the rivers and streams near paper mills. 160 fungal strains were isolated from water samples and incubated on potato dextrose agar (PDA). The solid media were prepared with PDA medium and the addition of lignin to a total concentration of 100 mg/L. A mycelium plug obtained from the edge of fungal strains were transferred to the center of a solid medium plate and inoculated at 25°C. Lignin degradation activity was determined by the formation decolorization zone in the solid media plate. After 7 days of incubation, 11 fungal isolates including Garnoderma carnosum, Phlebiopsis crassa showed lignin degradation activity.

[This work was supported by grants from NNIBR]

Keywords: Lignin, Fungi, Biodegradation, Fresh water

B025

Microbial Community Structure in a Nitrification Reactor Treating Livestock Wastewater

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The purpose of this study is to analyze the variation of microbial community structure in nitrfication and denitrification tanks and to identify the dominant nitrifying and denitrifying bacteria in a livestock wastewater treatment plant and ultimately to increase the efficiency of nitrogen reduction. In a livestock wastewater treatment plant, ammonium is oxidized to nitrate through nitrite in nitrification tank, and then nitrate is reduced to nitrogen gas in denitrification tank, which is emitted into atmosphere. This process reduces the total nitrogen content in effluent. It is known that many microorganisms are involved in the process of nitrification and denitrification. The first sampling was conducted in the nitrification tank in a livestock wastewater treatment plant in February 2018. The bacterial and archaeal 16S rRNA gene sequences were analyzed using Illumina SBS technology. In this analysis, *Thauera humireducens* was the most dominant bacterium in the nitrification tank, occupying 22.7% of the bacterial community. *Nitrospira lenta* was found to be the most dominant of the known nitrifying bacteria, occupying 5.0% of the bacterial community. Thus it is supposed that *Nitrospira lenta* is important in the nitrification process. *Nitrosophaera viennensis* known to be ammonia-oxidizing archaeon was also found, making up 63.0% of the archaeal community.

Keywords: Nitrification, Microbial community, Livestock manure, Denitrification

B026

Culture Condition of Antimicrobial Activity Producing Strain of *Paenibacillus* sp. against Soft Rot Disease

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[1]Department of Medicinal Plant Resources, Andong National University, [2]Institute for Development of Bioindustrial Materials, BHNBIO Co., LTD.

Chemical pesticides may pollute the ecosystem and environmentally hazardous, as the chemicals accumulate in the soil. Biological control is a frequently-used environment-friendly alternative to chemical pesticides in phytopathogen management. Therefore, biopesticide has become an important approach to sustainable agriculture. Soft rot disease has a wide host range, difficult due to the ability of the bacterial to survive in soil. Soft rot disease has caused by *Pectobacterium carotovorum* subsp. *carotovorum* (PCC) which is gram-negative bacteria. The economic importance of losses caused by Pcc can be very great, depending on the value of the crop and the severity of the attack. We isolated anti-PCC microorganisms from soil samples and investigated the characteristics of microorganisms according to culture conditions. Oxygen concentration and incubation temperature have been to the different results. Also, the inoculum volume did not significantly affect microbial growth. However, the results of mass culture showed different results depending on the cultivation volume.

Keywords: Biological control, Biopesticide, Soft rot disease, *Paenibacillus* sp.

B027

Inhibition of Quorum Sensing by Bacterial of AM-249

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Several types of bacterial cell-cell communication signals have been identified in the last two decades, such as quorum sensing that it's to take the upper hand in a competitive environment. Single-celled bacteria have adopted a community genetic regulatory mechanism, known as quorum sensing (QS). It is looked like phenomenon that collective activity system by network. But it's controlled by depending on bacterial population density. There are many phenomenon that in first, bioluminescence of *Vibiro fischeri*, many bacteria come out to induce activated autoinducer (AI) like switch that transfer gene by performing the biofilm that has virulence food poison and bacterial soft rot like a chili pepper, tomato, potato, radish and virulence by bacterial infection in human body. For this study, we isolated the microorganisms from the soil to inhibit quorum sensing. The present time, those are compared for optimization at the growth temperature 28℃ to 37 in cultures grown in aeration effects LB (Luria-Bertani broth), based on bioassay of *Agurobaterium tumefaciens* NT1. At last, we refer to significance and impact of this study: The results suggest the bio-active constituent from edible and these could be interfered with bacterial quorum-sensing system, regulate its associate function and prevent bacterial pathogenesis from bacteria using quorum sensing, evaluate them as quorum sensing inhibitor and analyze the exact mechanism of action.

Keywords: Quorum sensing, Bacterial cell-cell communication, Autoinducer

B028

Biodegradation of Organochlorine Pesticides by Actinomycetes

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Organochlorine pesticides have been widely used throughout the world for the control of numerous insects in a wide variety of food and non-food crops. Due to their stable structure, organochlorine pesticides was ubiquitously detected in the soils, water, and other surrounding environments. For isolation of endosulfan degrading bacteria, 230 strains isolated in several domestic farms were cultured in agar media containing 100 mg/L of endosulfan as carbon or sulfur source at 30°C for 7 days. Compared with the control without endosulfan, 23 strains were selected for their good growth in the presence of endosulfan. The selected 23 strains were cultured to confirm endosulfan degradation ability in liquid culture media under the same conditions. After cultured for 6 days, culture broth were centrifuged at 8500 rpm for 10 minutes. The cell growth were monitored by dry well weight, and the supernatant was extracted with EtOAc and analyzed by GC chromatography. As a result, 11 strains showed excellent cell growth in the presence of endosulfan, and the maximum biodegradation ability was obtained by *Streptomyces* sp. MJM14747 which degraded 95% of 100 mg/L of endosulfan. Although 0.250776 mg/L of α isomers and 0.564268 mg/L of β isomers of endosulfan were remained, there was no accumulation of intermediates or end products known to be toxic.

Keywords: Actinomycetes, Endosulfan, Biodegradation

B029

Variation of Microbial Community Structure in Agricultural Lands according to Soil Organic Carbon Content

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It is known that because atmospheric concentrations of greenhouse gases are already too high, only the reduction of greenhouse gas emission can't prevent climate change crisis and it is needed to pull down them. Soil is attracting a lot of interest as a huge reservoir to store atmospheric carbon and mitigate climate change. Soil carbon sequestration is to store atmospheric CO_2 as stable organic or inorganic carbon in soil. Some scientists advocate that 0.4% increase of organic matter in soil each year would compensate for the global emission of greenhouse gases. Although management practices such as reduced or no tillage, crop rotation, cover crops, and application of compost are known to increase soil carbon sequestration, the roles of soil microorganisms in soil carbon sequestration are now being explored. In this study, we investigated variation of microbial community structure in agricultural lands according to soil organic carbon content to identify the microbial groups important in soil carbon sequestration. To accomplish this, stable organic carbon content was determined using selected soil samples and the community structures of prokaryotes and fungi were determined using DNA and RNA extracted from the soils. The fungal/bacterial ratio, known as an important factor affecting soil carbon sequestration, was also determined based on real-time PCR results.

Keywords: Soil carbon sequestration, Prokaryotes, Fungi

B030

Biological Control Strategy Using Fungal Pathogens Specific for Exotic Weeds

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Due to active international trades and human movements, various exotic weeds have been introduced into agricultural lands of Korea as well as natural ecosystems. Three alien plants, *Amaranthus patulus*, *Ambrosia artemisiifolia*, and *Lactuca serriola*, were widely naturalized in Korea, and because of the possible negative effects on the ecosystem, they have been designated as harmful nonindigenous plants by the Korean Ministry of Environment. As various strategies so far applied to control these weeds have been unsuccessful, we conducted biological control treatments using pathogenic fungi specific towards the alien plants. As a preliminary procedure, various fungal pathogens were isolated from the three weeds in farm fields in 2018, and as a result, powdery mildew on *L. serriola* and leaf spot of *A. trifida* were collected. On the basis of morphological and cultural characteristics, they were identified as *Golovinomyces orontii* and *Septoria epambrosiae*, respectively, for which host specificity and biocontrol ability should be further tested.
[This study was supported by a research project of Rural Development Administration, Republic of Korea (Project no. PJ01347602)]

Keywords: *Ambrosia artemisiifolia*, *Lactuca serriola*, *Amaranthus patulus*, Alien weed, Biocontrol

B031

Response of *Bacillus mesonae* H20-5 to Abiotic Stresses

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Global warming and drastic climate change are the greatest threat to the world. Plants survive in these adverse conditions with a variety of abiotic stress (salt, high/low temperature, drought) response. *Bacillus mesonae* H20-5 is a beneficial trait which alleviates abiotic stress of plant. The genome of H20-5 contains genes for biosynthesis of spermidine and proline, which are known as abiotic stress relieving substances. Experiment were carried out and analyzed by qPCR and HPLC to investigate the response of H20-5 under abiotic stresses. Specific primer was in qPCR to analyze the expression level of proline biosynthetic genes. H20-5 cells were separated from the culture broth by centrifugation and used for analysis. The HPLC result showed that spermidine was produced in very low amount in control and decreased in salt treatment. Inverse expect observed on proline content in salt concentration and higher in salt treated supernatant than control. Quantitative analysis results showed that the expression level of proline was higher in -1,000 kPa treatment at 10℃ as compared to spermidine which was not detectable due to its low expression level. These results suggest that H20-5 produce proline as response to abiotic stresses. Further, we are planning to extend this research to clarify the plant-microbe interaction.
[Supported by grants from RDA]

Keywords: *Bacillus mesonae*, Abiotic stress, Spermidine, Proline

B032

Impact of *Bacillus velezensis* GH1-13 on Rhizosphere Bacterial Communities of Pepper under Different Fertilization Regimes

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The strain, *Bacillus velezensis* GH1-13 promotes plant growth and has strong antagonistic activities against pathogens, leads to enhance crop productivity. The field experiment was carried out to evaluate the impact of inoculation (seed dipping) with GH1-13 culture broth on rhizosphere bacterial community structures of pepper (*Capsicum annuum* L., chili pepper) under different fertilization regimes (no fertilizer, compost, chemical fertilizer, and compost+chemical fertilizer). Bacterial diversity of pepper rhizosphere decreased in fertilized plots compared with non-fertilized plot and decreased in plots inoculated with GH1-13. Rhizosphere bacterial community was significantly altered by applying chemical fertilizer and by inoculation with GH1-13. The relative abundance of the phylum *Proteobacteria* increased in the plots treated with chemical fertilizer but the relative abundance of the phyla *Planctomycetes* and *Parcubacteria* decreased significantly. The phyla *Planctomycetes*, *Parcubacteria* and *Nitrospirae* had higher relative abundance in all plots inoculated with GH1-13 than no-inoculated plots. These results suggest that application of chemical fertilizer and inoculation with beneficial microbes can decrease rhizosphere bacterial diversity and change the rhizosphere bacterial community structure.
[Supported by grants from RDA]

Keywords: *Bacillus velezensis*, Fertilization, Rhizosphere, Bacterial community

B033

Three New Records of *Penicillium* Species Isolated from Rhizosphere of *Cypripedium guttatum* in Korea

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To investigate mycorhyzal fungi of *Cypripedium guttatum*, we collected soil sample from the plant grown area (Mountine Hambaek, Korea). As a result, we isolated two unrecorded *Penicllium* species – *Penicillium soppii* in section *Ramosa* and *Penicillium yezoense* in section *Aspergilloides* - and one novel *Penicillium* species in section *Lanata-Divaricata*. Idenfitication of these strains were based on morphological characterization and phylogenetic analysis using ITS, beta-tubulin and calmodulin. Here, we present their mycological characters - phylogeny, mycelial growth on CYA, MEA, YES and CREA, and morphology of conidiophore.

Keywords: *Penicillium*, Rhizosphere, New records

B034

Understanding of Soil Microbiome by Host Genotype

Kyu-Chan Lee, Hyelim Keum, and Woo Jun Sul*

Chung-Ang University

In rhizosphere, the interaction of microbiome and plant root exudates are associated with plant growth and health. The plant root exudates are chemical compounds which influence the structure of root microbiome and are secreted by exocytosis. Vesicle-associated membrane protein (VAMP) plays a role of vesicle fusion and exocytosis. Particularly, VAMP721 and VAMP 722 plays important roles such as plant growth, immunity, and abiotic stress resistance. In this study, we analyzed the root microbiome community and functions (metagenome) for the understanding of soil microbiome from a total of three types of Arabidopsis thaliana (wild type, *vamp721* mutant, and *vamp722* mutant). The microbiome and the composition of gene products were different by the *A. thaliana* type. LEfSe (Linear Discriminant Analysis Effect Size) discovered that *Flavobacterium*, *Nitrospira*, and *Gemmata* were enriched in the wild type compared to the mutants. Genes from rhizosphere metagenome were compared, and distinct gene products were related to different types of *A. thaliana*. We found that genes for adaptations to environmental changes were related to the *vamp*721/722 mutants, while those for nutrient transports and carbon metabolism were related to the wild type. Thus, we suggest that the improvement of plant growth, immunity, and stress resistance are involved in the interactions of microbiome and plant root exudates, and enriched microbes in the wild type are key beneficial bacteria in the root ecosystem.

Keywords: Symbiotic microorganisms, Rhizosphere microbiome, Community analysis, Meta-dielectrics

B035

Genomic and Metabolic Analysis for Novel BTEX Compounds Degradation Bacterium Which Isolated from Nakdong River

Hyun Mi Jin

National Nakdonggang Institute of Biological Resource

The purpose of this study was to investigate what novel BTEX (Benzene, Toluene, Ethylbenzene, o-,m-,p-Xylene) biodegradation pathway from a bacterium, *Rhodococcus* sp. ABRD24, responsible for bioremediation of contaminated freshwater sediment. An enrichment culture was established using freshwater containing BTEX compounds to isolate a BTEX degrading bacterium from contaminated freshwater. The genomics and metabolomics analysis were also established for the BTEX-degrading bacterium. As the results, strains ABRD24 was isolated from enrichment system and degradation tests revealed that isolated strain had a great degradation ability for BTEX compounds in freshwater sediment. In addition to physiological analysis, the monoaromatic compounds degradation pathway was estimated from genomics and GC/MS based metabolomics analysis. Complete genome sequence and detected metabolites showed that strains harbors genes encoding many oxygenase such as toluene mono-oxygenase and hydroxylating enzymes responsible for degradation of various aromatic hydrocarbons. Further studies may be characterize the new gene cluster of the new pathway and discuss their functions in BTEX biodegradation of this strain.

Keywords: Biodegradation, Rhodococcus, BTEX

B036

Isolation and Identification of *Alternaria* Pathogen on Compositae in China

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Alternaria is a ubiquitous fungal genus that includes saprobic, endophytic and pathogenic species. This study aims to the diversity of the pathogenic *Alternaria* species on Compositae. In 2015-2018 years, disease samples were collected from 29 cities, 18 provinces, around China. 398 strains with *Alternaria*-type spores were obtained by single spore isolation. Among them, 17 strains were selected to characterize based on morphology, sequence analyses and pathogenicity test. Six genes of rDNA ITS, GAPDH, EF-1α , RPB2, Alt-a1 and ATPse were used for PCR amplification. During investigation, 3 new species and 4 new records (*A. argyranthemi*, *A. calendulae*, *A. cinerariae*, *A. tillandsiae*) were found, 5 known species (*A. helianthificiens*, *A. leucanthemi*, *A. sonchi*, *A. tagetica*, *A. zinnae*) were described. The pathogenicity tests revealed that the 12 species were pathogenic to their hosts. This article focused on the diversity and phylogenetic relationship of the pathogenic *Alternaria* species on Compositae in China, laid foundation on plant disease prevention and control.

Keywords: *Alternaria*, Taxonomy, Compositae, Morphology, Phylogenetic

B037

A New Record of *Alphamyces chaetifer* Isolated from a Freshwater Sample Collected at Sejong, Korea

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In a survey of indigenous fungal diversity in Korea, a strain of EML-GBW1-1 belonging to a family Alphamycetaceae within Rhizophydiales was isolated from freshwater samples collected at a pond located in Sejong, using a bait method. To identify the fungus at the species level, detailed morphological studies and rDNA sequence analyses were performed. BLASTn search indicated that the identity values of 28S rDNA sequences of EML-GBW1-1 isolate represented 99.5% (826/830 bp). Based on the phylogenetic analyses, EML-GBW1-1 strain was closest to *Alphamyces chaetifer* (GenBank accession no. NR_060383). On artificial medium, mature sporangia were spherical and ranged 13.4–24.8 μm in diameter. Hair-like rhizoids which emanated from the sporangial wall were abundant, slender, branched or unbranched and ranged 29.5–52.3 μm in length. Spores were released through a discharge pore. Upon discharged, zoospores were usually germinated in clusters. Germlings developed a rhizoidal axis and ranged 0.8–1.0 wide x 3.2–5.3 μm long. Our study shows that the EML-GBW1-1 strain is an undescribed species, *Avachytrium chaetife* in Korea. This finding is very significant in diversity study of undiscovered taxa, Chytridiomycota.

Keywords: Alphamycetaceae, *Alphamyces*, Chytridiomycota, Freshwater

C001

Plant Growth-promoting Activity of Extracellular Beta-propeller Protein YxaL Conserved in Bacillus Species

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The protein YxaL is conserved within the *B. subtilis* species complex, whose members are proficient for plant and soil. The amino acid sequence of YxaL contains a signal peptide (1-44 amino acid residues) and the mature portion (45-415 a.a.) of YxaL contains a repeated beta-propeller domain. However, the subcellular location and function of YxaL was not defined. The *yxaL* gene in *B. velezensis* strain GH1-13 was cloned and transformed in *E. coli* to overproduce the protein. Proteomic analysis and western blotting revealed that YxaL was constutively expressed and secreted into the medium out of the cells. When treating seeds with purified YxaL, a sub-nM range of YxaL showed a significant effect on the development of plant roots after seedling emergence and increased tolerance against drought stress during seedling growth of pepper (*Capsicum annuum* L.). YxaL induced effects on the growth of the lateral roots and root hairs (later stage of seedling), but not of the primary roots (early stage of seedling), were shown with the gene expression patterns of key enzymes in auxin, ethylene, and abscisic acid biosynthesis pathways. We present the first evidence that the extracellular beta-propeller protein YxaL is released into the medium and exerts a strong impact on plant root growth and tolerance against stress.
[This study was supported by Agricultural Science & Technology Development (PJ012467062018), Rural Development Administration, Republic of Korea.]

Keywords: Bacillus, Extracellular beta-propeller protein, Plant growth-promoting activity, Stress tolerance

C002

Phosphoproteome Analysis for Identification of Sexual Development Factors in *Aspergillus fumigatus*

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Aspergillus fumigatus is one of the opportunistic fungal pathogens, which can cause aspergillosis in human. The first report on induction of sexual development, which takes 6 months to produce sexual organs (cleistothecia), was published in 2009. Supermater pair, which produces cleistothecia in 4 weeks, was identified in 2011. In contrast to asexual development and virulence, however, no report on the molecular mechanism of sexual development has been published largely because of the lack of method suitable for omics studies. In order to overcome these limitations, we developed a novel procedure called 'Vegetative Mass Mating (VeM)', by which sexual stage-specific homogeneous cultures can be obtained. The VeM procedure also gives money- and time-saving advantages by producing cleistothecia on small size of agar plate within 2 weeks. Our improvement makes omics approach for sexual development feasible, therefore, phosphoproteome analyses were performed. The initial attempt revealed 43 phospho-spots from *MAT1-1*, 31 from *MAT1-2*, and 70 from mating culture. Among those from mating culture, 26 spots were mating-specific. Additional of phosphoproteome analyses and functional analysis of the identified proteins are under performing. Although further experiments are required, we established novel experimental procedure suitable for omics approaches, which will provide new insight into the sexual differentiation and virulence of the opportunistic fungal pathogen, *A. fumigatus*.

Keywords: *Aspergillus fumigatus*, Vegetative mass mating, Sexual development, Phosphoproteome

C003

Changes of Free Amino Acids and Flavor Components of Cultured Mycelium of *Lentinus edodes*

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Lentinula edodes (LE) were mushrooms belonging to the mushroom family of mycorrhizal fungi. Mycelium was cultured mainly in PDA (Photato Dextrose Agar) or PDB (Photato Dextrose Broth) medium. Changes in the amino acid content of these cultivars affected the growth and quality of fruiting bodies. In this study, the changes of the flavor components and the changes of 20 free amino acids were observed during the incubation period in the cultivation of LE in PDB (Photato Dextrose Broth) medium. The total cultivation period was 90 days and the total height of the free amino acids and the change of the fragrance components were observed in 45 days. As a result, 10 amino acids which were increased compared to the control were identified, among which Cystein increased from 9,889 ± 3 μg/L at first to 12,909 ± 2 μg/L at 45 days and 29,256 ± 4 μg/L at 90 days. The amino acids with decreased expression patterns were identified as 5 kinds. Arginine decreased to 83,751 ± 2 μg/L after 45 days at the first 161,787 ± 1 μg/L and decreased to 79,055 ± 7 μg/L at 90 days. In addition, one of the reduced amino acids was identified after the decrease and after the increase. Citruline was not expressed on the initial medium but was produced at 7,646 ± 6 μg/L on the 45th day of cultivation, but not on the 90th day. The change of amino acid was considered to affect the fragrance component, and the fragrance component analysis was performed by electronic nose analysis. Methanethiol, propanal, 1,2-butanediol, Gamma-terpinene, and Myristicin were identified immediately after shiitake mushroom application and Trimethylamine, Heptane, 2-Methylthiophene, Methyl 2-methylbutanoate, 2,3-Butanediol and Methyl nonaonoate The fragrance was confirmed. Trimethylamine, 1-butanamine, and 1-chloropentane were found on the 90th day of culture. The above results showed that the change of the free amino acid will affect the change of the fragrance component and the correlation with the changed amino acid and fragrance component will be further confirmed.

Keywords: *Lentinus edodes*, Free amino acids, Flavor components, Mycelium

C004

Characterization of Eukaryotic Proton Pumping Rhodopsin

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Microbial rhodopsins as a kind of photoreceptor harvest light energy for chemical energy synthesis and photo-signal transduction. Those genes were discovered in various species not only prokaryotic cells but eukaryotic species such as green algae and fungi. Microbial rhodopsin consists of seven transmembrane helices connected with retinal chromophore.
In this study, we chose a putative Bacteriorhodopsin-like gene from terrestrial algae which includes conserved amino acids for proton pumping activity. Chemically synthesized new algae rhodopsin was connected to the N-terminus Intein protein in pTWIN vector and successfully expressed in *E. coli* BL21(DE3) pLysS cell. After purification, the photochemical properties of rhodopsin were determined by UV-VIS spectroscopy. The absorption maximum of new algae rhodopsin was shown in 540 nm at alkali condition and two pKa value were calculated through pH titration. In case of proton donor mutant, the photochemical properties were similar with wild type. Furthermore, we tried to determine the light dependent chromophore configuration and proton pumping activity.
[This research was supported by NRF-2017R1A6A3A11036336 and NRF-2016R1D1A1B03934398]

Keywords: Photoreceptor, Proton pump, Eukaryotic rhodopsin, Membrane protein expression

C005

Biochemical Study of Putative Carotenoid-binding Rhodopsin

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Rhodopsin is composed of seven transmembrane helices with retinal as chromophore. Some microbial rhodopsin has the function of light-driven proton pump and it widely spread among freshwater bacterioplankton. The putative carotenoid-binding rhodopsin (PCBR) gene was chemically synthesized, cloned to pKA001 vector and expressed in UT5600 *E. coli* expression system. The all-*trans* retinal was treated to generate photoreactive chromophore bound rhodopsin, and expressed PCBR showed red. PCBR was purified with Ni-NTA resin by the batch method, and final yield was around 2 mg/ml. The characterization of purified PCBR was performed by proton pumping activity assay and retinal configuration assay in the light-dependent manner. In compare with *Gloeobactor* rhodopsin (GR) gene, PCBR has the potential residue to bind with carotenoid. To investigate the interaction between PCBR and carotenoid, UV/V is spectroscopy was used. The UV/Vis spectra of PCBR/carotenoid complex showed a peak shift toward red wavelength relative to PCBR without carotenoid. In this study, PCBR was acquired with carotenoid and recognized evidence of molecular relationship between PCBR and carotenoid existence. Based on the results, we will further try to figure out the molecular relationship between carotenoid and PCBR in various conditions.
[This research was supported by NRF-2016R1D1A1B03934398 and NRF-2017-R1A6A3A11036336]

Keywords: Rhodopsin, Caretenoid, Photoreceptor, Membrane protein

C006

A Physiological Study of *Faecalibacterium prausnitzii*

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Faecalibacterium prausnitzii (*FP*) is one of the most abundant bacteria in the human intestinal microbiota and regarded as a major effector in human intestinal health because of its anti-inflammatory effects. It produces butyric acid which has beneficial effect on human gut health. However, the extreme oxygen sensitivity has been a major obstacle to cultivate and study physiological characteristics of this organism. To investigate physiological characteristics of *FP*, its growth on various types of sugars was tested and the results showed that glucose was the most preferred sugar. Butyric acid was accumulated to 120–160 ppm in the growth supernatant, when glucose, fructose, or *N*-acetylglucosamine was supplemented. The genes encoding Butyryl-CoA dehydrogenase (BCD) and Butyryl-CoA: Acetate-CoA-transferase (BUT) in *FP* were cloned and expressed in *E. coli* to determine the effect of butyric acid production on intestinal health. The two genes were cloned individually or together under the control of the constitutive *cat* promoter into the plasmid pACYC184 and pBR322. According to the results of butyric acid production in wild-type *E. coli* expressing BCD, BUT or both, BCD was shown to be essential, while BUT was dispensable, for the production of butyric acid. The anti-inflammatory effects of butyric acid production were tested by administrating these strains into DSS-induced colitis and oxazolone-induced atopic dermatitis model mice.
[Supported by grants from KIST (2Z05310)]

Keywords: *Faecalibacterium prausnitzii*, Sugar, Butyric acid, Anti-inflammatory

C007

Putative APSES Transcription Factors, Swi4 and Swi6 are Involved in Osmotic Stress Response, Gliotoxin Biosynthesis and Conidial Hydrophobicity in *Aspergillus fumigatus*

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The APSES protein group is a transcription factors having a domain of basic helix-loop-helix (bHLH). Previously reported members (APSES: Asm1, Phd1, Sok2, Efg1 and StuA) were used to refer to this protein group and were known to be the key regulators of fungal development and other biological processes. StuA, an APSES transcription factor of *Aspergillus nidulans*, is known to regulate the expression of genes related to asexual development such as *abaA*. Another APSES gene, *rgdA* has been reported to involved in mycelial growth and conidia production. In this study, novel functions of putative APSES transcription factors, *swi4* (Afu3g13920) and *swi6* (Afu7g05620) in *Aspergillus fumigatus* were identified by knock-out and over-expression mutation. Both of their deletion mutants show increased production of gliotoxin compared to WT (AF293), whereas over-expression mutations reduced. In addition, the hydrophobicity of conidia in these deletion mutants was significantly reduced, whereas the over-expression mutants were observed that the hydrophobicity of the conidia was higher than WT. On the other hand, Δ*swi6* was sensitive to osmotic stress but Δ*swi4* was not affected. RNA-seq analysis of the Δ*swi4* and Δ*swi6* strains showed that the transcripts of the *gli* gene cluster were increased in both mutants while the expression of hydrophobin genes, including *rodA*, was significantly reduced.
[This work was supported by grant from the National Research Foundation (NRF).]
Keywords: APSES, *Aspergillus fumigatus*, Gliotoxin, Hydrophobin

C008

Improved Functionality of *Leuconostoc mesenteroides* NK-3 by Addition of the Extracts Derived from Oyster Mushrooms

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The aim of this study was to investigate various functionalities of lactic acid bacteria (LAB) isolated from oyster mushrooms and to assess the efficacy of enhanced activities by addition of mushroom extracts. Initially, NK-3 bacterium isolated from the mushroom was obtained via the enrichment culture technique. With use of BIOLOG system and 16S rRNA sequencing, the isolate was identified and assigned to *Leuconostoc mesenteroides* NK-3. Phylogenetic tree of *Leuco. mesenteroides* NK-3 was plotted based on 16S rRNA sequence comparisons. Various functionalities (*e.g.*, tyrosinase inhibitory activity, ACE inhibitory activity, SOD-like activity, antioxidant activity, depletion of sodium nitrite, production of exopolysaccharide, antibacterial activity) of *Leuco. mesenteroides* NK-3 cultures in the presence as well as in the absence of mushroom extracts were evaluated and compared. According to the results, all of the functionalities evaluated in this study were considerably higher in the cultures with 10% mushroom extracts than in the cultures without the extracts. This study demonstrated that mushroom extracts would be effective for improving the functionalities of LAB.

Keywords: Lactic acid bacteria, *Leuconostoc mesenteroides*, Functionality, Oyster mushroom

C009

Cellular Responses of *Legionella pneumophila* Exposed to Epigallocatechin Gallate (EGCG) Derived from Green Tea

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The aim of this study was to examine the cellular responses of *Legionella pneumophila* exposed to a major green tea component, EGCG (epigallocatechin gallate). EGCG showed a dose-dependent bactericidal effect on *L. pneumophila*. Western blot using anti-DnaK and anti-GroEL monoclonal antibodies was performed to investigate the expression of stress shock proteins (SSPs) in *Legionella pneumophila* exposed to EGCG. The amount of SSPs was induced as the exposure time increased and decreased. The molecular weights of DnaK and GroEL were 70 kDa and 60 kDa, respectively. SDS-PAGE with silver staining revealed that the amount of lipopolysaccharides increased or decreased in the strain treated to different concentrations and exposing periods of EGCG. Scanning electron microscopic analysis demonstrated the presence of perforations and irregular rod forms with wrinkled surfaces in cells treated with EGCG. These results suggest that EGCG is effective against *L. pneumophila* and has potential for use in phytochemical detergents.

Keywords: *Legionella pneumophila*, Epigallocatechin gallate, Green tea, Cellular responses

C010

Identification of the Components of the Phosphotransferase System in *Faecalibacterium prausnitzii*

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Faecalibacterium prausnitzii, an extremely oxygen sensitive Gram-positive bacterium, is known to be one of the most abundant bacteria in the human intestinal microbiota of healthy adults. This obligate anaerobe produces substantial amounts of butyrate, which has anti-inflammatory effects in the gut. In bacteria, the phosphoenolpyruvate-dependent phosphotransferase system (PTS) is the predominant mechanism used for the efficient uptake of carbohydrates. The phosphorylation status of the PTS components reflects the availability of carbohydrates and the energy conditions of the cell. While studies have been conducted on this bacterium's importance for human health, little is known about its PTS. Here, we identify all of the 18 PTS components in the *F. prausnitzii* A2-165 genome, categorizing each into their proper sugar family. A unique feature of the *F. prausnitzii* PTS is that it possesses two paralogs of EI, HPr, and $EIIBC^{glc}$. Through *in vitro* cross-phosphorylation assays with *Escherichia coli* EI and HPr, we characterize each of the *F. prausnitzii* PTS components, renaming certain components based on their ability to do phosphorelay and transport sugars.
[This work was supported by grants from the BK21 plus program through the National Research Foundation (NRF) funded by the Ministry of Education of Korea]

Keywords: *Faecalibacterium prausnitzii*, Phosphotransferase system, Sugar transport

C011

Neuroprotective Effect of *Rumminococcus albus* against Beta-amyloid-induced Apoptosis in SH-SY5Y Cell

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In recent years, research has focused on the interaction between gut microbiota and brain. Beta-amyloid ($A\beta$) is known as the main component of amyloid plaques found in the brains of Alzheimer patients. $A\beta$ peptides in brain generate reactive oxygen species, resulting in the neuronal cell death and DNA damage. The aim of this study was to investigate the protective effect of intestinal *Ruminococcus albus* on $A\beta$-induced neuronal SH-SY5Y cell death. To investigate neuroprotective effects of heat-killed *R. albus* (HKR) on SH-SY5Y cell viability was measured by MTT assay. HKR increased SH-SY5Y cell viability compared with the negative control. SH-SY5Y cell survival against $A\beta$ was measured by soft agar colony formation assay and DNA damage was measured by comet assay. HKR protected SH-SY5Y cells from $A\beta$-induced cell death. HRK significantly decreased DNA damage in $A\beta$-treated SH-SY5Y cells. Expression ratio of *bax* and *bcl-2* genes known to be associated with apoptosis was measured by real-time PCR. HKR significantly decreased the bax/bcl-2 ratio in $A\beta$ -treated SH-SY5Y cell compared with the negative control. In conclusion, HKR has protective effect against $A\beta$-induced SH-SY5Y cell death.
[Supported by grants from Korea University]

Keywords: *Ruminococcus albus*, Beta-amyloid, Apoptosis, SH-SY5Y cell

C012

Effect of Treatment of Useful Microbial Culture on Growth of Lettuce

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In this research, we wanted to compare the effect of microorganisms developed recently on the growth after treating useful microorganisms to lettuce seeds to improve efficiency. The useful microorganisms used in this experiment were photosynthetic bacteria (PS-2) and pseudomonas (GHR1-1), two patent strains of the Rural Development Agency. Each microorganism was diluted to 1×10^7, 1×10^6, 1×10^5 concentration on the basis of 1×10^9 CFU/ml and processed into seeds. As for the treatment method, after sowing lettuce seeds on Petri dish with filter paper put in it, treat 2 kinds of useful microorganisms with pipettes, treat amount like concentration, then keep at 25℃ and set germination rate and rooting Was measured. As a result of the experiment, GHR1-1 was good in the order of 1×10^6, 1×10^7 in the order of germination rate and roots length at 1×10^5 concentration. In the case of PS-2, at the concentrations of 1×10^5 and 1×10^6, the germination rate and the length of roots were similar, the results were similar, but the 1×10^7 concentration showed a tendency of the result value to drop a little. In other words, the lower the concentration of microorganisms, the better the germination rate and root length.

[This study was supported by Rural Development Administration 's joint research project (Project No: PJ0108252018)]

Keywords: Effective microorganism, Growth promotion, Lettuce

C013

Lifespan Extension of *Caenorhabditis elegans* by *Bacillus* Species through Stimulation of Innate Immune System

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Bacillus species are found in Korean traditional fermented foods such as kimchi and soybean paste. *Bacillus* species are known to contribute to human health. In this study, *Bacillus subtilis* and *B. amyloliquefaciens* were isolated from soybean paste and investigated *Bacillus*-dependent lifespan extension in *C. elegans*. *B. subtilis*, *B. amyloliquefaciens* or *Escherichia coli* OP50 was administered to *C. elegans* and lifespan was measured. To evaluate the effect of retarding aging, locomotory activity and body size were measured. Lifespan in loss-of-function mutants was measured to elucidate the target of *Bacillus* species. *B. subtilis* strain JB15 significantly extended the mean lifespan of *C. elegans* compared with *E. coli* OP50; while *B. amyloliquefaciens* JB19 did not extend lifespan of *C. elegans*. Administration of *B. subtilis* JB15 increased locomotory activity and decreased body size compared with *E. coli* OP50-fed worms. Lifespan was not extended in loss-of-function mutants of *daf-12*, *dbl-1, sma-4, pmk-1, daf-7, sma-9, daf-2* and *daf-16*, which highlights the potential role of these genes in *Bacillus*-induced longevity in *C. elegans*. In addition, *B. subtils* JB15 protected *C. elegans* from *S. typhimurium* infection. Interestingly, *B. amyloliquefaciens* JB19 protected *C. elegans* from *S. typhimurium* infection. In conclusion, *B. subtilis* JB15 might extend lifespan of *C. elegans* and increase pathogen resistance of *C. elegans* by overall enhancing innate immunity.

[Supported by KU]

Keywords: Bacillus, Innate immune, Lifespan, *C. elegans*

C014

Isolation and Characterization of Protease Producing *Bacillus* sp. DP1-8 from Landfill Soil

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Microbial strains exhibiting proteolytic activity were isolated from landfill site in Jeju, Republic of Korea. Then, the physiological properties and 16S rRNA sequences of isolated microorganisms were analyzed. All of the isolated 50 bacterial strains formed clear zones around their colonies when grown on NA supplemented with skim milk. Strain DP1-8 showed the highest protease activity among them. The strain was identified as *Bacillus* sp. based on morphological, physiological characteristics and 16S rRNA. The isolated The Optital Density (O.D) was recorded every 24 h for three days. As shown in most of the culture achieved their maximum growth within the first 24 h. Although further studies are needed to characterize the protease and enhance its activity, the newly isolated protein-degrading *Bacillus* sp. DP1-8 can be applicable for the production of peptides and for the degradation of proteins in various industries.

Keywords: Protease activity, Bacillus, 16S rRNA, Landfill, Degradation

C015

Iron Regulates Zinc Metabolism in *Aspergillus fumigatus*

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Aspergillus fumigatus, one of the most common causes of fungal pathogenesis, requires essential metal nutrients for growth. Zinc and iron are indispensable metals that are required for the maintenance of cellular homeostasis and are important virulence factors of fungal pathogenesis. Recently it has been reported that there are close relationships between iron and zinc metabolisms. From microarray analysis, we determined that the deletion of *Afmac1* resulted in the downregulation of *ZrfA*, *ZrfB*, and *ZrfC*, which encode zinc transporters, and confirmed this finding by northern blot analysis in *A. fumigatus*. The expression of zinc transporters was downregulated under iron-limited conditions, even when zinc was limited. However, copper limitation did not affect the gene expression of zinc transporters. Furthermore, deletion of *HapX* resulted in the downregulation of *ZrfA*, and the expression of *ZrfA* was upregulated in an iron-dependent manner. Higher zinc uptake activity was investigated at high concentrations of iron, and the deletion of *HapX* resulted in lower zinc uptake activity than in wild-type *A. fumigatus*. The conserved iron-binding motif REXXE was found from ZafA, and mutation of the iron-binding motif resulted in lower zinc uptake activity than in the wild type. Furthermore, gene expression of *ZafA* and *ZrfA* were downregulated by mutation of the REXXE motif, especially when the first glutamic acid was mutated to alanine (E1A). Taken together, these results suggest that iron regulates zinc metabolism by modulating ZafA expression, and iron and zinc regulation is coordinated in *A. fumigatus*.

Keywords: Zinc transporter, Iron-binding motif, Uptake assay, *A. fumigatus*

C016

Identification of Siderophores Produced by Microorganisms Using *Saccharomyces cerevisiae* Mutants

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The separation and identification of siderophores produced by microorganisms is a time-consuming and high cost procedure. We have developed a fast and low cost method to identify siderophores using well-established *Saccharomyces cerevisiae* deletion mutants. The *Δfet3,arn* strains fail to sustain growth, even when specific siderophores are supplied, and those mutants are siderophore-specific; *Δfet3,arn2* for TAFC, *Δfet3,arn1,sit1* for FC, and *Δfet3, sit1* for FOB. The culture broth of *Fusarium graminearum* was separated by HPLC, and each peak was subjected to a plate assay using *S. cerevisiae* mutants. We have found that each peak contained specific siderophores produced by *F. graminearum*, and these coincided with reference siderophores. This method will save time and cost in the identification of siderophores produced by microorganisms.

Keywords: Siderophore, Transporter, Iron, *S. cerevisiae*

D001

Ferment Extract of *Aureobasidium pullulans* GJW Induce Migration of Human Keratinocytes

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AMOREPACIFIC R&D Unit

Aureobasidium pullulans is a black-yeast-like fungus used for production of the polysaccharide pullulan, a neutral polysaccharide of repeating maltotriose units, which has numerous applications in medicine, cosmetics, the food industry, and other fields. Ferment extracts of *A. pullulans* strains isolated in Jeju Island were evaluated properties as active ingredients for cosmetics. *A. pullulans* strain named GJW had abundant productivity of crude exopolysaccharides (EPS, 3.25 mg/ml of culture broth), and the extract induced cell migration of human keratinocytes in dose-dependent manner. It was more effective than a fine pullulan or madecassoside, which had a wide range of reported biological activities including, anti-inflammatory, wound healing, and anti-oxidant activities. These results suggested that the ferment extract of *A. pullulans* GJW might be a potent therapeutic ingredient for skin care products.

Keywords: *Aureobasidium pullulans*, Ferment, Pullulan, Migration, Skin care

D002

Metabolic Engineering of *Ralstonia eutropha* for the Biosynthesis of 2-Hydroxyacid Containing Polyhydroxyalkanoates

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Polyhydroxyalkanoates (PHAs) bio-based polyesters synthesized by various microorganisms. Since material properties of PHAs are highly dependent upon their types and compositions of their monomers, microbial system to design polyesters containing novel monomers, which might confer superior material properties have been developed. Although PHAs consisting of 2-hydroxyacids as monomer units from *Escherichia coli* have attracted much attention, their production has not been efficient. Since *Ralstonia eutropha* is the one of the most efficient host strain for the production of PHAs from renewable resources, we report the metabolic engineering strategies for the development of recombinant *Ralstonia eutropha* strains to synthesize PHAs containing 2-hydroxyacids as monomers. This was achieved by the construction of base *R. eutropha* strains that express engineered PHA synthase able to use 2-hydroxyacyl-CoAs (2HA-CoAs) as substrates and engineered propionyl-CoA transferase to synthesize 2HA-CoAs. Detailed metabolic engineering strategies for the construction of versatile recombinant *R. eutropha* strains to produce PHAs containing various 2-hydroxyacid monomers will be presented.

[This work was supported by the Technology Development Program to Solve Climate Changes (Systems Metabolic Engineering for Biorefineries) from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012-M1A2A2026556)]

D003

Production of Poly(2-hydroxyisovalerate-co-lactate) through Engineered *Escherichia coli*

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Recently, metabolically engineered microorganisms has been successfully producing polyhydroxyalkanoates (PHAs) containing 2-hydroxyacids such as lactate (LA) and 2-hydroxybutyrate (2HB). Here, we have engineered *E. coli* for producing PHAs containing 2-hydroxyisovalerate (2HIV). *E. coli* strain expressing *ilvBNmut* (feedback resistant mutant), *ilvCD*, *panE* along with *pct540* gene encoding evolved propionyl-CoA transferase and *phaC1437* gene encoding evolved PHA synthase is able to produce a new polymer, poly(13.2 mol% 2HIV-*co*-7.5 mol% 2HB-*co*-42.5 mol% 3HB-*co*-36.8 mol% LA). Further deletion of *poxB*, *pflB*, *adhE* and *frdB* genes encoding enzymes involved in competing pathways allowed the *E. coli* strain to produce poly(20 mol% 2HIV-*co*-80 mol% LA), with the polymer content of 9.6% w/w. These results suggest novel PHAs containing 2HIV can be produced by engineering branched-chain amino acid metabolism.

[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A 2026556 and NRF-2012M1A2A2026557) and NRF grant funded by the MSIP (NRF-2016R1A2B4008707)]

Keywords: Polyhyroxyalkanoate, Metabolic engineering

D004

Engineered *Escherichia coli* Strains for the One-step Production of Aromatic Polyesters from Glucose

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Currently produced from petroleum, aromatic polyesters are widely used as commodity plastics. We have engineered *Escherichia coli* strains to produce aromatic polyesters from glucose through one-step fermentation. *Clostridium difficile* isocaprenoyl-CoA:2-hydroxyisocaproate CoA-transferase (HadA) and evolved polyhydroxyalkanoate (PHA) synthase genes are overexpressed in a D-phenyllactate-producing *E. coli* strain, poly(52.3 mol% 3-hydroxybutyrate (3HB)-*co*-47.7 mol% D-phenyllactate) is produced from glucose and sodium 3HB. Also, various poly(3HB-*co*-D-phenyllactate) polymers having 11.0, 15.8, 20.0, 70.8, and 84.5 mol% of D-phenyllactate is produced from glucose as a sole carbon source through additional expression of *Ralstonia eutropha* β-ketothiolase (*phaA*) and reductase (*phaB*) genes. The engineered bacterial system is a first attempt on the one-step fermentative production of aromatic polyesters from renewable resources.

[This work was supported by the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) and also by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation of Korea.]

Keywords: Metabolic engineering, Polyhydroxyalknoate

D005

Development of Feeds Using the Resource of Insects

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It is anticipated to be more challenging to secure biomass due to global warming and climate changes upon increasing the demand of food resources. It will be more difficult to secure the raw materials such as corn, especially, that is using as a feed. Hence, the applications and values of insect resource have been expanded more and they are utilized as the raw materials of general foods upon registration to expand the scope of insect resources. Fermentations using *Tenebrio molitor* (mealworm), *Hermetia illucens* (black soldier fly larvae), and *Gryllus bimaculatus* (two-spotted cricket) were performed to enhance the protein level and digestion absorption rate. The samples with fermentation showed the increased digestion absorption rate compared to the control group. In addition, the feed was produced upon proper mixture of mealworm, black soldier fly larvae and two-spotted cricket which were the source of protein, using fecal soil that was the high protein byproduct of insects and other substances. This utilization may be meaningful in terms of replacing effect of biological resources and development of biological materials under the unstable situation of future supply for crops due to the climate changes.

Keywords: Insects, Fermentation, Feed

D006

Selection of Lactic Acid Bacteria to be Used as Rice Straw Silage Fermentation

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Silage is feedstuff produced by controlled fermentation of the green fodder crop retaining the high moisture content. Lactic acid bacteria decrease the pH of the silage, thereby inhibiting the growth of spoilage bacteria and preserving the silage for a long period of time. In this study, we expand the application range of added lactic acid bacteria (LAB) for the production of high quality silage and investigated the possibility of the LAB strain as silage additives. Four strains selected by screening strains with good cellulase activity were applied to rice straw silage. As a result, the silage produced by *Leuconostoc mesenteroides* M17 strain was lower in pH, acid detergent fiber (ADF) and non detergent fiber (NDF) and higher in protein, lactic acid and relative feed value (RFV) than the silage produced by *Lactobacillus plantarum* CMRT strain. We also characterized its optimal growth conditions and determined cellulase activity to identify its potential for a silage additive.

Keywords: Silage, Cellulase activity, Lactic acid bacteria

D007

Characterization of *Weissella cibaria* JA1-1 as a Dextran Producer Isolated from Baechu Kimchi

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A strain of *Weissella cibaria* was isolated from Baechu kimchi that produced dextran and named as JA1-1. It was characterized in aspects of genetic relationships by RAPD-PCR and 16S rRNA sequencing. With primer LAB52, isolates such as *L. mesenteroides* JA2-3 and JB1-2 showed exactly same patterns of RAPD-PCR but *W. cibaria* JA1-1 showed a different pattern of RAPD-PCR comparing to *W. cibaria* JC2-3. With primer LAB65, both *L. mesenteroides* JA2-3 and *L. mesenteroides* JB1-2 revealed exactly same RAPD patterns however *L. mesenteroides* subsp. *mesenteroides* KCTC 3722 showed more typical RAPD DNA bands. RAPD DNA bands of *W. cibaria* JA1-1 were very different from others. This tendency was confirmed by other RAPD primers such as LAB74, 80, 86 and 94. Thus it is understood that RAPD-PCR is a valuable tool that can distinguish different species of lactic acid bacteria. Also phylogenetic analysis with 16S rRNA sequencing confirmed the results of RAPD-PCR. *L. mesenteroides* JA2-3 and *L. mesenteroides* JB1-2 were grouped into a same cluster, and both *W. cibaria* JA1-1 and JC2-3 were outgroup that was a branch of *W. cibaria*. From tests of dextran production, *W. cibaria* JA1-1 revealed its maximum dextran production in 20% sucrose (w/v) containing MRS media (8,220 $Pa.S^n$) rather than 30% sucrose (5,270 $Pa.S^n$). *W. cibaria* JA1-1 also showed sharp increase of viscosity under condition of treatment of skim milk while others did not show increase of viscosity.

Keywords: 16S rRNA, Baechu kimchi, Dextran, RAPD-PCR, *Weissella cibaria*

D008

Pan-genomic and Transcriptomic Analyses of *Lactobacillus sakei* during Kimchi Fermentation

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The genomic and metabolic characteristics of *Lactobacillus sakei*, one of the major lactic acid bacteria in kimchi fermentation, were analyzed by pan-genomic and transcriptomic analyses. *Lac. sakei* strains were identified based on 16S rRNA gene, average nucleotide identity (ANI), in silico DNA-DNA hybridization, molecular phenotype (presence or absence of genes) and core-genome of 36 strains of *Lac. sakei* strains from NCBI GenBank database. It was confirmed that they are separated into two types of phylogenetic lineages depending on the subspecies group. Pan-genome of *Lac. sakei* strains consist of 1,066 genes in core-genome, 1,546 genes in accessory-genome, and 707 genes in unique-genome, and their Clusters of Orthologous Groups (COG) of proteins and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed that *Lac. sakei* strains harbor different carbohydrate metabolism-related and intestinal adhesiveness related genes depending on each strain, and this probably indicates a high evolutionary genome change for the relevant genes. However, cofactors and vitamins metabolism-related genes are present in most strains; it is presumed to be a common feature of *Lab. sakei*. This study provides a new insight into the genomic and fermentation metabolic features of *Lac. sakei* in kimchi fermentation process through the pan-genomic and transcriptomic analyses.
[Supported by the World Institute of Kimchi (KE1802-2) funded by the Ministry of Science and ICT, Republic of Korea.]

Keywords: Kimchi, *Lactobacillus sakei*, Comparative genomics, Transcriptomic analysis, Fermentative metabolism

D009

Manufacture and Physicochemical Analysis of the Ginseng Vinegar Fermented with White-, Red-, and Black Ginseng

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Vinegar has various effects like anti-cancer and fatigue recovery as well as food sterilization effect. Different kinds of ginsengs, such as white ginseng (WG), red ginseng (RG) and black ginseng (BG), have different concentration of each ginsenoside and thus various biofunctional effects. The purpose of this study is to examine the effect of vinegar production from ethanol containing various ginsengs (WG, RG, or BG). Ethanol fermentation was carried out with rice and yeast containing white, red or black ginseng powders and then vinegar fermentation was performed with acetic acid bacteria to make ginseng rice vinegar. Changes in population of acetic acid bacteria, sugar contents, color and acidity during acetic acid fermentation were analyzed. The ginsenosides content of each ginseng vinegar was compared and analyzed through HPLC. And the contents of organic acids contained in ginseng vinegar were also measured by HPLC. These data can be helpful in the further development of new products using different ginsengs.
[This work was supported by grants from IPET. (No. 316014-03)]

Keywords: Fermentation, Ginseng rice vinegar, Ginsenoside

D010

Quantitative Validation and Analysis of Ginsenosides Composition of Ginseng Vinegar during Fermentation

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Ginsenosides, a bioactive compounds of ginseng, shows various beneficial effects on human body. They differ in absorption rate and efficacy according to the molecular form. To produce the ginseng rice vinegar, ginseng powder was mixed with rice and carried out the makgeolli fermentration and then vinegar production. Three different types of ginseng powder, such as white, red ginseng, and black ginseng, was used. *Acetobacter pasteurianus* was used as starter for vinegar production. In this study, we performed the qualitative and quantitative analysis of the ginsenosides from ginseng vinegar and validation based on accuracy, precision and reproducibility. And also, we determined the ginsenosides composition during fermentation by HPLC. As a part of result, selected ginsenosides including the main components, Rg1, Rb1, Rg3(S), as well as Re, Rf, Rg2(S), Rh1(S), Rh2(S), and compound K were found in the products of ginseng. In the HPLC validation experiment, the linearity of total ginsenosides were established by R2 values of more than 0.999 within the test ranges, and the recovery rate ranged from 98.2–105.3%. This study was demonstrated the production of naturally fermented ginseng rice vinegar, especially white, red or black ginsengs.
[This work was supported by grants from IPET. (No. 316014-03)]

Keywords: Ginsenoside, Ginseng rice vinegar, HPLC validation experiment

D011

Lichens Bioresource as a Novel Anti-adipogenic Agent

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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 16 of the extracts decreased the oil red O stained 3T3-L1 adipocytes. 16 lichen extracts were screened again by the expression of adipogenic transcriptional factors. 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. The results indicated that some lichen extracts are capable of inhibiting the differentiation process and lipids accumulation in 3T3-L1 preadipocytes, suggesting its potential function of anti-obesity.
[Supported by grants from Korea Forest Service and National Research Foundation of Korea]

Keywords: Anti-adipogenic effect, Anti-obesity, Adipocyte, Lichen substances, Lipids accumulation

D012

Ethyl Acetate Extract of the Endolichenic Fungus EL000327 Isolated from *Graphis* Exhibit Selective Cytotoxicity on Human Colorectal Cancer Cells

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Endolichenic fungi (ELF) are microbes which have capability to produce unique secondary metabolites with pharmaceutical properties while residing inside the lichen thalli. Here, 12 Ethyl acetate (EA) extracts of secondary metabolites of ELF isolated from Korean lichens were screened out to identify cytotoxic compounds against human colorectal cancer (CRC) Cells. EA extract of EL000327 isolated from *Graphis* collected from Hallasan in Jeju Island, South Korea in 2009 showed highest selective cytotoxicity against Caco2, human colorectal cancer cells, and CT26, mouse colon cancer cells. Green fluorescence-based active caspase 3/7 staining showed EL000327 crude extract induce the apoptosis in Caco2 cells. Active compounds were isolated from the extracts of endolichenic fungi by column chromatography and reverse-phase HPLC. Two active compounds within EL000327 crude extract were identified based on selective cytotoxicity and potency against Caco2 and MDCK (Madin-Darby canine kidney) cells. These results suggest that crude extract of EL000327 shows anti-cancer effect on Caco2 cells by induction of apoptosis and active compound in EL000327 have different mechanism of action on Caco2 and MDCK cell lines.
[Supported by a grants of Korea Forest Service and National Research Foundation of Korea]

Keywords: Endolichenic fungi, Secondary metabolites, Apoptosis, Human colorectal cancer

D013

Physciosporin Suppresses the Colorectal Cancer Cells

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Lichens, which represent symbiotic associations of fungi and algae, are potential sources of numerous natural products. Physciosporin (PHY) is a potent secondary metabolite found in lichens and was recently reported to inhibit the motility of lung cancer cells via novel mechanisms. Here, we investigated the effects of PHY on colorectal cancer (CRC) cells. PHY reduced the viability of various CRC cell lines (Caco2, CT26, DLD1, HCT116 and SW620). Moreover, PHY elicited cytotoxic effects by inducing apoptosis at toxic concentrations. At non-toxic concentrations, PHY dose-dependently suppressed the invasion, migration and colony formation of CRC cells. PHY inhibited the epithelial-mesenchymal transition (EMT) markers. Moreover, PHY modulated KAI1 C-terminal-interacting tetraspanin and KAI1 expression, and suppressed the downstream transcription factors c-jun and c-fos. Finally, PHY administration effectively decreased the growth of CRC xenografts in mice without causing toxicity. Together, these results indicate that PHY suppresses the growth and motility of CRC cells via novel mechanisms.
[Supported by grants of Korea Forest Service and National Research Foundation of Korea]

Keywords: Anticancer, Colorectal cancer, Metastasis, Tumourigenesis, Lichen, Natural product

D014

Characterization of Genome Structure and Flavor Profiles of Halotolerant Yeast Species Isolated from Korean Traditional Fermented Soybean Products

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Fermented soybean products have been getting the spotlight in the international market due to their nutritive value and many health benefits. During soybean fermentation, yeasts play salient roles in the production of diverse flavor compounds that are important to the quality of the soybean products. In this study, we analyzed the genome structure and flavor profiles of several yeast species isolated from Korean traditional fermented soybean products, called "Jang". Most yeast species isolated from "Jang" showed much higher halotolerance than *Saccharomyces cerevisiae*. The ploidy analysis by FACS and whole genome sequencing revealed the diverse ploidy: One of the *Debaryomyces* species, D-2 strain is haploid with the genome size of approx. 13 Mb, whereas the other C0-11-Y2 strain harbors the genome of 26 Mb, indicating diploid. Interestingly, C0-11-Y2 was halophilic, which grew better in the presence of salt. Moreover, these two strains exhibited different flavor profiles in the SPME-GC/MS analysis. The genome of *Wickerhamomyces anomalus* A30-7-Y4 appears as hybrid diploid and that of *W. subpelliculosus* SMY-04 as haploid. These two *Wickerhamomyces* yeasts generated significant amounts of acetate esters, an important aroma group, such as isoamyl acetate and phenethyl acetate. This study could help to develop multiple yeast starter cultures to improve and globalize Korean traditional Jang products with high quality and functionalities.
[Supported by a grant no. NRF-2017M3C1B5019295]

Keywords: Halotolerance, Yeasts, Fermented soybean products, Genome, Flavor

D015

In vitro Antimicrobial Activity of Active Compound of *Pseudomonas aeruginosa* BCNU 1204

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In the course of screening novel methicillin-resistant *Staphylococcus aureus* (MRSA) drugs, one bacterial isolate showed antibiotic activity to both Gram-positive and Gram-negative bacteria, and showed antibacterial activity even to MRSA. This strain was identified as *Pseudomonas aeruginosa* using phenetic and phylogenetic analysis. Dichloromethane (DCM) and ethylacetate (EA) extracts of *Pseudomonas aeruginosa* BCNU 1204 exhibited strong antimicrobial activity against Gram positive bacteria, and especially its EA extract exhibited strong inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA). One bioactive compound obtained by recycling preparative HPLC and preparative TLC was identified as phenazine-1-carboxylic acid belonging to phenazine derivatives using GC-MS analysis, and its minimum inhibitory concentration (MIC) values were determined to be below 50 μg/ml for MRSA strains. Therefore, further research is required to obtain various phenazine compounds from *Pseudomonas aeruginosa* BCNU 1204 and to use as a potential resource for development of antibiotics and antifungal agents.

Keywords: Antimicrobial activity, Methicillin-resistant *Staphylococcus aureus* (MRSA), Phenazine compounds, *Pseudomonas aeruginosa*

D016

Verification of Physiological Activity Transformation for Fermented Thistle (*Cirsium japonicum*) Roots by *Lactobacillus rhamnosus* BHN-LAB 105

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Thistle (*Cirsium japonicum*), commonly used to cooked potherb or tea, contains about 78 kinds of flavonoid compounds in a wild plant of Asteraceae in Korea. It has been reported to have excellent physiological effect such as an anti-microbial, anti-inflammatory, anti-cancer, detoxification, and immunity enhancement activities. Especially the roots of thistle, so called 'Daegyegeun' was reported to effect on an anti-diabetic, anti-obesity effects and used to treat for hematuria, hepatitis and hypertension. Besides, Lactic acid bacteria was informed to be involved to fermentation of foods and also having various physiological efficiencies such as entrails elutriation, anti-cancer effect. Fermentation of lactic acid bacteria could have benefit to elevate the nutrition or functionality to foods as well. It have been actively conducted on various studies to increase of the main active ingredients contents and the physiological activity for natural extracts through lactic acid fermentation. We investigated the fermentation of thistle roots using isolated *Lactobacillus rhamnosus* BHN-LAB 105 and change of physiological activity for fermented thistle roots extract. We confirmed that the fermented thistle roots showed advanced physiological activity through in this research.

Keywords: Fermentation, Thistle, *Cirsium japonicum*, Physiological activity, *Lactobacillus rhamnosus*

D017

Anti-diabetic Effect and Antioxidative Activity of Rice Fermented by *Monascus purpureus* BHN-MK 01

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Anti-diabetic effect of rice by fermented *Monascus purpureus* BHN-MK 01 as Red-yeast-rice for studies on the functional food materials. *Monascus* belongs to the phylum Eumycota, subphylum Ascomycotina, class Ascomycetes, order Eurotiales and the family Monascaceae. *Monascus* sp. has been used to produce for natural colourants, food supplements food colourant, preservative, food supplement, and traditional medicine over 1,000 years in East Asia. Currently, red yeast rice has become one of the major cholesterol-lowering ingredients used in food supplements worldwide. Red-yeast-rice has become one of the leading cholesterol-lowering ingredients used in food supplements throughout the world. Red-yeast-rice has been used in Chinese medicine to strengthen the spleen, promote or improve digestion, eliminate dampness and phlegm, promote or improve blood circulation, and remove blood stasis. In this study, fermented rice from *M. purpureus* BHN-MK 01 was investigated anti-diabetic effect. As a result, we confirmed that fermented rice by *M. purpureus* BHN-MK 01 can use functional food materials and an excellent strain to producing for useful substances.

[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (117104-02-1-CG000)]
Keywords: Anti-diabetic effect, Fermentation, *Monascus*

D018

Biosynthesis of α, ω-Dicarboxylic acids for Polymeric Monomers from Plant Oil by *Yarrowia lipolytica*

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Growing environmental concerns have stimulated attention on the efforts toward producing bio-based plastics from renewable sources. However, past efforts have focused sugar-based short-chain polymeric monomers. Plant oils represent renewable sources of long-chain hydrocarbons, many of them able to emulate the properties of today's petrochemicals, or to enable new industrial chemicals. In this study, we metabolically engineered cheese-rispening yeast *Yarrowia lipolytica* blocked β-oxidation metabolism by the deletion of 6 acyl-CoA oxidase genes (*POX1-6*) to produce long-chain α,ω-dicarboxylic acids from plant oil-derived fatty acids and their derivatives. Thus, this mutant was further engineered to overexpress w-oxidation by inserting one of 12 cytochrome p450 genes (ALK1), NADPH-cytochrome P450 reductase gene (CPR1) and fatty alcohol oxidase gene (FAO). As the results, this engineered strains produced more α,ω-dicarboxylic acids than Δ*POX1-6* strains. This insight marks the first step toward the biotechnological production of long-chain α,ω-dicarboxylic acids, the monomers of high performance polymers, in a cost-effective manner from renewable plant oils with the nonconventional yeast *Y. lipolytica*, and makes it possible that yeast produces the long chain monomers for polyamide and polyester according to the corresponding substrates.

Keywords: Yeast, *Yarrowia lipolytica*, ω-oxidation, α,ω-dicarboxylic acids, Polymeric monomers

D019

Bioconversion of Plant Biomass into Flavonoids by an Endophytic Fungus *Phomopsis* sp. XP-8 Isolated from *Eucommia ulmoides* Bark

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Flavonoids is a kind of valuable natural compound in plants. It has diverse pharmacological activities including anti-hypertension, anti-tumor, antioxidant and anti-inflammatory activities. Traditional method of flavonoids acquisition is plants extracting. Considering its long period, plant resources waste and low efficiency, some novel methods need to be discovered. Previous studies showed that an endophytic fungus *Phomopsis* sp. XP-8 was isolated from *Eucommia ulmoides* bark and we gained its genomic data by *de novo* sequencing. Based on genome annotation, we found that *Phomopsis* sp. XP-8 possessed complete flavonoid biosynthesis pathway and 23 feruloyl esterases. Thus we could infer that this fungus was able to converse plant biomass into flavonoids. Then, we used the 5 days cultured fungus to treat the waste tea that had been extracted tea-polyphenols overnight. As a result, additional 10% tea-polyphenols was detected and flavonoids content increased. Besides, 5 feruloyl esterases were induced to express abundantly. We cloned hypothetical 4-coumarate-CoA ligase, chalcone synthases and chalcone isomerase from *Phomopsis* sp. XP-8, and co-expressed them in *Saccharomyces cerevisiae* INV*Sc*1. After 48 h fermentation, LC-MS confirmed these 3 enzymes could converse *p*-coumaric acid into naringin. These experimental results show that *Phomopsis* sp. XP-8 has real potential to degrade plant biomass and catalyze them to flavonoids. Our studies shed new light into the way to make the best of the plant waste and acquire flavonoids more effectively.

Keywords: *Phomopsis* sp. XP-8, Flavonoid, Plant biomass, Ferulic acid esterase, Bioconversion

D020

Antioxidative Activity and α-Glucosidase Inhibition Activity of Solvent Fraction from Red-Yeast-Rice

Jung-Bok Lee[1], Byung-Hyuk Kim[1], Jung-Kyu Kim[1,2], Jun-Hyeong Lee[1,2], Yeo-Cho Yoon[1,2], Ye-Eun Park[1], Gu-Hyun Beck[2], Eul-Won Seo[3], Hye-Suk Park[1], Hak-Soo Hwang[4], and Gi-Seok Kwon[2]*

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Red mold-fermented products have been used in Asia for centuries to enhance the flavor of food, as well as serving as a traditional medicine for the treatment of digestive disorders, vascular function, and blood. The antioxidative and α-glucosidase inhibition activity of various solvents extract from Red-Yeast-Rice (RYC) have been studied. In this study, we prepared the ethanol extract of RYC and its subsequent solvent fractions. The ethanol extraction yield of RYC was 10%, and the fraction yields of n-hexane, ethylacetate, butanol and water residue were, 5, 8.3, 11.33 and 14.3%, respectively. Analysis of DPPH radical scavenging activity and SOD-likely activity showed that the EtAC and Hexane fraction had the highest content (72.58% and 54.78%) amongst the fractions. And, antid-diabetic activity as, α-glucosidase inhibition activity of n-hexane, ethylacetate, butanol and water residue were, 92.16, 69.67, 66.11 and 58.05%, respectively. Our results suggest that RYC was developed as a functional food anti-diabetic ingredient.
[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA)(117104-02-1-CG000)]

Keywords: Monascus, Antioxidative activity, α-Glucosidase inhibition activity

D021

Antioxidative Activity and α-Glucosidase Inhibition Activity of Solvent Fraction from Fermented Barley with *Monascus perpures* BHN-MK01

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Traditionally, Monascus-fermented rice is produced by solid state fermentation with rice as the substrate. Other cereal grains i.e. corn, wheat, barley, finger millet and adlay, and those cereal residues e.g. rice bran and wheat bran have been used as Monascus fermentation substrate. In this study, we prepared the ethanol extract of the fermentation Barley with *M. perpures* BHN-MK01, and its subsequent solvent fractions. The ethanol extraction yield of fermentation Barley with *M. perpures* BHN-MK01 was 22.26%, and the fraction yields of n-hexane, ethylacetate, butanol and water residue were, 6.67, 1.33, 5.33 and 32%, respectively. Analysis of DPPH radical scavenging activity and SOD-likely activity showed that the EtAC fraction had the highest content (63 and 50%) amongst the fractions. And, antid-diabetic activity as, α -glucosidase inhibition activity of n-hexane, ethylacetate, butanol and water residue were, 89.59, 49.21, 67.58 and 73.59%, respectively. Our results suggest that fermentation Barley with *M. perpures* BHN-MK01 was developed as a functional food and ingredient.
[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA)(117104-02-1-CG000)]

Keywords: Monascus, Red-barley-yeast, Fermentation

D022

Quality Characterization of Rice Beer Fermented by Rice Cultivar and Local Yeast

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We have studied mashing (saccharification) efficiency, wort and beer quality characteristics of rice cultivars (Hangaru, Seolgaeng, Palbangmi, Brown Rice) in order to determine optimal rice cultivar for rice beer. The rice mixing ratio with malt was set to 40% and the enzyme was compensated for a sufficient reaction with rice mixed at 40%. As a result, the mashing efficiency was improved in wort mixed with rice except for brown rice rather than when malt was solely used. Especially, the mashing efficiency of the wort used with Seolgaeng and Hangaru which are soft rice varieties, was 10.9% and 8.5% higher than that of malt solely, respectively. Meanwhile, the FAN of wort decreased to 80% in brown rice, 72% in Hangaru, Seolgaeng and Palbangmi were compared with malt solely. It should be noted that the decrease of the FAN of wort slows the fermentation speed in the rice beer, compared with malt solely beer. In this regard, it is necessary to supplement FAN in the rice beer. The quality of rice beer used with Hangaru and Seolgaeng was a very weak off-flavor (rice-smell, bitterness), and mild taste. Taken these results, it was concluded that Hangaru and Seolgaeng were optimal rice cultivars for rice beer. In addition, the optimum two yeasts among 9 local yeasts were selected in terms of fermentation characteristics and quality of rice beer.
[This work was supported by grants from Rural Development Administration (RDA) AGENDA R&D project.]
Keywords: Rice, Beer, Rice beer, Yeast

D023

Chemical Constituents of the Culture Broth of *Perenniporia medulla-panis*

Ji-Yul Kim, E-Eum Woo, Kyeong-Woon Jeong, Lee Su Ha, In-Kyoung Lee, and Bong-Sik Yun*

Division of Biotechnology, Chonbuk National University

Mushrooms are valued as a nutritionally functional food and also as an important source of beneficial medicinal components. They produce various primary and secondary metabolites which have interesting biological activities and unusual chemical structures. During the search for new secondary metabolites from the culture broth of fungal strains of mushroom origin, we isolated three new compounds from the culture broth of *P. medulla-panis*. *P. medulla-panis* is a wood-rotting fungi belonging to the family polyporaceae, which comprised of approximately 100 species. *P. medulla-panis* was cultured in potato dextrose broth medium on stationary condition at 27°C for four weeks. The culture broth was extracted with acetone at room temperature and filtered. The filtrate was concentrated to remove acetone and then partitioned between ethyl acetate and water. The ethyl acetate-soluble portion was separated by silica gel column chromatography, MPLC, and preparative reversed-phase HPLC to afford three compounds. Their structures were determined by spectroscopic methods including NMR and mass analysis.

Keywords: Mushroom, *Perenniporia medulla-panis*, Secondary metabolites

D024

Chemical Constituents of the Culture Broth of the Fungus *Panus neostrigosus*

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Mushrooms are nutritionally functional foods and important sources of physiologically beneficial medicines. They produce various secondary metabolites which have interesting biological activities and unique chemical structures. As part of our ongoing investigation on chemical constituents of the fungi of mushroom origin, *Panus neostrigosus* was analyzed. HPLC analysis of the culture broth of *P. neostrigosus* revealed that this strain produced diverse secondary metabolites in its culture broth. In this study, we isolated two compounds from the culture broth of *P. neostrigosus* and determined their chemical structures. Fermentation was carried out in potato dextrose broth medium for 4 weeks at 27°C on the rotary shaker of 120 rpm, and the fermentation broth was extracted with acetone and filtrated. The filtrate was concentrated and partitioned with chloroform. The chloroform-soluble layer was subjected to silica gel column chromatography, MPLC, and preparative HPLC, consecutively, to provide two compounds. Chemical structures of these compounds were determined by spectroscopic methods.

Keywords: Fungus, *Panus neostrigosus*, Spectroscopic

D025

Chemical Constituents of the Culture Broth of *Oudemansiella mucida*

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Mushrooms are valued as a nutritionally functional food and important sources of physiologically beneficial medicines. They produce various secondary metabolites of significant biological activity with unusual chemical structures. In ongoing efforts for secondary metabolites with antifungal activity from the culture broth of the fungal strains of mushroom origin, we found that *Oudemansiella mucida* exhibited significant antifungal activity against *Alternaria solani*, *Diaporthe* sp., and *Botrytis cinerea*. *O. mucida* is a slimy wood-rot fungus belonging to the family Physalacriaceae. The fungus *O. mucida* was cultured in potato dextrose broth medium for 4 weeks at 27°C with agitation of 120 rpm, and the culture broth (12 L) was extracted with acetone at room temperature. The acetone extract was concentrated under reduced pressure to eliminate acetone. The aqueous concentrate was separated by solvent partition, silica gel column chromatography, MPLC, and Sephadex LH-20 column chromatography, consecutively. Finally, oudemansin A and two compounds were purified by preparative HPLC. The structures of these compounds were established by spectroscopic methods, mainly 1D and 2D NMR and ESI-mass measurements.

Keywords: Mushroom, *Oudemansiella mucida*, Antifungal activity

D026

Verification of Anti-obesity and Anti-diabetes Activities of Red Pepper (*Capsicum annuum* L.) Fermented with *Lactobacillus plantarum* BHN-LAB 33

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Red pepper (*Capsicum annuum* L.) is one of the important vegetables in traditional Korean foods and it has many vitamin A, vitamin C, vitamin E, polyphenol, and flavonoids. Also, red pepper has a high anti-oxidant ability and it is known to be effective in preventing obesity, diabetes, hypertension, digestive disorders, stress, and aging. We investigated the anti-obesity and anti-diabetic activities of fermented red pepper with *Lactobacillus plantarum* BHN-LAB 33. Fermented red pepper has been effective for anti-obesity and anti-diabetes. As result, we suggest that fermented red pepper could use the health functional food materials.
[This work was supported by the Technology development Program (S2537226) funded by the Ministry of SMEs and Startups (MSS, Korea).]

Keywords: Red pepper, Fermentation, Lactic acid bacteria, Obesity, Diabetes

D027

The Increase of Aglycon Isoflavone through Intestinal Fermentation System with *Lactobacillus rhamnosus* BHN-LAB 76 in the Intestinal Anaerobic Conditions

Byung-Hyuk Kim[1], Jong-Ok Jang[2], Ye-Eun Park[1], Yeo-Cho Yoon[1,2], Jung-Kyu Kim[1,2], Jun-Hyeong Lee[1,2], Hye-Suk Park[1], Hak-Soo Hwang[3], Gi-Seok Kwon[2], and Jung-Bok Lee[1]*

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Phytochemical isoflavones, which exert a weak estrogenic activity, were reported extensively for their potential role in preventing chronic disease, cancer, osteoporosis, and postmenopausal syndrome. However, the major sources of isoflavones as daidzin and genistein from the soy and puerarin, they occur mainly as unabsorbable and biologically inactive glycosides. The bioavailability of glucosides is increased by hydrolysis of the sugar moiety using β-glucosidase. The water solubility and chemical stability of aglycone forms make them effective for detoxifying endogenous metabolites and xenobiotics, including defense against mycotoxins. Also, isoflavone itself is less available in the body without the aid of intestinal bacteria. Lactic acid bacteria (LAB) are typical probiotic microorganisms that are used in various industries including fermented foods, feed additives, and pharmaceuticals. Isoflavone-transforming activity as the production of daidzein was assessed by TLC and we found *Lactobacillus rhamnosus* BHN-LAB 76. The *Pueraria lobata* extract was fermented with *L. rhamnosus* BHN-LAB 76 for 72 h at 37°C. The *L. rhamnosus* BHN-LAB 76 has converted about 25% of daidzin to daidzein in the anaerobic fermentation conditions. These results confirmed the potential of *L. rhamnosus* BHN-LAB 76 as a probiotic culture that can be utilized in the manufacturing of fermentation foods and functional materials.

Keywords: Aglycon, *Lactobacillus rhamnosus*, Anaerobic conditions, Intestinal fermentation system

D028

Gas stripping Integrated Fermentation for the Production of Isobutanol in Metabolically Engineered *Escherichia coli*

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Isobutanol as higher alcohol have several beneficial properties such as low hygroscopicity, vapor pressure, corrosivity and high energy density, enabling more convenient and efficient use, when compared with the conventional biofuel ethanol. However, fermentative production of isobutanol is limited by the toxicity of the final product itself. To overcome isobutanol toxicity, we removed the isobutanol from fermentation broth using gas stripping system. To produce isobutanol, pET28b(+) and pCDFDuet-1 plasmid that harbored ilvC, ilvD, alsS, kivD, and adhP gene were introduced into *Escherichia coli* BL21 strain. By removing the isobutanol from fermentation broth using gas stripping, the concentration of isobutanol in broth was maintained below 4 g/L during 66 hours of cell culture. This result shows that gas stripping integrated fermentation can overcome isobutanol toxicity in *E. coli* cultivation.
[This research was supported by grants from Ministry of Trade, Industry and Energy.]

Keywords: Gas stripping fermentation, Isobutanol, Toxicity, *Escherichia coli*

D029

Anti-obesity Effects of the Fermented Ethanol Extracts from White Jelly Fungus (*Tremella fuciformis* Berk) with *Lactobacillus rhamnosus* BHN-LAB 76

Yeo Cho Yoon[1], Byung-Hyuk Kim[1], Jung-Kyu Kim[1], Jun-Hyeong Lee[1], Ye-Eun Park[1], Hye-Suk Park[1], Hak-Soo Hwang[2], Gi-Seok Kwon[3], and Jung-Bok Lee[1]*

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White jelly fungus (*Tremella fuciformis* Berk; TF) has been used as a traditional medicine in Asia and known to prevent hypertension, aging, cancer, and arteriosclerosis. We investigated the effects of the anti-obesity activity of Fermented *Tremella fuciformis* Berk (FTF), which ethanol extracts from fermented with *Lactobacillus rhamnosus* BHN-LAB 76. The FTF has been to the increases α-glucosidase inhibitory activity and suppresses adipogenesis of 3T3-L1 adipocytes. These inhibitory effects of FTF are accompanied by regulating phosphorylation of AMPK and Akt. As a result, we suggest that TF fermented with *L. rhamnosus* BHN-LAB 76 suppress adipogenesis by affecting the adipogenic signaling.

Keywords: *Tremella fuciformis* Berk., Lactic acid bacteria, Anti-diabetic activity

D030

Glucose Repression can be Alleviated by Reducing Glucose Phosphorylation Rate in *Saccharomyces cerevisiae*

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Microorganisms commonly exhibit preferential glucose consumption and diauxic growth when cultured in mixtures of glucose and other sugars. Although various genetic perturbations have alleviated the effects of glucose repression on consumption of specific sugars, a broadly applicable mechanism remains unknown. Here, we report that a reduction in the rate of glucose phosphorylation alleviates the effects of glucose repression in *Saccharomyces cerevisiae*. Through adaptive evolution under a mixture of xylose and the glucose analog 2-deoxyglucose, we isolated a mutant strain capable of simultaneously consuming glucose and xylose. Genome sequencing of the evolved mutant followed by CRISPR/Cas9-based reverse engineering revealed that mutations in the glucose phosphorylating enzymes (Hxk1, Hxk2, Glk1) were sufficient to confer simultaneous glucose and xylose utilization. We then found that varying hexokinase expression with an inducible promoter led to the simultaneous utilization of glucose and xylose. Interestingly, no mutations in sugar transporters occurred during the evolution, and no specific transporter played an indispensable role in simultaneous sugar utilization. Additionally, we demonstrated that slowing glucose consumption also enabled simultaneous utilization of glucose and galactose. These results suggest that the rate of intracellular glucose phosphorylation is a decisive factor for metabolic regulations of mixed sugars.

D031

Antioxidative Effects and Antimicrobial Activities of Isolated Fungi *Monascus* spp.

Ku Hyeon Baek[1], Jung-Bok Lee[2], Jong-Ok Jang[1], Chun-Pyo Jeon[3], Jung-Yup Lee[1], Ha-Young Ji[1], Hon-Sol Kim[1], Yeo-Wool Seok[1], and Gi-Seok Kwon[1]*

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Red yeast rice is made by fermenting rice with red colored filamentous fungus, which is discovered by the Japanese scholar as lovastatin and used as anti-hyperlipemia medicine. Red yeast rice contains a high pigment and monacolin K and GABA. Red yeast rice has attracted attention as a natural pigment which can replace synthetic tar pigment. Red yeast rice produces various kinds of pigments such as red pigment, orange pigment, and yellow pigment, and there are six kinds of pigment such as monascarbin, which is an anthraquinone derivative. In this experiment, we examined the chromaticity of extracts from the Monascus species the Monacolin K content, the anti-bacterial activity, and the activity of antioxidant. Its Antibacterial activity and antidiabetic activity were higher in the higher color values. This study will provide basic data for the industrial application of red yeast rice.

Keywords: *Monascus*, Pigment, Anti-bacterial

D032

Antibacterial Activity of Isolated Bacteria *Paenibacillus* spp. against the Plant and Human Pathogen

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Plant pathogenic microorganisms affect food production and ecosystem stability worldwide. The various ingredients released by the pathogen cause extensive tissue damage to the body cells, resulting in soft roots, odor and withering plants. Moreover, an increasing number of phytopathogens develop resistance to the chemical pesticide. However, increasing use of chemical pesticide causes negative effects. In addition, there have recently been diseases that cannot be cared by using antibiotics. The reason is that the bacteria are resistant to antibiotics through genetic mutation. Improper use of antibiotics is one of the causes. Disease caused by antibiotic resistance is two to three times more likely to die than general disease. In this study, we selected a microorganism with antibacterial properties for plant and human pathogen. In addition to KACC 1025, KACC 10342, KACC 10371 and KACC 10458, two other types of plant pathogens were used. The human pathogens used four other things besides *S. aureus*, *B. cereus*, and *P. aeruginosa*. As a result of the experiment, *Paenibacillus* spp. showed a high level of anti-bacterial activity for plant pathogen as well as for human pathogens.

Keywords: Antibotic-resistant, Anti-bacterial, *Paenibacillus* spp., Human pathogen, Plant pathogen

E001

Genome Analysis of *Streptomyces* sp. BH38 Exhibiting Broad Spectrum Antimicrobial Activity

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The phylum Actinobacteria, the genus *Streptomyces* in particular, are important producers of bioactive compounds, accounting for about 75% of the naturally derived antibiotics. In this study, an actinobacterium designated *Streptomyces* sp. BH38 exhibiting significant antimicrobial activities against a wide range of bacteria and fungi was isolated from soil and characterized. Strain BH38 was mostly related to the type strain of *Streptomyces hundungensis*, showing 99.5% 16S rRNA gene sequence similarity. The complete genome sequence of strain BH38 was determined, and the resultant data indicated that the genome consists of 8,393,044 bp with a linear chromosome, 7432 protein-coding sequences, 85 tRNAs and 24 rRNAs. Among the protein-coding genes, 2.55% were predicted to encode secondary metabolites biosynthesis, transport and catabolism. Genomic sequencing and the secondary metabolite cluster analysis demonstrated that strain BH38 possessed a total of 35 biosynthetic gene clusters associated with the production of secondary metabolites. Among these predicted biosynthetic gene clusters, five clusters showed 100% similarities with the compositions of known gene clusters for bioactive compounds such as venezuelin, melanin, desferrioxamine B, ectoine and chloramphenicol. The bioactive fractions were collected and subjected to HPLC-MS analysis, which would be combined with genome data for the elucidation of the nature of antimicrobial compounds produced by BH38.

Keywords: *Streptomyces*, Antimicrobial activity, Genome

E002

Distinct Genotypes of Seoul Virus in Humans and Rodents Using Multiplex PCR-based Next-Generation Sequencing

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Seoul virus (SEOV) poses a critical public health threat worldwide. This virus, harbored by brown rat (*Rattus norvegicus*) and black rat (*R. rattus*), is the causative agent of urban HFRS. Recently, SEOV outbreaks have been reported in the United Kingdom and United States. Defining SEOV genome sequences plays a critical role in development of preventive and therapeutic strategies against SEOV outbreaks. Next-generation sequencing (NGS) is a robust method to delineate genomic sequences and characteristics of the virus in endemic outbreaks. To enrich low copies of viral genomes from the HFRS patients and rodents, we designed multiplex PCR primers to amplify 150-bp length reads for the entire SEOV tripartite genome. Multiplex PCR–based NGS was applied to whole-genome sequencing of retrospective HFRS patients and seropositive *R. norvegicus* rats. Using nearly whole-genome sequences of SEOV, comparative genomic analyses demonstrated the global distinct genotypes of SEOV and possible genomic configuration of genetic exchanges. Thus, this study provides useful insights into genomic-based surveillance, disease risk assessment, and mitigation against hantavirus outbreaks.
[Supported by grants from ADD and NRF]

Keywords: Hantavirus, Seoul virus, Next-generation sequencing, Phylogenetic analysis

E003

Development of Multiplex PCR-based Next-generation Sequencing for Hantaan Virus in Humans and Rodents

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Hantaviruses (Family *Hantaviridae*) are negative-sense single-stranded RNA virus containing tripartite genomes. Hantaan virus (HTNV), harbored by *Apodemus agrarius*, is the causative agent of hemorrhagic fever with renal syndrome (HFRS) 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 robust approach to define genomic sequences and characteristics of the virus. However, acquisition of viral genome sequences from clinical specimens is a challenge due to low copies of the virus genomes. To enrich the viral genomes from clinical specimens and rodents, we performed multiplex PCR by using the primer set designed to amplify 150-bp length reads. The multiplex PCR-based NGS enabled the recovery of nearly whole-genome sequences of HTNV from clinical specimens and rodents. In combination with whole-genome sequences of HTNV from HFRS patients and rodents, phylogeographic analysis demonstrated genetic clusters of HTNV strains from clinical specimens with the HTNV circulating in rodents on each of exercising sites of the patients. Overall, multiplex PCR-based NGS was a potent method to obtain whole-genome sequences of viruses from clinical specimens and rodents. This study provides significant insights for the whole-genome sequencing, genomic-based surveillance, and genetic diversity of hantaviruses.
[Supported by grants from ADD and NRF]

Keywords: Hantavirus, Hantaan virus, Next-generation sequencing, Phylogenetic analysis

E004

Complete Genome Sequence of *Bacillus subtilis* subsp. *inaquosorum* KCTC 13429^T

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Bacillus subtilis subsp. *inaquosorum* is facultatively anaerobic and non-pathogenic. The type strain of *B. s. inaquosorum* KCTC 13429T are rod-shaped, Gram-positive and motile. The genome of *B. s. inaquosorum* KCTC 13429T was sequenced by PacBio RS2 and the libraries were prepared using PacBio_20K. Genome assembly was performed with PacBio SMRT Analysis 2.3.0 and gene calling was done by Prodigal 2.6.2. The coverage of the genome is 209.44. The genome size of *B. subtilis* subsp. *inaquosorum* KCTC 13429T is 4,350,498 bp and its G+C contents is 43.82%. The number of total genes are 4,274 while the number of protein coding genes are 4,023 genes. The numbers of RNA genes and pseude-genes are 118 and 233 respectively. COG classification of the genes was performed with eggNOG. The most abundant COG categories were E (Amino acid transport and metabolism, 8.44%), K (Transcription, 8.34%) and G (Carbohydrate transport and metabolism, 8.11%).
[This research was supported by a grant from the National Research Council of Science & Technology (NST) by the Korean government (MSIP) (No. CRC-16-01-KRICT).]

Keywords: *Bacillus subtilis* subsp. *inaquosorum*, Complete genome sequence, PacBio sequencing

E005

Cross Breeding of Homokaryotic Protoclones of *Ganoderma lucidum* and *G. applanatum* via Protoplasting

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Breeding of mushrooms is a recent applied science compared to plant or animal breeding. The large breeding scientific projects were set up for the *Agaricus* mushroom cultivation. The ultimate goal of researchers and cultivators is to produce high yield and high-quality mushrooms, as well as reduce the cost of production. Protoplast fusion and cross-breeding of homokaryotic protoclones is an efficient method to generate a novel mushroom from two different species. The lytic enzyme was one of the key factors for protoplasting. Yatalase (10 mg/ml) was the most efficient enzyme for *G. lucidum* (Korea) and *G. applanatum* (Brazil) protoplasting. The success of protoplasting depends on the optimum condition for the efficient regeneration of protoplast. The regeneration frequency of *G. lucidum* and *G. applanatum* were 0.26% and 0.22%, respectively. Mating of monokaryotic mycelia by hyphal fusion is a unique method to generate new dikaryotic strains. The pairing of incompatible isolates of *G. lucidum* and *G. applanatum*, a mutual repulsion and antagonistic reaction is evident which leads to the formation of a line of segregation. The hyphal tips may branch profusely and a clear line of the contract appears with increasing age of the culture. *G. lucidum* and *G. applanatum* strains were hybridized by dual culture technique of homokaryotic protoclones cultures were successfully hybridized and produced heterokaryotic mycelium in the success rate was 13.33%.
[Supported by grants from RDA, PJ012057]

Keywords: Homokaryotic protoclone, *Ganoderma lucidum*, *Ganoderma applanatum*, Protoplasting

E006

Complete Genome Sequence of *Salmonella enterica* subsp. *enterica* serovar Enteritidis Strain Isolated from Korean Food-borne Outbreak

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Salmonella enterica subsp. *enterica* is an infectious food-borne pathogen and causes public health problem worldwide. Here, we present complete genome sequence of *Salmonella enterica* subsp. *enterica* serovar Enteritidis strain MFDS1004838 isolated from the food-borne illness outbreak in 2014. The genome was sequenced using single molecule real-time (SMRT) sequencing system on the PacBio RS II. And it was *de novo* assembled using the HGAP/Quiver software package in SMRT Analysis version 2.3.0. Also, the functions of the predicted proteins had been annotated with homology search against Swiss-prot, EggNOG 4.5, SEED, and KEGG databases. Two circular contigs were de novo assembled as mean coverage of 242X. The complete genome sequence of Salmonella strain MFDS1004838 contains a 4,692,509-bp-length chromosomal DNA and a 101,582-bp-length plasmid, with G+C contents of 52.2% and 49.1%, respectively. The genome contains 4,602 genes consist of 4,496 protein, 84 transfer RNA, and 22 ribosomal RNA coding sequences. Especially, putative two prophage regions had been identified, which consist of 42 and 70 CDSs, respectively. Also, the genome includes several genes involved in Salmonella infection and beta-lactam resistance. Thus, we propose that the genome information of this strain has a potential usefulness for understanding the Salmonella Enteritidis infection as a food-borne pathogen.
[This work was supported by a grant (17161MFDS033) from Ministry of Food and Drug Safety in 2018.]

Keywords: *Salmonella* Enteritidis, Food-borne pathogen, Complete genome sequence

E007

Complete Genome Sequences of *Azoarcus* sp. TSPY31 and *Azoarcus* sp. TSNA42 Bio-transforming of Indole to Indigo

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Gyeongbuk Institute for Marine Bio-Industry (GIMB)

The *Azoarcus* sp. TSPY31 and TSNA42 strains, which produce indigo, were isolated from oil-contaminated marine tidal flats. The genomes of *Azoarcus* sp. TSPY31 and TSNA42 were all composed of one complete chromosome. The TSPY31 genome contains 4,572,082 bp with a G+C content of 63.2% and TSNA42 contains 4,886,934 bp with a G+C content of 62.8%. The genomes of TSPY31 and TSNA42 were found to contain two styrene monooxygenase that convert indole to indigo, respectively.
[This work (Grants No. C0563841) was supported by Business for Cooperative R&D between Industry, Academy, and Research Institute funded Korea Small and Medium Business Administration in 2017 and Gyeongsangbuk-do R&D Program.]

Keywords: *Azoarcus*, Indigo, Complete genome

E008

Complete Genome Sequence of Indigo Producing *Yangia* sp. TSBP01

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Yangia sp. TSBP01, isolated from oil-contaminated marine tidal flats, bio-transformed indole to indigo. Indigo is produced from the indole via indoxyl by oxidizing enzymes. The genomic analysis of *Yangia* sp. TSBP01 revealed 2 chromosomes and 5 plasmids, totaling 5,165,974 bp and a G+C content of 66.5%. YSBP01 strain has several oxygenases such as indole oxygenase involved in the conversion of indole to indoxyl.

[This work (Grants No. C0563841) was supported by Business for Cooperative R&D between Industry, Academy, and Research Institute funded Korea Small and Medium Business Administration in 2017 and Gyeongsangbuk-do R&D Program.]

E009

Genomic Characterization and CRISPR-Cas9 Based Gene-editing of Industrial Yeast Strains Used for Production of Korean Rice Wine

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The traditional yeast *Saccharomyces cerevisiae* has long been used for fermentation of traditional alcoholic beverages. In this study, we characterized the whole genome structure and sequences of two *S. cerevisiae* strains, KSD-YC and KFRI 98-5, which are used for commercial production of Korean traditional rice wine, "Makgeolli". The ploidy analysis indicated the genomes of both strains are diploid. The whole genome sequencing revealed that KSD-YC carries two copies of almost identical genome, whereas KFRI 98-5 has two copies of genomes showing significantly high heterozygosity. Notably, the phylogentic tree analysis indicated that KSD-YC is evolutionary very close to a Japanese sake strain K7, while KFRI 98-5 is rather close to the YJM1381 strain used for Rum fermentation in Cuba. Furthermore, we evaluated the feasibility of CRISPR/Cas9-based gene editing to introduce stop codons in *URA3*. Selection of Ura^- mutants either on the 5'-FOA plate or on the NAT plate generated efficiently Ura^- mutants in KSD-YC but not in KFRI 98-5. The difference in the feasibility of Cas9 based-gene editing might reflect the distictive genome structure and sequences between two strains. The availability of genome information and a gene editing technology in the "Makgeolli" yeast strains would facilitate applying advanced omics technologies and metabolic engineering strategies to develop starter strains with improved function and flavor.

[Supported by IPET grants No. 914007-4 and No. 918010-4]

Keywords: Industrial yeasts, Makgeolli, Ploidy, Whole-genome analysis, Gene-editing

E011

Transcriptional Regulation of CRISPR-Cas System by H-NS in *Acinetobacter baumannii* ATCC 19606

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Acinetobacter baumannii is an opportunistic bacterial pathogen primarily associated with hospital-acquired infections. it is top of the global priority list of antibiotic-resistant bacteria reported by the WHO in 2017. Most of pathogenic bacteria have a CRISPR-Cas system system against invading bacteriophages. Recently, CRISPR/Cas9 technology for gene editing is highlighted. However, the molecular mechanism of transcriptional regulation from CRISPR/Cas system is not clear. We found CRISPR-Cas type I-F system in *A. baumannii* ATCC 19606. Cas3 is a key protein in the CRISPR-Cas system. To identify transcriptional regulator on Cas3 promoter. We performed DNA affinity chromatography-pulldown assay. We found DNA binding proteins, H-NS, IHF, Hu and IclR, on promoter region of Cas3 through identification using LC MS/MS. We focused on transcriptional regulation of Cas3 by histone-like nucleoid-structuring (H-NS). We purified AbH-NS protein in *E. coli* BL21 DE3 star. We tested the degree of oligomerization of H-NS using size exclusion chromatography and cross-linking experiments. We also confirmed transcriptional repression by H-NS using in vitro experiments. In addition, Cas1 and Cas3 repressed by H-NS as a RNA level. This study demonstrates that a global repressor, H-NS is strong repressor of CRISPR-Cas system from *A. baumannii*.

Keywords: *A. baumannii*, CRISPR-Cas system, H-NS

E012

Virulence Characteristics and an Action Mode of Antibiotic Resistance in Multidrug-resistant *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa displays intrinsic resistance to many antibiotics and known to acquire actively genetic mutations for further resistance. In this study, we attempted to understand genomic and transcriptomic landscapes of multi-drug resistant (MDR) *P. aeruginosa* clinical isolates. We also aimed to reveal a mode of antibiotic resistance by elucidating transcriptional response of genes conferring antibiotic resistance. To this end, we sequenced the whole genomes and RNA transcripts of three different MDR clinical isolates that are phylogenetically distant from one another. Multi-layered genome comparisons with genomes of antibiotic-susceptible strains and 70 other antibiotic-resistant strains revealed both well-characterized conserved gene mutations and distinct distribution of antibiotic-resistant genes (ARGs) among strains. Transcriptions related to quorum sensing and type VI secretion systems were invariably downregulated in the MDR strains. Virulence-associated phenotypes were further examined and results indicate that our MDR strains are clearly avirulent. Transcriptions of 64 genes, logically selected to be related with antibiotic resistance in MDR strains, were active under normal growth conditions and remained unchanged during antibiotic treatment. These results propose that antibiotic resistance is achieved by a "proactive" response scheme, where ARGs are constitutively expressed even in the absence of antibiotic stress, rather than a "reactive" response.

Keywords: *Pseudomonas aeruginosa*, Multidrug-resistance, Virulence, Genomics, Transcriptomics

E013

Whole Genome Sequence of *Paucibacter aquatile* CR182^T, a Strain with Antimicrobial Activity, Isolated from Freshwater of the Nakdong River

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A Gram-negative, aerobic, motile, and rod-shaped bacterial strain designated CR182^T was isolated from freshwater of the Nakdong River, Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequence indicates that the strain CR182^T belongs to type strains of genus *Paucibacter*. Strain CR182^T showed 98.0% 16S rRNA gene sequence similarity with *Paucibacter oligotrophus* CHU3^T and formed a robust phylogenetic clade with this species. Here we describe the features of strain CR182^T, together with the whole genome sequence information and annotation. The whole genome of strain CR182^T consists of 5,523,543 bp with 66.3% GC content. The genome contained 4,544 protein-coding sequences, 28 rRNAs (5S, 16S, and 23S), and 72 tRNAs. Analysis using the Antibiotic Resistance Genes Database (ARDB) led to the identification of several genes (marB, mecR, pbp2b, catB2, bacA, smeD, and oprM) putatively involved in antibiotic resistance, some conferring specific resistance to macrolides, methicillin, penicillin, chloramphenicol, bacitracin, fluoroquinolone, and/or aminoglycosides. These genome data provide insights into the genetic basis of this strain's antibacterial activity and adaptive mechanisms.

Keywords: Antimicrobial activity, Nakdong River, *Paucibacter aquatile*

E014

Characterization of the Velvet Regulators in *Aspergillus flavus*

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Fungal development and secondary metabolism are intimately associated via the activities of novel regulators, called the Velvet proteins that are highly conserved in filamentous fungi. Here we investigated the roles of the *velvet* genes in *Aspergillus flavus*. Unlike other *Aspergillus* species, *A. flavus* genome contains five *velvet* genes including *veA*, *velB*, *velC*, *velD* and *vosA*. To study the function of the *velvet* genes, we generated individual deletion mutants in *A. flavus*. The mutant strains were examined for asexual spore production and sclerotia formation. We also found that the absence of *veA*, *velB* and *velD* block the production of aflatoxin B1. The deletion of *vosA* and *velB* caused reduced trehalose amounts and increased sensitivity to thermal stress on conidia. To further study we generated Δ*vosA*Δ*velB* double deletion mutant and we examined gene expression using RNA sequencing in WT, Δ*velB* and Δ*vosA* backgrounds. These results are consistent with the current model of the *velvet* genes in *A. nidulans*, indicating that their function may be highly conserved among the *Aspergilli*.

E015

Regulation of the Encapsulin Gene Expression in *Myxococcus xanthus*

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Encapsulins are spherical protein nanocompartments found in many bacteria. The encapsulin genes of *Myxococcus xanthus* DZ2 consist of *encA* encoding a capsid protein, *encB-D* encoding cargo proteins and associated genes, *encE-G* and *MXAN_2409*. A set of mutants carrying *lacZ* fusions with each of the *encA-D* genes were created and used to analyze the expression of the genes. It appeared that the encapsulins containing the EncC and EncD proteins were mainly produced at the early stage of vegetative growth and the encapsulins containing the EncB protein were produced at the stationary and developmental phase of the cells. Expression of the fusions in the mutants carrying a deletion of the *encA, encB, encE, encF* or *encG* gene indicated that the expression of the *encA-lac*Z fusion required an intact *encA* gene. The expression of the *encB-lac*Z fusion required intact *encB* and *encG* genes, but was inhibited by the *encC* gene. The expression of the *encC-lac*Z fusion required an intact *encA* gene, but inhibited by *encE* and *encG* genes. The expression of the *encD-lac*Z fusion required an intact *encE* gene, but was inhibited by the *encA* and *encF* genes. Based on the results, a model for the regulation of the encapsulin gene expression in *M. xanthus* was proposed.
[Supported by a research grant from the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A3B-03030500)]

Keywords: Myxobacteria, *Myxococcus xanthus*, Encapsulin

E016

Characterization of VosA-VelB Target Genes *vidA* in *Aspergillus nidulans*

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The velvet family proteins regulate fungal growth, asexual development, and spore viability in *Aspergillus nidulans*. The VosA-VelB hetero-complex acts as a transcription factor that regulates mRNA expression of the genes related to sporogenesis in *A. nidulans*. Previous our study identified 111 direct target genes of VosA-VelB in conidia. To study the roles of VosA-VelB targets, we generated several deletion mutant strains and examined their phenotypes. Among them, *vidA* (VosA/VelB-inhibited developmental gene, AN2498) is involved in fungal growth and development. VidA is a transcription factor containing C_2H_2 zinc finger protein. Deletion of *vidA* led to decrease colony growth. In addition, the *vidA* deletion mutant strain exhibited defects in sexual fruiting bodies production. Complemented strain of *vidA* recovered growth and asexual and sexual development. Overall, these results suggest *vidA* plays crucial role in fungal growth and development in *A. nidulans*.

E017

The Role of VosA/VelB-down Regulated Gene *vidB* in *Aspergillus nidulans*

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The filamentous fungus *Aspergillus nidulans* mainly reproduced by forming asexual spores called conidia. The formation of conidia, called conidiation, is governed by the NF-κB type velvet regulators VosA and VelB. During conidiation, the VosA-VelB hetero-complex regulates the expression of spore-specific structural and regulatory genes. Previous our study identified 111 potential genes that are the target of the VosA-VelB, and then we characterize one of VosA/VelB-inhibited developmental gene *vidB* (AN5859). The *vidB* gene encodes an ortholog of *Saccharomyces cerevisiae* PDR8 (pleiotropic drug resistance) that is a Zn_2Cys_6 transcript factor. Mutation of *vidB* exhibited less growth and conidiation than WT. Also, the mutants showed resistance to H_2O_2 and neomycin. These results imply that VidB is required for fungal growth, development, conidiation and sensitivity by stress conditions in *A. nidulans*.

E018

Cultural Characteristics (VII) of Hybrid Strains of *Lentinula edodes* on Sawdust Medium

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The parental strains of *Lentinula edodes* were selected to develop new cultivar of *Lentinula edodes* with good quality. A total of fifty hybrid strains were obtained by mono-mono hybridization between thirteen different strains of *L. edodes* including new cultivar "Sanbaeghyang". We investigated on cultural characteristics and fruiting body productivity of the hybrid strains on sawdust media. For sawdust cultivation of hybrid strains, we used the polypropylene bags with square bottom that filled with 2 kg sawdust medium containing 20% of wheat bran.
The average weight loss of sawdust media of 50 strains was 16.8% after 100 days of incubation. And nineteen strains (38%) of them showed above-average rates of weight loss in the media. NIFoS 3465 had the highest weight loss rate (24.9%) among all hybrid strains, followed by NIFoS 3454•NIFoS 3455 (24.4%), NIFoS 3464 (23.1%), NIFoS 3468 (21.5%), NIFoS 3466 (20.6%) and NIFoS 3460•NIFoS 3461 (20.7 %). After the third production of fruiting body, the average weight of mushrooms was 209 g per 2 kg sawdust medium. Eleven strains (34%) showed above-average productivity of the fruiting body. The productivity of fruiting body was the highest in NIFoS 3495 (1435 g), followed by NIFoS 3464 (1070), NIFoS 3496 (936 g), NIFoS 3491 (758 g), NIFoS 3485 (570 g), NIFoS 3477(564 g), NIFoS 3472(544 g), NIFoS 3497(510 g).

Keywords: Cultural characteristics, Hybrid strains, *Lentinula edodes*, Production, Sawdust media

E019

Unraveling the Molecular Mechanism of Zur Oligomerization in *Streptomyces coelicolor*

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Metal ions are important for reactions such as respiration, photosynthesis, and nitrogen fixation, but excess metals can be toxic. As metals cannot be degraded or synthesized, bacterial metalloregulators regulate the expression of genes involved in metal transport, metal storage, and metal usage when metal levels deviate from optimal set point. These metalloregulators are generally multimeric DNA-binding proteins whose DNA-binding activity is modified upon metal binding, causing changes in gene expression either via co-repression or co-activation. The Zn(II)-specific sensor Zur (Zinc uptake regulator) is known to express its regulons in two phases in response to zinc concentration in *Streptomyces coelicolor*. Dimeric Zur binds to the Zur-box motif at sub-femtomolar zinc concentration resulting in the low *zitB* expression while dimeric Zur binds to Zur-box motif and forms oligomerization toward the upstream of the Zur-box motif at micromolar zinc concentration resulting in high *zitB* expression. Here, we aim to elucidate the mechanism of Zur oligomerization. We performed electrophoretic mobility shift assay (EMSA) with DNA fragment containing Zur box and found Zur variants that showed different binding patterns compared to wild type Zur. Additionally, footprinting analysis showed that these variants were unable to form oligomerization. Further works regarding this topic are in progress.
[This work is supported by BK21-Plus fellowship]

Keywords: Zur, Oligomerization

E020

Unravelling of the Polysaccharide Capsule Regulatory Signal Pathways in the Opportunistic Pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic pathogen that causes fungal meningitis, which is responsible for more than 600,000 deaths worldwide annually. The polysaccharide capsule of *C. neoformans* is a key virulence factor which interferes with the phagocytosis by host innate immune cells. The cAMP/PKA and HOG pathways are the central signal transduction systems to control the capsule formation. In our previous studies, we measured the ability of each TF or kinase mutant to produce capsule under Dulbecco's Modified Eagle's (DME) solid medium at 37°C and found that 50 TFs and 51 kinases appear to be negatively or positively involved in capsule production. This result suggests that cryptococcal capsule production requires direct or indirect involvement of complex signaling pathways. Nevertheless, it still remains elusive how these complex signaling components and pathways are coordinated during capsule induction and formation.
To obtain the holistic view of core capsule signaling pathways, for 50 TF and 51 kinase mutants exhibiting altered levels of capsule production, we examined their capability to produce capsule under other capsule inducing conditions, such as Littman's medium and fetal bovine serum (FBS) medium. Here we found that 11 signaling components were found to be critical for capsule production in all capsule induction media we tested. These include 7 kinases and 4 TFs. This study will allow us to reveal the capsule production related mechanisms in *C. neoformans.*

Keywords: *Cryptococcus neoformans*, Polysaccharide capsule

E021

The TOR (Target of Rapamycin) Signaling Pathway Governs Growth and Pathogenicity of Fungal Meningitis Pathogen *Cryptococcus neoformans*

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Tor1 is a serine/threonine protein kinase. Tor pathway was known as it has been implicated in regulating cellular responses to nutrients, including proliferation, translation, transcription, autophagy, and ribosome biogenesis. Here we identified two homologues of *S. cerevisiae TOR*, CNAG_06642 (*TOR1*) and CNAG_05220 (*TLK1, TOR*-like kinase 1), in *Cryptococcus neoformans*. Both *TOR1* and *TLK1* has rapamycin binding domain but *TLK1* has truncated form of that. To study the TOR1 signaling pathway, we attempted to construct the *tor1*Δ and *tlk1*Δ mutants. But we could construct only *tlk1*Δ mutant. So we confirmed the essentiality of *TOR1* by using a promoter of copper transporter (CTR4) and diploid strain. As a result, *TOR1* is an essential gene for viability in *C. neoformans*. Although we could not construct the *tor1*Δ mutant, we constructed *TOR1* overexpression mutant using a promoter of histone H3 in *C. neoformans*. We found that Tor1 overexpression mutant was resistant to rapamycin but *tlk1*Δ did not affect to resistance of rapamycin. And we also identified that Tor1 is involved in response to diverse stresses including genotoxic stress, oxidative stress, thermo-stress, antifungal drug treatment, and production of melanin. Deletion mutant library of transcription factors in *C. neoformans* that we constructed in our previous study was screened to identify the downstream factors of Tor1. So we identify that Atf1, Crg1 and Bzp3 might be a downstream factor of Tor1.

Keywords: Cryptococcus, Serine/threonine kinase, Target of rapamycin

E022

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

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

Keywords: Pseudouridylation, *Cryptococcus neoformans*, Fungal pathogen

E023

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

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Cryptococcus neoformans causes fatal cryptococcal meningoencephalitis in immunocompromised patients as well as immunocompetent individuals. Despite its clinical importance, the signaling networks governing its virulence remains elusive and therapeutic options for treatment of systemic cryptococcosis are limited. Here, to understand signaling networks regulating the virulence of *C. neoformans*, we aim to identify and functionally characterize the 139 putative phosphatases, which are major signaling components. 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 other fungal species and human. We classified putative phosphatases into 16 groups based on InterPro phosphatase domain annotation. Thus far, we have successfully constructed 228 signature-tagged gene-deletion strains representing 114 putative phosphatases through homologous recombination methods. We are in the middle of examining their phenotypic traits under 32 different *in vitro* and *in vivo* conditions, including growth, differentiation, stress response, antifungal resistance, virulence-factor production and virulence potential in animal 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 signaling networks.

Keywords: Phosphatase, Cryptococcus

E024

Crosstalk between Hog1 and Mpk1 MAPK Pathways Coordinately Regulate the Fungal Cell Wall Integrity

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

Keywords: Human fungal pathogen, *Cryptococcus neoformans*, MAPK, Cell wall integrity

E025

Identifying the Signaling Networks Associated with the Developmental Process of *Cryptococcus neoformans*

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The fungal pathogen *Cryptococcus neoformans* causes cryptococcosis by inhalation of infectious spores generated by unisexual/bisexual reproduction. To understand signaling networks modulating this process, understanding of 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 showed altered mating efficiency. To elucidate the mating regulatory mechanism, we constructed knockout mutants of the mating-regulating TFs and kinases in YL99 strain—MATa isogenic strain of H99 strain—to monitor unilateral and bilateral mating, and to perform an analysis of their function. We constructed 22 gene-deletion strains representing 11 TFs and are currently constructing gene-deletion strains for the remaining TFs and kinases. For confirmed mutant strains, we are examining mating phenotypes during bilateral mating: mating pheromone production and filamentous growth. Furthermore, we are examining transcript profiles of mating-regulating TFs and kinases at different developmental stages. 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

E026

Genetic Characterization of Influenza C Virus in Korea

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Background: Influenza viruses are classified into A, B, C, and D types, and are known to cause respiratory symptoms with fever in cases of A and B infected patients. Influenza C virus has seven Negative-sense RNAs and only one glycoprotein (Hemagglutinin esterase glycoprotein) unlike types A and B. Influenza C viruses are known to be predominantly in pediatric patients infected by upper and lower respiratory tracts, unlike types A and B which are mainly infected by upper respiratory tract. In this study, we tried to detect influenza C virus in influenza-like illness patients, and finally isolated the virus.

Methods: The 5,533 upper respiratory specimens of influenza-like illness patients through Korea Influenza and Respiratory Surveillance System (KINRESS) were used. After RNA extraction from specimens, the positive specimen was screened by real-time RT-PCR designed primers and probe specific for HE of Influenza C virus. Influenza C virus was isolated via amniotic inoculation in 10 days old embryonated eggs. HE sequence of the isolated influenza C virus analyzed to determined clade in phylogenetic tree. The virus was quantitated by hemagglutination assay with 0.5% turkey's red blood cell and $TCID_{50}$/ml infected in MDCK (Madin-Darby canine kidney) cell.

Results: The 76 (1.37%) influenza C viruses were detected in 5,533 specimens using real-time RT-PCR and highly detected in 0 to 6 years of age. The HE gene was sequenced directly from 9 specimens determining 6 number of Sao Paulo/82 lineage and 3 number of Kanagawa/76 lineage. Only one influenza C virus was isolated in eggs via amniotic inoculation and it was identified Sao Paulo/82 lineage by HE gene analysis. The isolated influenza C virus showed 2^6HA titer and $10^{2.83}TCID_{50}$/ml.

Conclusions: Influenza C virus was confirmed in 1.37% of influenza-like illness patients in Korea. Their lineages were identified Kanagawa/76 and Sao Paulo/82 in 9 number of influenza C viruses by HE gene analysis. Despite of difficulty of isolating influenza C virus in classical MDCK cell and embryonated egg inoculation, fortunately we succeed in isolation of influenza C virus from amniotic inoculation in eggs. Development of real-time RT-PCR based diagnostic method to detect influenza C virus will help to identify pathogen in influenza-like illness patients. Moreover, the genetic characterization will help to understand influenza C virus in Korea.
[This study was supported by the intramural funds (4845-300-210) from the Korea CDC.]

Keywords: Influenza, Influenza C virus, Genetic characterization

E027

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

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Moraxella osloensis, a Gram-negative bacterium, is identified in diverse environments including human skin, soil, wet household surfaces and nematode. Here, we report isolation of four *M. osloensis* strains of human skin origin for their capability to grow on *t*-octylphenol polyethoxylates (Triton-100), a common surfactant, as sole source of carbon and energy. Of the four *M. osloensis* strains, strain TT16 are most resistance to Triton X-100 and strain NP7 strain showed resistance to β-lactam and aminoglycoside antibiotics. The other three strains are sensitive to the antibiotics tested. We also compared complete genome sequences of four strains. TT16 and KSH strains have four plasmids, and YHS and NP7 strains have three and seven, respectively. The strain NP7 has the shortest chromosome with a size of 2.39 kb and the chromosomes of other strains are 2.48–2.57 kb in length. The G + C contents of four genomes are in a range of 43.6–43.9% but those of plasmids are highly variable. All four genomes contain four ribosomal RNA operon copies and forty-seven tRNA genes. Remarkably, gene rearrangements in chromosomes and acquisition of novel genes with plasmids are evident. For instance, the genes conferring resistance to β-lactam and aminoglycoside antibiotics are predicted to reside in the plasmid of NP7 strain. Our results showed that genome plasticity is a key element for *M. osloensis* to adapt to a wide range of environments including skin surface.

[This research is supported by the Basic Science Research Program and CK-1 program through the NRF of Korea.]

Keywords: *Moraxella*, Skin, Genome, Alkylphenol polyethoxylates

E028

MpkB MAP Kinase is Dispensable for Mycotoxin Production but is Required for Sexual Development in *Aspergillus nidulans* and *Aspergillus flavus*

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MAP kinase pathways regulate growth, development and stress responses in most eukaryotic systems. MpkB MAP kinase in a model fungus *Aspergillus nidulans* has been known to coordinate sexual development as well as secondary metabolism, including sterigmatocystin (ST) production. In this study, however, the results of the TLC analysis of wild type and *mpkB* deletion mutants showed that the *mpkB* gene did not affect the ST production and ST related gene expression especially in *veA*$^{+}$ genotypes that showed ST production of Δ*mpkB*, Δ*mkkB* and Δ*mpkB*Δ*mkkB* mutants in the *veA*$^{+}$ background were similar with wild type. Furthermore, MpkB constitutive activation or inactivation mutants also showed no significant effect on the ST production. Some genes required for ST production (*aflR, stcE* and *stcU*) were constitutively expressed in each mutant, but, interestingly, ST production of *mpkB* and *mkkB* mutants was remarkably delayed in the *veA1* background, suggesting that the ST production is affected primarily by the *veA* gene. Similarly, in *Aspergillus flavus*, MpkB ortholog *AflmpkB* mutant couldn't produce any sclerotia, but it produced aflatoxin B1 normally. Taken together, the *mpkB* gene alone does not affect the expression of genes involved in mycotoxin production such as ST in *A. nidulans* or aflatoxin B1 in *A. flavus*, indicating that the signaling of MpkB MAP kinase and mycotoxin production were governed by independent pathways.

Keywords: Aspergillus, Differentiation, MAP kinase, Mycotoxin, Transcriptome analysis, Secondary metabolites

E029

Defense Mechanism against Zinc Using Alternative Zur Box

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In *Streptomyces coelicolor*, Zur, the zinc-specific regulator of Fur family, regulates many target genes to maintain zinc homeostasis. Both zinc uptake system and zinc exporter are regulated by Zur with different concentration of zinc. Novel regulation pattern of exporter and other genes by Zur suggests a different kind of DNA consensus sequence. We found a new Zur binding consensus sequence and confirmed binding patterns between Zur and artificially generated DNA consensus sequences. Transcriptome analysis using WT and *zur* mutants was performed to confirm the Zur regulation controlled by the new consensus sequence. The transcriptome analyzes show clues explaining toxicity by unconjugated intracellular zinc.

Keywords: Zinc, Zur, Regulon, Toxicity, New consensus

F001

Determination of Novel Flagellar Proteins in *Borrelia burgdorferi*

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Motility of *Borrelia burgdorferi* (*Bb*) was reported to be critical for the pathogenic life cycle of this Lyme spirochete. Unlike externally flagellated bacteria, *Bb* possesses periplasmic flagella that contain a unique structural component called the collar. The collar structure is hypothesized to be critical for flagellar assembly as well as for providing proper rigidity and flexibility of flagella within the periplasmic space during rotation. However, nothing is known about the proteins encoding the collar or their function in any spirochete. We discovered for the first time that the *flbB* and *bb0236* are involved in collar structure assembly, and these collar proteins are crucial for orientation of periplasmic flagella, motility, and assembly of the motor structures. Because collar is a spirochete-specific structure, the knowledge obtained in these studies can be directly applied to understand the structure and function of flagellar motors of other medically significant spirochetes including the syphilis-causing *Treponema pallidum* which cannot yet be cultivated *in vitro* for molecular analysis. Because the collar is essential for motility, and motility for host infection and bacterial transmission, these studies can also lead to applications in structure-based drug design to disrupt motor assembly, therefore blocking the *Bb* dissemination, and preventing the spread of Lyme as well as other spirochete-borne diseases.

[Supported by grants from NIAID, Welch fdn., NIAMS, and KMOU]

Keywords: *Borrelia burgdorferi*, Collar, Lyme disease, Periplasmic flagella, Spirochete

F002

Subinhibitory Concentrations of Trimethoprim and Sulfamethoxazole Prevent Biofilm Formation by *Acinetobacter baumannii* through Inhibition of Csu Pili Expression

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Acinetobacter baumannii (*Ab*) is an increasingly prevalent multi-drug resistance nosocomial pathogen. A chaperone/usher pili, designated Csu pili, have been identified and studied in *Ab* 19606, but a thorough understanding of these cell surface appendages is still limited. While the Csu pili are important for biofilm formation in *Ab* 19606, its functionality and role in other *Ab* strains has not been studied. Herein, we show that Csu pili are important for biofilm formation not only in *Ab* 19606 but also in *Ab* 17978. Interestingly, this Csu-dependent biofilm formation is blocked by subinhibitory concentrations of anti-folate antibiotics, sulfamethoxazole and trimethoprim, which abolish the folate-derived one carbon metabolism and inhibit bacterial DNA synthesis. We hypothesized that the repression of Csu pili and biofilm formation were induced by folate stress, and that folate pathway intermediates may relieve the stress as a supplement. Indeed, addition of folate or tetrahydrofolate are able to partially restore Csu pili expression and biofilm formation phenotypes in *Ab* 17978 under anti-folate treatment. This is the first demonstration of a linkage between folate stress and Csu pili mediated biofilm formation in *Ab* 17978. Our findings provide the evidence of regulation of virulence factors in *Ab* is dependent on folate stress, and are the starting point for understanding the folate pathway and its regulation mechanisms in this bacterium.

[Supported by grants from NIAID and KMOU]

Keywords: *Acinetobacter baumannii*, Biofilm, Csu pili, Folate stress

F003

The Predominant 16S RMTase Genes of Amikacin-resistant Bacteria in Korea

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Pathogenic Gram-negative bacteria with amikacin resistance have already been distributed all over the world. A major mechanism of high-level resistance to amikacin, one of aminoglycoside, is related with exogenously acquired 16S ribosomal RNA methyltransferases (16S RMTases). To investigate the predominance of amikacin resistance associated with 16S RMTases in Korea, we collected a total of 222 amikacin resistant Gram-negative clinical isolates from patient specimens between 1999 and 2015 from three hospital banks across Korea. *ArmA* and *rmtB* was the predominant 16S RMTase genes responsible for amikacin-resistant isolates circulating in Korean community settings.

Keywords: Amikacin resistance, 16S RMTase, ArmA, RmtB

F004

Dendritic Cell-based Therapeutic Vaccine for Hepatitis B Virus Using CTP Technology

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Hepatitis B Virus (HBV) is one of the most common liver diseases that can lead to hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Over 70% of the patients with liver cancer in Korea are caused by HBV. Although HBV drugs are developed, current standard therapeutics have their own limitations such as a low rate of clearance. Therefore, unmet needs still exist unceasingly, like a safe and effective therapeutic vaccine. In the last decade, dendritic cell (DC) based vaccines are regarded a very beneficial arm to activate the immune system in immune therapy strategies for cancer. DCs are known to be the most powerful of the antigen-presenting cells (APCs), are very efficient at generating potent immune responses. Additionally, cytoplasmic transduction peptide (CTP) technique was applied to induce a strong CTL response with high efficiency. We purified CTP conjugating hepatitis B core (HBc) protein, CTP-HBc Ag, using *E. coli* overexpression system. We differentiated hematopoietic stem cells from mouse bone marrow into DCs to generate DCs for use in vaccines. In this study, the CTP-HBc Ag pulsed DCs were accompanied by enhanced MHC I and co-stimulatory molecule expression such as CD86, CD80, CD40, and showed a capacity of DC for migration and T cell activation, which induced proliferation of more $CD8^+$ T cell than $CD4^+$ cell in compare to imDC. These data suggest that CTP-HBc Ag facilitates DC maturation into a strong stimulator of CTLs.

Keywords: Hepatitis B virus, Dendritic cell, Cytoplasmic transduction peptide, Therapeutic vaccine

F005

A Revised Method for Preparation of Lipopolysaccharide and Bio Assay

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Lipopolysaccharide (LPS) calls great attention because it protects Gram-negative bacteria, activates host immunes, and causes endotoxin shocks. LPS preparation is needed to study its organization and function in Gram-negative bacteria and hosts. However, LPS prepared by a conventional method often has low yield and contamination that can reduce its potency and interfere with its activity. We introduced a revised method for extraction of LPS with high yield and low contamination. After cell disruption, RNase, DNase, and proteinase were treated in a mild buffer condition containing 0.1% SDS. LPS was then extracted using a Trizol reagent and precipitated by 70% ethanol. The purity and concentration of LPS determined by absorbance at 204 nm were validated by SDS PAGE followed by Alcian blue-silver staining method. Biological activity of purified LPS was evaluated by activation of monocyte-like RAW 264.7 cells to macrophages. The purified LPS was characteristic for each strain. The LPS components from *Salmonella* and *Acinetobacter* strains had different effective doses (ED50) for activating RAW 264.7 macrophages. In conclusion, we developed a simple method for high purity LPS, which is useful for structural and functional studies on Gram-negative pathogens and host cells.
[Supported by the NRF of Korea (2016R1A2B2014493)]

Keywords: Lipopolysaccharide, Macrophage activation, Gram-negative bacteria, *Salmonella typhimurium*, *Acinetobacter* spp.

F006

Identification and Characterization of Acinetobacter Isolates from the Oral Cavities of Cambodian Children

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Strains CAM121 and CAM180-1 were isolated from two Cambodian children with different stages of caries. These strains belonged to *Acinetobacter junii* and *A. baumannii* based on their 16S rRNA gene and multilocus sequence types. We characterized their antibiotic susceptibilities, growth rates, cell aggregation, sedimentation and adhesion in comparison with *A. baumannii* strain ATCC 19606. During cultivation in LB medium, strain CAM180-1 ($\mu' = 1.86\ h^{-1}$) grew faster in the exponential phase than strains CAM 121 ($1.5\ h^{-1}$) and ATCC 19606 ($1.38\ h^{-1}$). Strains CAM180-1 and ATCC 19606 were resistant to chloramphenicol and cephalexin but were susceptible to colistin. In contrast, strain CAM121 was susceptible to most antibiotics, but for colistin resistance. Oral strains CAM121 and CAM180-1 did not adhere to microplate surfaces, whereas strain ATCC 19606 formed a biofilm. Particularly, strain CAM180-1 can form strong cell aggregates with rapid sedimentation, while strains ATCC 19606 and CAM121 grew in a dispersed manner. Their LPS profiles were characteristically different from each other. We presented that strain CAM180-1 has different properties for cell aggregation and sedimentation, seemingly associated with the risk of severe caries among children in Cambodia.
[Supported by the NRF of Korea (2016R1A2B2014493)]

Keywords: *Acinetobacter* spp., Children caries, Cell aggregation, Adhesion, Lipopolysaccharide

F007

Mycobacterium tuberculosis Acyl Carrier Protein Inhibits Macrophage Apoptosis by Down-regulating the Reactive Oxygen Species/c-Jun N-Terminal Kinase Pathway

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Mycobacterial acyl carrier protein (AcpM; Rv2244) is a meromycolate extension acyl carrier protein of *Mycobacterium tuberculosis*, which participates in multistep mycolic acid biosynthesis. However, the function of AcpM in host-mycobacterium interactions during infection remains largely uncharacterized. Here we show that AcpM inhibits host cell apoptosis during mycobacterial infection. To examine the function of AcpM during infection, we generated a recombinant AcpM (rAcpM) protein, which was expressed from *E. coli* BL21. We proved that treatment of rAcpM up to 20 μg/ml had no cytotoxic effect to macrophages. In addition, rAcpM treated-primary bone marrowderived macrophages (BMDMs) expressed both pro- and anti-inflammatory cytokines. Its inflammatory response was Toll-like receptor 2 (TLR2) dependent, contrary to LPS, which has TLR4-dependent activity. Importantly, treatment of rAcpM significantly decreased *M. smegmatis*-induced apoptosis of infected BMDMs. It also down-regulated reactive oxygen species (ROS) generation and activation of c-Jun N-terminal kinase (JNK) signaling. Moreover, rAcpM enhanced intracellular survival of H37Rv when treated to infected BMDMs. Taken together, these results indicate that AcpM plays a role as a virulence factor by modulating host cell apoptosis during mycobacterial infection.
[This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIP) (No.2017R1A5A2015385).]

Keywords: Acyl carrier protein, *Mycobacterium tuberculosis*, Apoptosis, Reactive oxygen species, c-Jun N-terminal kinase

F008

Assessment of Bacterial Contamination of Mobile Phone of Dentists and Dental Hygienists by Illumina MiSeq

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In a clinical environment, mobile phones often come into contact with the hands of medical staff and can be contaminated by bacteria. There is a possibility that cross-contamination occurs because the bacteria are transported from the contaminated hands of the healthcare worker to the patient. In this study, we used Illumina MiSeq, a molecular biology technique, to identify bacterial diversity in mobile phones of dentists and dental hygienists. We surveyed five dentists and five dental hygienists working in a dental hospital. In the dentist's mobile phone, *Pseudomonas* (52.52%), *Janthinobacterium* (14.21%), *Enterococcus* (9.66%), *Stenotrophomonas* (5.68%), *Streptococcus* (4.29%), *Acinetobacter* (3.15%) were identified, *Enterococcus* (32.02%), *Pseudomonas* (23.76%), *Streptococcus* (22.44%), *Lactobacillus* (8.77%), *Janthinobacterium* (3.54%), *Acinetobacter* (1.7%), *Stenotrophomonas* (1.22%) were found in the mobile phone of dental hygienists. In addition, 38.09% of the total genus number in the dentist's mobile phone and 25.74% in the mobile phone of the dental hygienist were identified as the genus containing the pathogenic species. Bacteria associated with oral diseases such as *Streptococcus, Actinomyces, Porphyromonas*, and *Fusobacterium* were also found in both groups. This study not only proves that mobile phones used by dentists and dental hygienists show high contamination rates, but also shows the possibility of contamination of infectious pathogens.

Keywords: Mobile phone, Contamination, Bacteria, Illumina Miseq

F009

Assessment of Bacterial Contamination of Toothbrush by Illumina MiSeq

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In a clinical environment, mobile phones often come into contact with the hands of medical staff and can be contaminated by bacteria. There is a possibility that cross-contamination occurs because the bacteria are transported from the contaminated hands of the healthcare worker to the patient. In this study, we used Illumina MiSeq, a molecular biology technique, to identify bacterial diversity in mobile phones of dentists and dental hygienists. We surveyed five dentists and five dental hygienists working in a dental hospital. In the dentist's mobile phone, *Pseudomonas* (52.52%), *Janthinobacterium* (14.21%), *Enterococcus* (9.66%), *Stenotrophomonas* (5.68%), *Streptococcus* (4.29%), *Acinetobacter* (3.15%) were identified, *Enterococcus* (32.02%), *Pseudomonas* (23.76%), *Streptococcus* (22.44%), *Lactobacillus* (8.77%), *Janthinobacterium* (3.54%), *Acinetobacter* (1.7%), *Stenotrophomonas* (1.22%) were found in the mobile phone of dental hygienists. In addition, 38.09% of the total genus number in the dentist's mobile phone and 25.74% in the mobile phone of the dental hygienist were identified as the genus containing the pathogenic species. Bacteria associated with oral diseases such as *Streptococcus, Actinomyces, Porphyromonas*, and *Fusobacterium* were also found in both groups. This study not only proves that mobile phones used by dentists and dental hygienists show high contamination rates, but also shows the possibility of contamination of infectious pathogens.

Keywords: Toothbrush, Contamination, Bacteria, Illumina Miseq

F010

Screening of Inhibitors against a Variety of ß-Lactamases

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ß-Lactamase-mediated resistance to ß-lactam antibiotics has been significantly threatening the efficacy of these clinically important antibacterial drugs. Although some ß-lactamase inhibitors are prescribed in combination with ß-lactam antibiotics to overcome this resistance, the emergence of enzymes resistant to current inhibitors necessitates the development of novel ß-lactamase inhibitors. In this study, we screened dinucleotides to investigate their inhibitory effects on a variety of ß-lactamases through nitrocefin-hydrolyzing assay, inhibition kinetic studies, and *in vitro* susceptibility of ß-lactam antibiotic resistant *E. coli*. Interestingly, some dinucleotides decreased an extended-spectrum class C ß-lactamase. These results may offer the potential of the dinucleotide scaffold for the development of novel ß-lactamase inhibitors.

Keywords: Lactamase, Dinucleotide, Inhibitor

F011

Sirtuin 3 is Required for Antimicrobial Responses against Mycobacterial Infection *in vivo* and in Macrophages

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Sirtuin 3 (SIRT3), a mitochondrial protein deacetylase, maintains respiratory function, but its role in the antimicrobial defense remains unclear. Herein, we examined the role for SIRT3 in the innate host defense against mycobacterial infection. To investigate this, *Sirt3*$^{+/+}$ and sirt3$^{-/-}$ mice were intranasally infected with *Mycobacterium tuberculosis* (Mtb) or *M. bovis* Bacillus Calmette-Guérin (BCG). We examined the bacterial loads in the infected lungs and found them to be significantly increased in the lungs of Mtb- or BCG-infected *sirt3*$^{-/-}$ mice compared to *Sirt3*$^{+/+}$ mice. In addition, Mtb or BCG infection induced significantly more granulomatous lesions in the lungs of *sirt3*$^{-/-}$ mice, which were dominated by neutrophilic infiltrates and necrotic cells, than in *Sirt3*$^{+/+}$ mice. Moreover, *sirt3*$^{-/-}$ bone marrow-derived macrophages (BMDMs) exhibited a significant increase in intracellular Mtb or BCG compared to *Sirt3*$^{+/+}$ BMDMs. Collectively, these data indicate that SIRT3 contributes to antimicrobial activity and promotes host survival during mycobacterial infection.

[This work was supported by National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIP) (2017R1A5A2015385), and by grants from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (HI15C0395).]

Keywords: Sirtuin, *Mycobacterium tuberculosis*

F012

Lipoteichoic acid of Probiotic *Lactobacillus plantarum* Inhibits the *Enterococcus faecalis* Biofilm Formation

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Enterococcus faecalis, a Gram-positive bacterium commonly isolated in patients with refractory apical periodontitis, easily invades dentin tubules and forms biofilms. Bacteria in biofilm are more resistant to antimicrobial agent than planktonic cells and avoid phagocytosis, which contributes to recurrent or chronic inflammatory diseases. Although, *Lactobacillus plantarum* lipoteichoic acid (LTA) has been known to regulate the inflammatory response, little is known about the effect of *L. plantarum* LTA on *E. faecalis* biofilm formation. In this study, we investigated whether *L. plantarum* LTA inhibited the *E. faecalis* biofilm formation using purified *L. plantarum* LTA. Biofilm formation of *E. faecalis* was reduced by *L. plantarum* LTA in dose-dependent manners. *L. plantarum* LTA inhibited in the initial stage of biofilm development of *E. faecalis* without affecting bacterial growth. Interestingly, *L. plantarum* LTA destructed the preformed *E. faecalis* biofilm. Furthermore, these inhibitory effects were also observed on the surface of human dentin slices. Other Lactobacillus LTA such as *Lactobacillus acidophilus*, *Lactobacillus casei*, or *Lactobacillus rhamnosus GG* also inhibited *E. faecalis* biofilm formation. Collectively, these results suggest that *L. plantarum* LTA inhibits *E. faecalis* biofilm formation. Therefore, *L. plantarum* LTA could be used as anti-biofilm agents for treatment of prevention of *E. faecalis*-associated diseases.
[This work was supported by grants from KHIDI]

Keywords: Biofilm, *Enterococcus faecalis*, Lipoteichoic acid, *Lactobacillus plantarum*, Apical periodontitis

F013

Prevalence of Methicillin-resistant *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* among Healthy Dogs and Dogs with Otitis Externa

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Staphylococci have been considered as an opportunistic pathogens of canine skin infections. Recent emergence of *S. pseudintermedius* and *S. schleiferi* among dogs with otitis externa and skin & soft tissue infections has become one of the significant issues in veterinary medicine due to high level of antimicrobial resistance along with various virulence factors. In this study, we investigated major genotypic and phenotypic characteristics (i.e. detection of *mecA* gene & SCC*mec* typing, antimicrobial resistance profiles and biofilm formation) using *S. pseudintermedius* and *S. schleiferi* strains isolated from healthy dogs or dogs having otitis externa. Among the 41 *S. pseudintermedius* strains, 24 strains (58.5%) were resistant to methicillin (MRSP), while 7/31 *S. schleiferi* strains were resistant to methicillin (MRSS) (22.6%). Overall, *S. pseudintermedius* strains tended to have higher level of resistance to most antibiotics tested versus the *S. schleiferi* strains, regardless of infection status. In addition, 78% of *S. pseudintermedius* strains carried *mecA*, while 35.5% of *S. schleiferi* strains harbored *mecA*. However, most of the *mecA*-positive *S. pseudintermedius* and *S. schleiferi* strains were found to have SCC*mec* V (87.5% and 100% for MRSP and MRSS, respectively). The presence of MRSP and MRSS, especially with multidrug resistant phenotypes, necessitates further investigation of antimicrobial resistance mechanisms among the *S. pseudintermedius* and *S. schleiferi* in companion animals

Keywords: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), Methicillin-resistant *Staphylococcus schleiferi* (MRSS), Antimicrobial resistance, Canine otitis externa

F014

Protective Effects of Small RNA RaoN against Nitrosative and Oxidative Stress in *Salmonella enterica* serovar Typhimurium

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RaoN is a *Salmonella*-specific small RNA that is encoded in the *cspH-envE* intergenic region on *Salmonella* pathogenicity island-11. We previously reported that RaoN is strongly induced under oxidative stress conditions and nutrient limitation, contributing to the intramacrophage survival of *Salmonella*. Here we investigated the effect of *raoN* mutation on production of reactive oxygen species (ROS) by murine macrophages. Flow cytometry analysis revealed that the inactivation of *raoN* leads to an increase in intracellular ROS levels in *Salmonella*-infected macrophages, suggesting that RaoN is involved in the inhibition of ROS production. We further show that *raoN* mutant strains have increased susceptibility to nitrosative stress by using a nitric oxide generating acidified nitrite. To assess the effect of *raoN* deletion on systemic infection, we performed the virulence assays in the mouse model. All C3H/HeN mice that were infected with the wild-type strain became ill and died within 15 days, while mice infected with the *raoN* mutant demonstrated 70% survival until 15 days. Our findings support the concept that small RNA RaoN has a role in virulence.

Keywords: Small non-coding RNA, RaoN, Oxidative stress, Nitrosative stress, Mouse virulence

F015

Live *Staphylococcus aureus* Induces the Granulation of the Rat Basophilic Leukemia Mast Cell

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Apotic dermatitis (AD) is in a condition showing a complex, up still now, not fully understood etiological disease in worldwide. Atopic individuals are susceptible to allergic sensitization with microbial colonization as well. About 90% of AD patients are colonized by *S. aureus*, while the healthy subjects of this rate is ranging from 20% to 40%. The research suggesting that the skin colonization by *S. aureus* is a primary factor in AD pathogenesis is still unclear. Mast cells (MCs) are activated for degranulation and mediators released by allergens that cross-linked with IgE molecules or by microbial products. Therefore, MCs might critically be involved in the pathogenesis of AD. However, understanding mechanisms of MC degranulation by *S. aureus* in relation to AD have still not been fully elucidated. We found that both live and heat-killed *S. aureus* displayed a high adherence and invasive abilities and were able to trigger proinflammatory cytokine releases from RBL 2H3 cells. However, RBL 2H3 cell degranulation was induced by live *S. aureus*, whereas heat-killed *S. aureus* failed to do so. These result suggest that MC degranulation could be induced by various pathways.

Keywords: Atopic dermatitis, *Staphylococcus aureus*, Mast cell, Rat basophilic leukemia cell line

F016

Characterization of Plasmid Profiles of Carbapenem-resistant *Klebsiella pneumoniae* Isolated from Hospital Patients

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National Institute of Health

In recent decades, the global spread of antibiotic resistance among Gram-negative bacterial pathogens has become a major threat to public health. WGS-based plasmid analysis can provide the information of characteristic, diversity, and its mobility. In this study, we aimed to annotate the entire mobile genetic element content (mobilome) of whole plasmid sequence of 19 carbapenem-resistant *Klebsiella pneumoniae* isolates from different patients using Illumina Miseq platform. Analysis platform was established for assembly and annotation of short lead sequence data. Most isolates carried 5 different plasmids and the plasmid sequences was shown the highest identity (about 99%) to those of NDM-1 and OXA-232 co-producing *K. pneumoniae* as a representative in the previous report from India. Various genes of antibiotic resistance (ARGs) including beta-lactamase genes and carbapenemase genes were carried on plasmids. In addition, mobilome were spotted in up- and downstream of ARGs, indicating potential spread of ARGs into other bacteria. Building-up of plasmid analysis platform is useful to obtain the fundamental structure of whole plasmid sequence of carbapanem-resistant *K. pneumoniae*. Further is needed to expand the clear evidence of explanation of persistence of plasmid-encoded ARGs in host.
[This study was supported by a grant of the Korea Centers for Disease Control and Prevention (2018-NI005-00).]

Keywords: Plasmid, *Klebsiella pneumonia*, Carbapenem resistance

F017

Investigation of Biofilm Formation in Clinical Isolates of *Staphylococcus aureus*

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Staphylococcus aureus is one of the most common human pathogens that frequently colonizes skin or mucosal membranes. It is of major clinical importance as a nosocomial and community-acquired bacterial pathogen which can cause a wide array of infectious diseases. The ability of *S. aureus* to form biofilm makes them resistant to antimicrobials, thereby limiting treatment options. Here, 16 clinical isolates of *S. aureus*, were investigated for biofilm production by 96-well microtiter plate assay. Bacteria were grown in TSB supplement with different concentration of glucose and NaCl and biofilm were quantified by CV staining. Result of the assay showed that out of 16 isolates, 4 (25%) MRSA forms strong biofilm in TSB. Moreover, a supplement of TSB with 0.5% and 1.0% glucose further enhanced the biofilm production. On the other hand, the addition of 2.0% NaCl in TSB slightly reduced the biofilm formation among the same strains. All the *S. aureus* isolate tested were strong biofilm producers in TSB+0.5% glucose-based media, but differences in biofilm formation were observed among them. Supplement of 2.0% NaCl strongly triggered the biofilm production in 9 (56%) isolates, including 6 MRSA and 3 MSSA. Almost 75% *S. aureus* isolates formed low biofilm in TSB, but glucose supplementation remarkably promoted biofilm production in all strains. However, biofilm phenotypes of *S. aureus* were distinct depending upon the composition of culture media.
[Supported by NRF2017M3A9E4077232]

Keywords: *Staphylococcus aureus*, Methicillin-resistant, Biofilm formation

F018

Clinical Epidemiology of HIV Infection in Korea HIV/AIDS Cohort Study of Multicenter (2006-2017)

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This study was to identify the clinic-epidemiologic characteristics of HIV infected persons in Korea.
Since 2006, we recruited HIV-infected patients for the Korea HIV/AIDS cohort study. 1,482 HIV-infected patients were registered in the cohort (2006-2017). Their mean age was 41.4±12.6 years and the male-to-female ratio was 14.0. For the AIDS defining disease, there was followed tuberculosis (11.2%, n=161), progressive multifocal leukoencephalopathy (11.0%. n=158), esophagus candidiasis (4.5%, n=65). For past history of disease, 223 (15.5%) were with tuberculosis, 533 (36.6%) were with venereal disease (syphilis, gonorrhea, nongonococcal urethritis, condyloma acuminatum). The CD4+ cell counts were as follows: 262 (21.9%), <200 cells/ml; 587 (49.1%), 200–500 cells/ml; 346 (29.0%), 500≤ cells/ml. The Viral load was as follows: 691 (60.3%), <1,000 copies/ml; 169 (14.8%), 50,000 copies/ml≤. History of smoking was 840 (57.7%) in the smoking (current smoking 614, past smoking 226) and 411 (28.2%) in the non-smoking. History of alcohol was 860 (59.0%) in the alcohol (current alcohol 648, past alcohol 212) and 363 (24.9%) in the non-alcohol. The distribution of body mass index (BMI) was 97 (8.8) in <18.5 kg/m^2, 807(73.5%) in 18.5–25.0 kg/m^2, 194 (17.7%) in 25.0 kg/m^2≤. This study showed the clinic-epidemiologic characteristics of the HIV-infected. This basic information will be used for strategies for prevention of HIV infection and disease progression
[Supported by grant from KCDC]

Keywords: HIV, AIDS, Korea HIV/AIDS cohort study, Clinical epidemiology

F019

Imaging of Bioluminescent *Acinetobacter baumannii* in a Mouse Pneumonia Model

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Animal models are essential to assess the role of virulence factors in the development of infectious diseases. Bioluminescence imaging has proven useful for the *in vivo* monitoring of bacterial infection in animal models. The outer membrane protein A (OmpA) is a major outer membrane protein that is responsible for the integrity of outer membrane and pathogenesis of *Acinetobacter baumannii*. In this study, we constructed bioluminescent reporter strains of *A. baumannii* ATCC 17978 and its Δ *ompA* mutant for the *in vivo* imaging assay and examined the *in vivo* monitoring of bacterial infection correlated with bacterial burden and histopathology in an acute murine pneumonia model. The bioluminescent *A. baumannii* strains were constructed by integrating a *luxCDABE* luciferase gene into the bacterial chromosome. The growth of two reporter strains was identical to that of the parent strains. The reporter strains expressed bioluminescence *in vitro* culture. The $\geq 10^4$ CFUs produced relative light unit values clearly above the background. Bioluminescence of two reporter strains was clearly visible in the lungs 30 min after infection and the bioluminescent signal increased over 24 h. Bioluminescence was correlated with bacterial burden and histopathology in the mice infected with reporter strains. The bioluminescent imaging assay using the reporter strains of *A. baumannii* inserted with a *luxCDABE* gene is useful tool for the real-time evaluation of bacterial infection in animal models.

Keywords: *Acinetobacter baumannii*, Bioluminescence, Pneumonia, *lux* gene, OmpA

F020

The Sensor Kinase BfmS Regulates the Secretion of OmpA through Outer Membrane Vesicles in *Acinetobacter baumannii*

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Acinetobacter baumannii is an important nosocomial pathogen worldwide. Among the virulence factors reported in *A. baumannii*, outer membrane protein A (OmpA) plays an important role in *A. baumannii* pathogenesis. OmpA is released from bacteria through outer membrane vesicles (OMVs) and the OMV-bound form of OmpA is responsible for the host cell death and inflammatory responses in the hosts. BfmRS is a two-component system in *A. baumannii* that controls biofilm formation and cellular morphology. The contribution of the sensor kinase BfmS to the virulence of this bacterium remains unknown. In this study, a bfmS knockout and complementation studies were performed to clarify the role of BfmS in the virulence of *A. baumannii*. The Δ*bfmS* mutant of *A. baumannii* ATCC 17978 displayed a reduction in biofilm formation compared with the parent strain. The expression of the *ompA* gene was not different between the wild-type and Δ*bfmS* mutant strains. However, the expression of OmpA in the outer membrane was significantly decreased in the Δ*bfmS* mutant, whereas the secretion of OmpA through the outer membrane vesicles (OMVs) was significantly increased in the Δ*bfmS* mutant. The OMVs from Δ*bfmS* mutant showed more cytotoxic effect in host cells than those from the wild-type strain. These results suggest that sensor kinase BfmS of two-component system BfmRS regulates the secretion of OmpA through the OMVs, which mediates OmpA-mediated pathogenesis of *A. baumannii*.

F021

Role of ppGpp in Antibiotic Suseptibility and Biofilm Formation of *Acinetobacter baumannii*

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Acinetobacter baumannii is an opportunistic pathogen in hospitals and is well known as an important multidrug resistant microorganism. The demonstrated ability of *A. baumannii* strains to grow as biofilm is believed to play a significant role in their antibiotic resistance. The treatment of antimicrobial peptide 1018 reduced the biofilm formation of various pathogenic bacteria via ppGpp (PLoS Pathog. 2014 May 22;10(5):e1004152). The (p)ppGpp are secondary messengers involved in growth control and various stress responses in bacteria. We investigated through biofilm assay whether ppGpp is involved in the pathogenesis of *A. baumannii*. Antibiotic susceptibility tests were conducted to determine the association between ppGpp and antibiotic resistance. The ppGpp-deficient *A. baumannii* strain reduced biofilm formation levels by more than 3-fold and was more sensitive to most antibiotics. We found that the expression of csu operon, an important pili biosynthetic gene for the early formation of biofilm, was remarkably reduced in ppGpp-deficient strain. And the antimicrobial susceptibility test showed the greatest difference in the level of the trimethoprim, indicating the difference in expression of gene related to the efflux pump. As a result, ppGpp regulates gene expression for biofilm formation and antibiotic resistance. This study is meaningful for the first time demonstrating the association between ppGpp and antibiotic susceptibility in *A. baumannii*.
[Supported by grants from NRF]

Keywords: ppGpp, Biofilm, MIC, *Acinetobacter baumannii*, Multi-drug resistance

F022

Porcine Epidemic Diarrhea Virus (PEDV) Infection Upregulates Heat Shock Protein 70 Expression in Infected Cells

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Viral replication completely depends on the host cellular factor. Many cell factors are necessary for viral infection in their life cycle. Heat shock protein 70 (HSP70,HSPA1A), a member of the heat shock protein family, has been known that it is related with various cellular processes. It is important for helping of refolding the protein which is misfolded or aggregated caused by cell stress. Porcine epidemic diarrhea virus (PEDV) infects Vero cells, and detailed infection mechanism to Vero cells is not clear yet. Correlation between heat shock protein 70 and virus infection has been reported in many other viruses. It is known that virus genome replication, viral protein synthesis and assembly is closely related with heat shock protein 70. In our studies, we found that PEDV infection is closely related with heat shock protein 70. We found that PEDV infection upregulates heat shock protein 70 expression in infected cells.

Keywords: Porcine Epidemic Diarrhea Virus (PEDV), Viral replication, HSPA1A

F023

N-Terminal Eukaryotic Extention Domain of Human Tryptophanyl-TRNA-Synthetase Activates Macrophage via TLR Signaling

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Tryptophanyl-tRNA synthetase (WRS) is an essential enzyme for protein synthesis as it catalyzes tryptophan ligation to its cognate tRNA. Interestingly, WRS undertakes several non-canonical biological activities beyond its catalytic reactions. While N-terminal truncated form of WRS (mini-WRS) functions as an angiostatic ligand, FL-WRS secreted from monocytes in response to pathogen infection works as an endogenous ligand of TLR4-MD2 for innate immune activation. Here, we show that the N-terminal eukaryotic extension domain, which consists of 154 amino acids in FL-WRS (N154) and is present in humans but not in prokaryotes, activated TLR signaling. N154 significantly induced chemokine and TNF-α secretion, comparable to the effect of FL-WRS in macrophage. FL-WRS and N154, but not mini-WRS, induced the activation of the NF-κB and ERK signaling pathways. Furthermore, blockade of TLR2 and TLR4-MD2 by siRNA abrogated N154-induced MIP-1α and TNF-α production. Finally, the protein-protein docking study proposed the interaction mode of N154 and TLR4-MD2 and mutational analysis further revealed that N-terminal 10 and 152 residues are critical for the macrophage activation. Taken together our data suggested that the N154 of WRS is sufficient to recapitulate the FL-WRS activity.
[This work was supported by Global Frontier Project grants (NRF-2015M3A6A-4065732) of Ministry of Science and ICT through the National Research Foundation.]

Keywords: Full-length tryptophanyl-tRNA synthetase (FL-WRS), Innate immunity, Endogenous ligand, TLR4-MD2

F024

Generation and Efficacy Evaluation of Inactivated Vaccine Candidate of West Nile Virus in Mouse

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West Nile virus (WNV) is a mosquito-borne pathogen worldwide that can cause to mild febrile illness, encephalitis or death in some cases. Currently, there is no specific vaccine and treatment available for humans. As one of measures on the preparedness of occurrence of human infection, we generated inactivated-vaccines and evaluated efficacy of vaccine candidates of West Nile virus in mouse in this study. The WNV lineage 1 and 2 strains were inactivated with 3% Formalin or 0.05% hydrogen peroxide at 37℃ for 7 days. Each vaccine candidates were evaluated for immunogenicity in mouse model. Six-week-old BALB/c female mice were intraperitoneally injected 3 times at 2 weeks intervals, and sera were collected on 0, 14, 28 and 42 days. The inactivated WNV linage 1(385-99) vaccine induced similar antibody titers compared with inactivated WNV linage 2(B956) vaccine groups. Antibody levels were similarly induced in both formalin-inactivated groups and hydrogen peroxide-inactivation groups, implying that there was no difference by methods of inactivation. Both inactivated 385-99 and B956 WNV vaccine candidates were showed a protective efficacy with the virus cross-challenge.
These results suggest that vaccination with both formalin or hydrogen peroxide-inactivated vaccines candidate strains were effective in preventing the infection of WNV. Those candidate strains could be further tested as vaccine candidates for WNV.
[Supported by grants from 2017-NI53005_00 of NIH, KCDC.]

Keywords: West Nile virus, Inactivated vaccine, Flavivirus

F025

Immunogenicity and Antibody Response of Recombinant Protein Vaccines Targeting Envelope Domain I and III of West Nile Virus

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West Nile Virus (WNV) is a causative agent of mosquito-borne disease. As the effective preventive and therapeutic measures, it is important to develop the safe and efficient vaccine in public health. For this purpose, the envelope (E) protein of flavivirus is considered to be the major target in the development of vaccine.
In this study, recombinant proteins of WNV envelop domain I (EDI) and domain III (EDIII) of two strains (lineage I, 385-99 strain; lineage II, B956 strain) were produced by *Escherichia coli* expression system. We evaluated the efficacy of the recombinant antigens (with alum) in mouse model. Six-week-old female Balb/C mice were injected 3 times intraperitoneally and their sera were collected at 14 days after final injection. The antibody activity were measured by Enzyme-linked immunosorbant assay and plaque inhibition assay. Mice immunized with the purified WNV recombinant EDI or EDIII antigens produced specific antibodies against WNV. Moreover, the antibodies of E protein were able to neutralize WNV *in vitro*. Taken together, the purified WNV E proteins could be promising vaccine candidates inducing neutralizing antibodies and could be further tested for the development of preventive measures against WNV infection. Increasing the immunogenicity of E domain proteins and evaluating the efficacy as potential vaccine candidates should be further studied.
[Supported by grants from 2017-NI53005_00 and 4837-301-210-13 of NIH, KCDC.]

Keywords: West Nile virus, Envelop protein, Recombinant protein, Vaccine

F026

Hepcidin-ferroportin Axis Controls the Iron Content of *Salmonella*-containing Vacuoles in Macrophages

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Macrophages release iron into bloodstream via the membrane-bound iron export protein, ferroportin (FPN). The hepatic iron-regulatory hormone hepcidin controls FPN internalization and degradation in response to bacterial infection. *Salmonella typhimurium* is capable of invading macrophages and proliferating in the *Salmonella*-containing vacuole (SCV). Hepcidin is reported to increase the mortality of *Salmonella*-infected animals by increasing the bacterial load in macrophages. Here, we assess the iron levels and find that hepcidin increases iron content in the cytosol but decreases it in the SCV through FPN on the SCV membrane. Loss of FPN from the SCV via the action of hepcidin impairs the generation of bactericidal reactive oxygen species (ROS) as the iron content decreases. We conclude that FPN is required to provide sufficient iron to the SCV, where it serves as a cofactor for the generation of antimicrobial ROS rather than a nutrient for *Salmonella*.

Keywords: *Salmonella typhimurium*, Hepcidin, Ferroportin, Iron, Reactive oxygen species

F027

Transmembrane Protein pUL50 of Human Cytomegalovirus Inhibits ISGylation by Downregulating UBE1L

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Interferon-stimulated gene (ISG) 15 encodes a ubiquitin-like protein that can be conjugated to proteins via an enzymatic cascade involving the E1, E2, and E3 enzymes. ISG15 expression and protein ISGylation modulate viral infection; however, the viral mechanisms regulating the function of ISG15 and ISGylation are not well understood. We recently showed that ISGylation suppresses the growth of human cytomegalovirus (HCMV) at multiple steps of the virus life cycle, and that the virus-encoded pUL26 protein inhibits protein ISGylation. In this study, we demonstrate that the HCMV UL50-encoded transmembrane protein, a component of the nuclear egress complex, also inhibits ISGylation. pUL50 interacted with UBE1L, an E1-activating enzyme for ISGylation, and to a lesser extent ISG15. However, pUL50 caused proteasomal degradation of UBE1L. The UBE1L level induced in human fibroblast cells by interferon-β treatment or virus infection was reduced by pUL50 expression. This activity of pUL50 involved the transmembrane (TM) domain, although pUL50 could interact with UBE1L independent of the TM domain. Consistently, colocalization of pUL50 with UBE1L was observed in cells treated with a proteasome inhibitor. Furthermore, we found that RNF170, an ER-associated ubiquitin E3 ligase, interacted with pUL50 and promoted pUL50-mediated UBE1L degradation via ubiquitination. Our results demonstrate a novel role for the pUL50 transmembrane protein of HCMV in the regulation of protein ISGylation.

Keywords: Human cytomegalovirus, pUL50, ISGylation, UBE1L

F028

Genome-wide Analysis of Regulatory G-quadruplexes Affecting Gene Expression in Human Cytomegalovirus

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G-quadruplex (G4), formed by repetitive guanosine-rich sequences, plays various regulatory roles in cells. We performed genome-wide analyses of G4s present in the putative promoter regions of a 235-kb human cytomegalovirus (HCMV) genome and investigated their roles in viral gene expression. We evaluated 36 putative G4-forming sequences associated with 20 genes for their ability to form G4 and for the stability of G4s in the presence or absence of G4-stabilizing ligands, by circular dichroism and melting temperature analyses. Most identified sequences formed a stable G4; 28 sequences formed parallel G4s, one formed an antiparallel G4, and four showed mixed conformations. However, when we accessed the effect of G4 on viral promoters by cloning the 20 putative viral promoter regions into the luciferase reporter and monitoring the expression of luciferase reporter gene in the presence of G4-stabilizing chemicals, we found that only 9 genes were affected by G4 formation, revealing promoter context-dependent gene suppression by G4 formation. Mutational analysis of G4s also demonstrated gene suppression by the sequence-specific G4 formation. Furthermore, the analysis of a mutant virus incapable of G4 formation in the UL35 promoter confirmed promoter regulation by G4 in the context of virus infection. Our analyses provide a platform for assessing G4 functions at the genomic level and demonstrate the properties of the HCMV G4s and their regulatory roles in viral gene expression.

Keywords: G-quadruplex, HCMV, Gene expression

F029

Analysis of IE62 Mutations Found in Varicella-Zoster Virus Vaccine Strains for Transactivation Activity

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Live attenuated vaccine strains have been developed for Varicella-Zoster virus (VZV). In the vaccine strains, ORF62 that acts as a transactivator for viral gene expression contains six non-synonymous mutations, but whether these mutations affect transactivation activity of IE62 is not understood. Here, we investigated the role of non-synonymous vaccine-type mutations (M99T, S628G, R958G, V1197A, I1260V, and L1275S) of IE62 in Suduvax, a vaccine strain isolated in Korea, for transactivation activity. In reporter assays, Suduvax IE62 showed 2- to 4-fold lower transactivation activity toward ORF4, ORF28, ORF29, and ORF68 promoters than wild-type IE62. Introduction of individual mutations into wild-type IE62 did not affect transactivation activity. However, the combination of M99T within the N-terminal Sp transcription factor binding region and V1197A/I1260V/-L1275S within the C-terminal serine enriched acidic domain (SEAD) significantly reduced the transactivation activity of IE62. The M99T/V1197A/-I1260V/ L1275S mutant IE62 did not show considerable alterations in intracellular distribution and Sp3 binding compared to wild-type IE62, suggesting that other alteration(s) may be responsible for the reduced transactivation activity. Collectively, our results suggest that acquisition of mutations in both Met 99 and the SEAD of IE62 is responsible for the reduced transactivation activity found in IE62 of the VZV vaccine strains and contributes to attenuation of the virus.

Keywords: Varicella-Zoster virus, Vaccine, IE62, Mutation, Suduvax

F030

Genetic Polymorphism of Plaque-purified Varicella Zoster Virus through Passaging *in vitro* Cell Culture

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Varicella-zoster virus (VZV) belongs to alphaherpesvirus and has a dsDNA genome of 125 kb. Previous studies suggested that VZV preparations are genetically polymorphic quasispecies containing mixed genotypes. This study attempted to analyze the genetic polymorphism of clinical and vaccine strains of VZV through plaque-purification and passaging *in vitro* cell culture. At low passage (p4), plaque-purified vaccine strain Suduvax (ppVac) contained approximated 4.8-fold more genetically polymorphic sites (GPS) than the plaque-purified clinical strain YC01 (ppCli). The number of GPS increased in higher passages (P30, P60) of ppCli, but did not change significantly in high passage of ppVac. Among the putative 24 vaccine-specific sites, 5 genetically polymorphic sites at low passage of ppVac became fixed to vaccine allele at high passage. These studies suggested that the high passage of plaque-purified vaccine strain might be a suitable candidate of additional VZV vaccine.
[Supported by grants from Green Cross]

Keywords: Varicella zoster virus, Genetic polymorphism, Vaccine

F031

Exploring Novel Inhibitors for HIV Tat-mediated Viral Transcription from LOPAC1280 with Dual Reporter Screening System

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Tat-mediated HIV-1 transcription is critical for HIV-1 life cycle, which is considered as a potent therapeutic target for inhibition of HIV-1. Recently, we developed a dual reporter screening system for precisely discriminating the inhibition of Tat-mediated transcription form off-target effects. In the present study, we performed the drug repositioning using the LOPAC1280 to identify novel Tat inhibitory compounds with our dual reporter screening system. Three compounds including CYC202, Quinacrine and Gemcitabine identified as specific inhibitors for Tat-mediated viral transcription without off-target effect. Two compounds, CYC202 and Quinacrine had been already known as viral transcription inhibitors, whereas, Gemcitabine was identified as a novel Tat inhibitory compound, which being used as an anti-cancer drug composed to nucleoside. To discover compounds having enhanced inhibitory effect among the several nucleosides, 3 derivatives of gemcitabine and 5 nucleoside analogues were tested in our system. Surprisingly, Gemcitabine much more strongly inhibited the viral infectivity than that shown in Tat inhibition.
Taken together, the results indicated that our dual reporter screening system was applicable to identify novel inhibitor for Tat-mediated viral transcription from a large number of compounds, and Gemcitabine and its similar compounds might be considered to therapy HIV-infection.
[This work was supported by intramural grants from KNIH (2017-NI51001-00).]

Keywords: HIV, Tat, Dual Reporter screening

F032

Reactive Oxygen Species Induced Apoptosis by ER-stress in Macrophage during *Mycobacterium smegmatis* Infection

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Mycobacterium smegmatis, a rapidly growing non-tuberculosis mycobacterium (NTM), is a good model for studying the pathogenesis of tuberculosis because of its genetic similarity to *Mycobacterium tuberculosis* (Mtb). In this study, we examined the role of apoptosis in *M. smegmatis* infected macrophage. Apoptosis is broadly accepted as a host defense mechanism against intracellular bacteria. We have previously reported that ER stress is important as a host defense mechanism against Mtb infection. *M. smegmatis*-induced ER stress was stronger than *M. tuberculosis*-mediated ER stress. We have shown that *M. smegmatis*-induced ROS play a critical role in induction of ER stress-mediated apoptosis. Pretreatment of ROS scavenger was effective to suppress *M. smegmatis*-induced ER stress. Elimination of ROS decreased ER stress responses and significantly increased the intracellular survival of *M. smegmatis*. These data suggest that *M. smegmatis*-induced ER stress plays an important role in growth suppression of *M. smegmatis*. Taken together, our results suggest that enhanced ROS generation decreases intracellular survival of *M. smegmatis* via ROS–mediated ER stress. Better understanding the role of ROS could provide new insights into the pathogenesis of tuberculosis.

Keywords: *Mycobacterium smegmatis*, ER stress, Apoptosis, ROS

F033

Transcriptional Elements of the Type VI Secretion System Hcp Gene Z0264 in Enterohemorrhagic *Escherichia coli* O157:H7

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Type VI secretion system (T6SS) is a novel secretion system found in many Gram-negative bacteria, with a potential role in bacterial competition and virulence. A promoter upstream of the T6SS gene cluster is affected by various biological signals. A previous report showed that enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 strain EDL933 contains a single T6SS gene cluster (Z0248-Z0264). In this study, we attempted to define transcription start sites (TSSs) and promoter regions for Z0264, the first gene from the T6SS gene cluster.
TSSs for Z0264 was identified using the ARF-TSS method. Transcriptional activity of predicted Z0264 promoter regions (P_{Z0264}) was analyzed by a truncated promoter-*lacZ* fusion assay.
As a result, we identified a guanosine residue located 117-bp upstream of the start codon with the highest frequency. P_{Z0264}-*lacZ* fusion assay also showed that the predicted region contained a working promoter. Notably, a P_{Z0264}-*lacZ* fusion harboring a 169-bp region showed stronger transcriptional activity than that of harboring a 591-bp region. In addition, cDNA qPCR assay showed that RNA transcript from P_{Z0264} did not cover the entire T6SS cluster.
Collectively, we identified a main TSS and functional promoter regions for Z0264 gene in EHEC O157:H7. Our results also suggest that negative regulation may occur upstream of Z0264 promoter.
[This study was supported by a grant from National Research Foundation (NRF-2017R1A2B4013056), Republic of Korea.]

Keywords: Enterohemorrhagic *Escherichia coli*, Type VI secretion system

F034

The Comparison of Clinical Characteristics in Three Types of Viral Acute Diarrhea in Infants and Toddlers and the Effect of *Lactobacillus acidophilus* on Rotaviral Diarrhea

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The most common causes of acute viral diarrhea in infants and toddlers are rotavirus, astrovirus and norovirus. The purpose of this study was to evaluate epidemiological data of pathogens obtained from stool exams and compare them with the clinical course in pediatric patients with symptoms of acute viral diarrhea and to investigate the clinical efficacy of *L. acidophilus* for acute diarrhea caused by rotavirus. Epidemiologic, clinical and laboratory data for three types of viral acute diarrhea were reviewed by enzyme-linked immunosorbent assay. Viruses were detected in 68% of subjects, with rotavirus being the most commonly reported in 50% of cases. To examine the characteristics of each virus, a clinical epidemiological study was performed. Noroviral infection symptoms included vomiting and diarrhea in patients of all age groups. Dehydration in noroviral acute diarrheal patients was less common than in rotaviral acute diarrheal patients. The clinical efficacy of orally administered *Lactobacillus acidophilus* in the treatment of acute viral diarrhea in infants and toddlers was also evaluated. *L. acidophilus* was an effective therapeutic adjuvant in acute viral diarrhea in infants and toddlers.

Keywords: Diarrhea, *Lactobacillus acidophilus*, Norovirus, Rotavirus

F035

Characterization of Antimicrobial Resistance and Virulence Factors in Zoonotic *Aeromonas* spp. Isolated from a Wild Nutria (*Myocastor coypus*) in South Korea

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The genus *Aeromonas* (*A.*) which belongs to the family *Aeromonadaceae* is widely distributed in aquatic environments. Interests in this genus have increased due to its zoonotic potential and the emergence of antibiotic resistance. Since 2016, we have investigated the occurrence of potential zoonotic aeromonads in animals to implement the one-health perspective on emerging public health threats. A total of 14 strains were isolated from a wild nutria (*Myocastor coypus*) collected at the Nakdong River (South Korea) and were identified based on 16S rRNA gene and *gyrB* sequence analyses, and biochemical characterizations. As a result, these isolates were classified as *A. hydrophila* (n = 8), *A. veronii* (n = 2), *A. caviae* (n = 2), *A. rivipollensis* (n = 1), and *A. dhakensis* (n = 1), and then, we conducted virulence gene screening and antimicrobial susceptibility tests. The identified aeromonads harbored several potential virulence-related genes and showed phenotypical resistance to several antibiotics such as β-lactams, quinolones, and carbapenems. In conclusion, our results indicated that wild animals could be one of the sources of transmission of zoonotic and antibiotic-resistant aeromonads to humans.
[This study was supported by the grants of the KRIBB Initiative program (KGM4691612) and the Global Frontier Program (2013M3A6B2078954) of the BioNano Health-Guard Research Center funded by the Ministry of Science and ICT of the Republic of Korea.]

Keywords: Aeromonas, Zoonotic potential, Antimicrobial resistance, Wild animal

F036

Heme Oxygenase-1 Regulates *Bacteroides fragilis* Enterotoxin-induced Dendritic Cell Maturation via a Mitogen-activated Protein Kinases- and Nrf2-dependent Pathway

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The *Bacteroides fragilis* enterotoxin (BFT) is a virulence factor of enterotoxigenic *B. fragilis* (ETBF) and causes colitis. Dendritic cells (DCs) play a major role in directing the nature of adaptive immune responses against intestinal infection and heme oxygenase-1 (HO-1) has been implicated in regulating function of DCs. We already demonstrated that HO-1 can be upregulated in intestinal epithelial cells stimulated with BFT. However, little is known about HO-1 induction in DCs in response to ETBF infection. This study was conducted to investigate the effect of BFT on HO-1 expression in DCs and their maturation. Stimulation of DCs with BFT resulted in upregulated expression of HO-1. HO-1 induction was dependent on Nrf2 activation in in BFT-exposed DCs. HO-1 induction via Nrf2 in DCs was regulated by mitogen-activated protein kinases (MAPKs) such as ERK and p38. Furthermore, HO-1 upregulation induced by BFT stimulation led to inhibit lipopolysaccharide (LPS)-induced DC maturation. These results suggest that signaling pathways involving ERK and p38 MAPK-Nrf2 in DCs is required for HO-1 induction during exposure to ETBF-produced BFT. Following this induction, increased HO-1 expression may regulate the DC maturation.
[Supported by grants from the Basic Science Research Program through NRF-2015R1D1A1A01058565 and 2018R1D1A1B07043350]

Keywords: *B. fragilis* enterotoxin, Dendritic cells, Heme oxygenase-1

F037

Parvulin 14 and Parvulin 17 Upregulate HBV Replication in a HBx-dependent Manner

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The parvulin 14 (Par14) and parvulin 17 (Par17) proteins, encoded by *PIN4* gene, play roles in protein folding, chromatin remodeling, DNA binding, ribosome biogenesis, and cell cycle progression. However, their effects on viral replication have never been explored. In this study, we found that, in the presence of HBx, either Par14 or Par17 could upregulate hepatitis B virus (HBV) replication, whereas in absence of HBx, neither Par14 nor Par17 had any effect on replication. Overexpression of Par14/Par17 markedly increased formation of covalently closed circular DNA (cccDNA), synthesis of HBV RNA and DNA, and virion secretion. Conversely, *PIN4* knockdown significantly decreased HBV replication in HBV-transfected and -infected cells. Par14/Par17 engaged in direct physical interactions with HBx in the cytoplasm, nucleus, and mitochondria, possibly *via* substrate-binding residues on Par14/Par17 (E46/D74 and E71/D99, respectively) and conserved $^{19}R^{20}P$-$^{28}R^{29}P$ motifs on HBx. These interactions enhanced HBx stability and promoted HBx translocation to the nuclear and mitochondrial fractions. In the presence of HBx, Par14/Par17 were efficiently recruited to cccDNA and promoted transcriptional activation via specific DNA-binding residues (S19/44). By contrast, in absence of HBx, Par14/Par17 bound cccDNA only at basal level. Taken together, our results suggest that knockdown or inhibition of *PIN4* represents a promising new therapeutic option to functionally cure chronic hepatitis B.

Keywords: PIN4, Par14, Par17, HBx, cccDNA, HBV replication

F038

A Case Report for Outbreak of Bovine Tuberculosis in Jeju Island

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Bovine tuberculosis (bTB) is cause by *Mycobacterium bovis (M. bovis)*, known as one of the chronic and wasting diseases, which affects cattle husbandry worldwide. In Korea, bTB has been known as endemic disease since first report in 1913, but only Jeju Island had only officially bTB free status in Korea. But from 2017 to 2018, some outbreaks of bTB were reported in Jeju Island. We tried to analyze the epidemiological study for these outbreaks.

We isolated and identified six *M. bovis* from four infected herds in Jeju Island and then, all isolates were analyzed by spoligotyping and 15 loci MIRU-VNTR.

Three of total herds were connected epidemiologically by cattle trade. Five *M. bovis* isolates from these herds shared same spoligotype and MIRU-VNTR. But the other herd showed no specific epidemiological data and link with other herds and *M. bovis* isolate had different molecular pattern.

These outbreaks were supposed to originate from the mainland because epidemiological molecular patterns by spoligotyping and MIRU-VNTR were already identified there although we don't know how to inflow from the mainland.

[This study was supported by Animal and Plant Quarantine Agency.]

Keywords: *Bovine tuberculosis*, Spoligotyping, MIRU-VNTR

F039

A Dual Regulator on *Salmonella*'s Phosphate Signaling

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Phosphate is known as an essential component for microorganisms to survive. A *Salmonella enterica serovar* typhimurium has an exclusive transport system for phosphate, Pho regulon. Pho regulon has seven components, PstS, PstA, PstB, PstC, PhoB, PhoR, and PhoU. PstS recognizes phosphate concentration of periplasm, and PstA and C, which are channel membranes, transport phosphates to cytoplasm. PstB transfers phosphate signal to PhoR. PhoB-PhoR is a two-component system, PhoR is a histidine kinase protein and PhoB is a response regulator protein. PhoR receives the signal and phosphorylates to its own histidine residue. Afterward, PhoR phosphorylates PhoB to make phospho-PhoB. Phospho-PhoB binds to promoter to transcript phosphate transport proteins. PhoU, a negative regulator, interacts with PhoR to inhibit the transport system. Here, we determined an additional interaction residue between PhoU and PhoR, suggesting a new role of PhoU on phosphate signaling.

[Supported by grants from NRF]

Keywords: Bacteria, Pathogen, Phosphate, Transporter, Pho regulon

F040

Evaluation of Addition of Interleukin-7 & 12 to IFN-γ Assay for Diagnosis of Bovine Tuberculosis

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Animal and Plant Quarantine Agency

Bovine tuberculosis (bTB) is known as one of the zoonotic diseases, which is chronic and it is difficult to be clean once infected. The IFN-γ assay is used as ancillary test with the intradermal tuberculin skin test depending epidemiological context. The aim of the study is to assess the addition of interleukin-7 & 12 to whole blood to prolong activity of T-lymphocyte for the IFN-γ assay.

We collected blood samples to from 100 bTB infected cattle and 119 non-infected cattle from slaughter house from 2017 to 2018. All whole blood samples after addition of candidates for preservative like interleukin were incubated on the same day or 30 h later for 24 h at 37℃. The titer for IFN-γ and the diagnostic result were compared between the control and the experimental groups.

Although when combination with Interleukin-7 & 12 to blood collected from bTB infected cattle boosted the IFN-γ production from 0.15 ng/ml to 0.2 ng/ml for each interleukin, there was no significant boosting and false positive results for non-infected cattle. Result of comparison between on the same day and next day incubation after collection of blood for 65 bTB infected cattle showed that 90.5% (59/65) of the experimental group was maintained the diagnostic result until 30 h incubation.

Our result showed the possibility of application of interleukin-7 & 12 to whole blood to extend the viability of T-cell.

[This study was supported by Animal and Plant Quarantine Agency.]

Keywords: Bovine tuberculosis, IFN-γ assay, Interleukin, T-cell

F041

The Prevalence and Characteristics of Enterohemorrhagic *Escherichia coli* (EHEC) Isolated in Korea from 2009 to 2018

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Enterohemorrhagic *Escherichia coli* (EHEC) has been recognized as a water and foodborne pathogen that causes several human gastrointestinal illnesses such as bloody or non-bloody diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). In this study, we reported the prevalence and characteristics of EHEC isolated from domestic residents from 2009 to 2018 in Korea. To characterize isolated EHEC, shiga toxin genes and O serotype of isolates was determined by multiplex-PCR and agglutination method with the available O antisera, respectively. And EHECwas analyzed according to isolated season, patients' age and sex.
A total of 386 EHEC isolates were collected from acute diarrheal patients. Isolation rate by patients' age showed highest ratio (70%) in children under age of 5but, there was no difference in sex. Among the 386 strains, the O157 (15%) was most prevalent serogroups and O103 (12%), O26 (6%), O111 (5%), O91 (4%), O8 (2%), O108 (2%) were followed. The profiles of shiga-toxin genes of O157 strains weredifferent form that of other serogroups (O103, O26 and O111). In O157 strains, *stx*1&*stx*2 was detected most frequently than *stx*1 and *stx*2 alone, while *stx*1 gene was most frequently present than *stx*2 and *stx*1&*stx*2 in non-O157 EHEC strains (O103, O26 and O111). For 10 years, the isolation ratio of EHEC was high inchildren under 5 years old. Therefore, EHEC Hygiene education should be addressed on diarrheal disease susceptible groups.

Keywords: EHEC, O157, Enter-Net

F042

The Prevalence and Characteristics of Bacteria Causing Acute Diarrhea in Korea, 2015-2017

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This study was performed to determine the characteristics of the diarrheal causing pathogens according to season, isolated regions, patient`s age and sex and to provideuseful data for the prevention of diarrheal disease. 33,677 stool specimens were collected from patients with diarrhea to identify the pathogenic bacteria from 2015 to 2017 in Korea. From the 33,677 stool specimens, 5,340 pathogenic bacteria were isolated and analyzed according to season, isolated regions, patients' age and sex. The proportions of isolated pathogenic bacteria were *Salmonella* spp. 1,100 (20.6%), pathogenic *E. coli* 1,487 (27.8%), *V. parahaemolyticus* 36 (0.7%), *Shigella* spp. 23 (0.4%), *Campylobacter* spp. 458 (8.6%), *Cl. Perfringens* 339 (6.3%), *S. aureus* 1,238 (23.2%), *B. cereus* 622 (11.6%), *L. monocytogenes* 10 (0.2%) and *Y. enterocolitica* 27 (0.5%). Isolation rate for most of pathogenic bacteria showed highest ratio in summer season, from June to August. Isolation rate of pathogenic bacteria by patients' age showed highest ratio at 0 to19 year for most of pathogenic bacteria. And Isolation rate by region, 51.1% isolated from cities and 48.9% isolated from rural provinces. Hygiene education should be addressed on diarrheal disease susceptible groups, such as age under 10, age of 10-19, and more than 70 years old, and ongoing monitoring for the pathogens is still required. In addition, efficient information system and surveillance project for infection prevention should be continued.

F043

The FgCYP51B Y123H Mutation Confers Reduced Sensitivity to Prochloraz and is Important for Conidiation and Ascospore Development in *Fusarium graminearum*

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Tyrosine 123 is an important amino acid in *Fusarium graminearum* CYP51B, which is predicted to lie in one of the substrate binding pocket based on the binding mode between demethylation inhibitors (DMIs) and CYP51B. Previous study suggests that resistance to DMI fungicides is primarily attributed to point mutations in the *CYP51* gene and that the Y123H mutation in *F. verticillioides CYP51* confers prochloraz resistance in the laboratory. To investigate the function of CYP51B Y123 residue in the growth and development, pathogenicity, and DMI-resistance of *F. graminearum*, the Y123H mutant was generated and analyzed. Results revealed that Y123H mutation led to reduce conidial sporulation and affect ascospore development and moreover, the mutation conferred reduced sensitivity to prochloraz. These results will attract more attention to the potential DMI-resistant mutation of *F. graminearum* and further deepen our understanding of the resistance mechanisms.

Keywords: Biological phenotype, CYP51B, Mutagenesis, Prochloraz, Resistance

F044

Characterization of Red Bread Mold Disease Caused by *Neurospora tetrasperma* in *Lentinula edodes*

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Gyeongnam Agricultural Research and Extension Services

Lentinula edodes has traditionally been cultivated on hardwood logs, mainly oak (*Quercus* spp.) in order to obtain fruiting bodies for human consumption. Recently sawdust cultivation is becoming more common and getting increase. Fungal contamination is an important factor affecting the stage of inoculation, spawn running, mycelia browing, and growth of fruit body. In 2018, unusual symptoms on *L. edodes* were observed in mushroom farm in JinJu-city of Gyeongnam Province. One of the main symptoms was the inhibition of mycelial growth and the formation of a large mass on the surface of media. When the red bread mold was contaminated at spawn running stage, a confrontation line was formed between the edges of mycelia. Finally, the mycelia of red bread mold was made into a large mass in the inoculated region. The fungal mycelium has the branched chains of macroconidia, roughly ellipsoidal cells containing nuclei. It is also produced the tiny microconidia. The optimum temperature for mycelial growth is 25℃. The phylogenetic tree obtained from the ITS sequence analysis showed that the isolated fungal pathogen corresponded to *Neurospora tetrasperma* (100.0%). This report will help the farmers to understand the characterization of red bread mold disease on *L. edodes* caused by *N. tetrasperma*.

F045

In vitro Pharmacodynamics of Colistin against Avian Pathogenic *Escherichia coli* (APEC)

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Colistin is an effective to treat infections of the stomach and the intestine caused by Gram-negative bacteria. To date, the emergence of mcr-1 has been reported in carbapenem-resistant *E. coli*. However, despite the absence of the mcr-1 gene, we found the susceptible APEC can be changed to the resistant after several time exposures at sub-MIC. So, the purpose of this study investigates colistin pharmacodynamics against the susceptible avian pathogenic *E. coli*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against APEC were tested according to CLSI 2016. The mutant prevention concentration (MPC) was defined as the lowest drug concentration that suppressed growth for 72 h. To determine the rate at which colistin kills APEC at a given concentration, time-kill assay was performed. On the other hand, inhibition of the formation of biofilm used as a means of survival of APEC was further studied. The APEC were susceptible to colistin (MIC value was >0.5 μg/ml). APECs were exposed six times at a half MIC and MIC by time-kill assay. During time-kill assay, strains showed inhibition after colistin exposure at 0.0126–1 μg/ml but regrowth after 12 h. However, strains exposed to colistin for several times survived in concentration of 4-8 μg/ml after 12 h, without mrc-1 gene (they didn't survive at 16 μg/ml). In this study, we found inappropriate exposure of colistin concentration induces colistin resistance without mrc-1 gene against APEC.

Keywords: Avian pathogenic *Escherichia coli* (APEC), Colistin, Pharmacodynamics

F046

Characterization of CTX-M-15 Producing *Salmonella enterica* Serotype Enteritidis from Food-producing Animals in Korea

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Salmonella enterica serotype Enteritidis (*S.* Enteritidis) is the one of main serovars related to salmonellosis in human. Furthermore, CTX-M-derived ESBL-producing *Salmonella* spp. has increasing worldwide. The aim of this study was to characterize CTX-M producing *S.* Enteritidis isolated from animals and animal products in Korea during 2010-2016. A total of 36.9% (80/217) of *S.* Enteritidis were resistant to the third generation cephalosporins. All of 80 resistant *S.* Enteritidis possessed the $bla_{CTX\text{-}M\text{-}15}$ gene. High resistance rates of $bla_{CTX\text{-}M\text{-}15}$ positive isolates were detected to tetracycline (92.5%), gentamicin (93.8%), neomycin (83.8%), and streptomycin (87.5%). IncHI2 and/or IncFIIs conjugative plasmids were identified in 18 strains that co-carried tetracycline and aminoglycoside resistance. In all transconjugants, the mobile elements IS*Ecp1* upstream and *orf477* downstream of $bla_{CTX\text{-}M\text{-}15}$ were found. PFGE identified 11 different profiles and one major clone. The presence and spread of $bla_{CTX\text{-}M}$ *Salmonella* isolates in food-producing animals may pose a potential treat for public health.
[Supported by a grant from the Animal and Plant Quarantine Agency, Republic of Korea (N-1543081-2015-24-01)]

Keywords: *S.* Enteritidis, ESBLs, CTX-M-15, Conjugative plasmids

F047

Evolution of *Klebsiella pneumoniae* with Mucoid and Non-mucoid Type Colonies within a Single Patient

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We obtained nine *Klebsiella pneumoniae* strains successively isolated from a single patient. Four pairs (M1–M4 and NM1–NM4) obtained simultaneously from the same site showed different colony types, mucoid and non-mucoid, while the final strain (M5) was isolated alone from the blood and showed a mucoid phenotype. The whole genome of strain M5 was sequenced *de novo* using the PacBio RSII system, while the others were sequenced with an Illumina Hiseq4000 and mapped to the genome sequences of M5. To identify insertions or deletions in the *cps* locus, we amplified and sequenced *cps* locus genes. We identified insertion sequence (IS) elements in several genes of the *cps* locus or one amino acid substitution in WcaJ in all non-mucoid strains. Based on the genome data and *cps* locus sequences, the mucoid phenotype may have been lost or converted into the non-mucoid phenotype because of the insertion of IS elements or amino acid alterations at this locus. We predicted a within-host evolutionary scenario, in which the mucoid strains evolved continuously from previous strains and non-mucoid variants emerged repeatedly from isogenic mucoid strains, but may be short-lived because of their low fitness. Bacterial within-host evolution may vary because of variations in the bacterial species, host conditions, bacterial features under selection pressure, and others.
[Supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF)]

Keywords: *Klebsiella pneumoniae*, Whole genome sequencing, Mucoid, *cps* Locus, Insertion sequence element

F048

Screening of Chemical Compounds for Anti-mycobacterial Activity *in vitro*

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Tuberculosis (TB) is a serious infectious disease worldwide. Recently, incident of multidrug resistance (MDR)-TB has been increased. However, there is little proper treatment of MDR TB. To investigate effective anti-mycobacterial therapy, we have examined inhibitory effects against mycobacterial infection among 134 chemical compounds which are based on structure of nutlin-3, an activator of P53. We performed intracellular survival (ICS) analysis for assessment of the antimycobacterial activity of these compounds. Among them, two compounds effectively suppressed intracellular survival of mycobacteria. The number 266 compound has 60% inhibitory efficiency to mycobacteria in macrophages, and the number 256 compound has 72% inhibitory efficiency to intracellular growth of mycobacteria compared to the control. Combined treatment of each isolated compound and antimycobacterial drugs such as isoniazid, rifampicin, and ethambutol synergically suppressed the growth of mycobacteria in macrophages. These results suggest that chemical compounds (No. 266 and 256) might be considered as a candidate of antimycobacterial drugs in future.

Keywords: Turberculosis, Intracellular survival, *Mycobacterium tuberculosis*, Screening, Chemical compound

F049

Comparison of Genetic Features and Virulence between Plasmid- and Chromosome-mediated Colistin-resistant *Escherichia coli*

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The report of plasmid-borne mobile colistin resistance gene *mcr-1* has been threatening human being with the idea of dissemination of colistin resistant bacteria. Here, we investigated the characteristics of *mcr-1* strains with colistin susceptible, colistin resistance-induced, chromosome-mediated colistin resistance and *mcr-1*-transferred strains.
Mutation and expression levels of the *basRS*, *phoPQ*, and *eptA* genes were detected. Several virulence assays were performed to determine the characteristics of each strain. And also, to find out the role of *pap2* gene, *mcr-1*- and *pap2*-transfered transformants were constructed.
The *eptA* expression levels of three colistin resistant-induced strains were significantly increased in comparison with those of colistin susceptible strains. *In vitro* competition assay, drosophila and macrophage infection experiments indicated that the *mcr-1*-harboring strains didn't lose the fitness. Also, in serum resistance assay, *mcr-1*-positive strains were more resistant in human serum. The *mcr-1-pap2*-transferred strain showed higher colistin MIC than parental strain.
In this study, we figured out that the *mcr-1*-harboring colistin resistant strains did not show the bacterial fitness cost. Therefore, we are facing a threat of dissemination of colistin resistant bacteria through the horizontal gene transfer (HGT). And also, we confirmed that the *pap2* supports the *mcr-1* when the *mcr-1*-mediated colistin resistance is occurred

Keywords: *Escherichia coli*, Colistin resistance, *mcr-1*, *pap2*

F050

Persister Cell Formation in *Pseudomonas aeruginosa* Clinical Isolates

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Pseudomonas aeruginosa is a Gram-negative bacterium and the major nosocomial pathogens. Persister cell stays in dormant form when high concentration antibiotics exist. These persister cells leads a state of dormancy, which are metabolically inactive. This phenomenon makes it difficult to remove pathogens completely from patients. This study characterized persister cell formation from mid-exponential phase cell. Antimicrobial susceptibility test was performed using Clinical and Laboratory Standard Institute recommendations. Persister cell formation assay was performed with four antibiotics. To further characterize the formation of persister cells, first persister cell formation rate was compared to the second rates from re-grown persister cell in the fresh media. During incubation time, the survival rates of persister cell was investigated on each term. This study determined to which combination therapy of antibiotics is more synergistic in *P. aeruginosa*. To understand the mechanism of persister cell formation, the expression level of *relA*, *spoT* gene was measured by quantitative reverse transcription polymerase chain reaction and measured the concentration of ATP. Various mRNA expression level was detected in each treated antibiotic and each strain. We observed the phenotype of persister cell, using Transmission electron microscopy. These results suggest that persister cells against each antibiotic have different mechanisms and phenotypes.

Keywords: *Pseudomonas aeruginosa*, Persister cell, Antibiotic combination

F051

Prevalence and Characterization of Erythromycin-resistant *Campylobacter coli* Isolated from Food-producing Animals in Korea, 2010 to 2016

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Macrolide antibiotics are primarily used for the treatment of *Campylobacter* infections. We studied the mechanism of macrolide resistance in *Campylobacter coli* from food animals. A total of 161 erythromycin resistant *C. coli* isolates were found in cattle (8.7%, 14/161), pigs (66.5%, 107/161), and chickens (24.8% 40/161) in Korea during 2010-2016. The isolates showed high resistance to nalidixic acid (85.1%, 37/161), tetracycline (80.7%, 130/161), clindamycin (64.6%, 104/161) as well as macrolide resistance. Sixty-six of the isolates were randomly selected and their macrolide resistance mechanisms were investigated. Sequence analysis of the 23S ribosomal RNA revealed an A2075G amino acid mutation in all isolates, with 33 of the isolates also harboring mutations in ribosomal proteins L4 and L22. The minimum inhibitory concentration of erythromycin was determined in the absence and presence of PAβN efflux pump inhibitor. More than two-fold decrease was observed in 21 of the 66 resistant *C. coli* isolates. The mutations in the 23S rRNA gene were primary responsible for macrolide resistance in *C. coli* in this study. However, further studies were necessary to investigate the roles of protein L4, L22 and efflux pump on macrolide resistance.
[Supported by a grant from the Animal and Plant Quarantine Agency, Republic of Korea (N-1543081-2015-24-01)]

Keywords: *Campylobacter*, Marolide resistance, Efflux pump

F052

Antiviral Effect of *Enterococcus canintestini* on Influenza A Viruses

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Pandemic influenza poses a serious threat to global health and the world economy. Here we tested engineered microbes for potential antiviral activity against influenza A viruses. In this study, we exploited engineered *Enterococcus canintestini* fused with sulfur to enhance its therapeutic efficacy.
Engineered microbe was constructed with fusing sulfur to *Enterococcus canintestini*. When orally administered into mice, prolonged survival of the mice by 50% after influenza virus infection. These results suggest that biological antiviral candidates using *Enterococcus canintestini* fused with sulfur were antiviral activities, represents a promising strategy.

Keywords: *Enterococcus canintestini*, Influenza A virus, Antiviral agent

F053

Biological Tick Management in *Haemaphysalis longicornis*: The Use of Entomopathogenic Fungi and Effective Application

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Longhorned tick, *Haemaphysalis longicornis* (Ixodida: Ixodidae) is a serious pest causing severe fever with thrombocytopenia syndrome (SFTS) in human. The ticks occurs in most grass fields, and pyrethroid insecticides cause insect resistance and environmental residual toxicity. Particularly the use of chemicals near residential areas where persons live become a big issue, so much environmentally safe control agents needs to be considered. Here in this work, our interest was given to the selection of highly virulent entomopathogenic fungi against *H. longicornis*. A total of 101 fungal pathogens collected were assayed by a dipping the nymph stage of ticks into a conidial suspension (1×10^7 conidia/ml). Interestingly among several species, *Metarhizium* species showed high virulence and mycosis were observed in 7–14 days. Most of the selected isolates produced a large amount of conidia in Italian millet, rice and millet with thermotolerance at 35–45°C. Based on these results, we selected a couple of isolates with high virulence against *H. longicornis*. This work suggests that selected isolates in this study can be used for the biological control of *H. longicornis*.

[Supported by grant from the Korea Centers for Disease Control and Prevention.]

Keywords: Entomopathogenic fungi, Fungal pathogen, *Haemaphysalis longicornis*, Longhorned tick, SFTSV

F054

Selection of Virulent Insect Killing Fungi to Respress the Population of Silverleaf Whitefly *Bemisia tabaci*

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Silver leaf whitefly, *Bemisia tabaci* has a broad host range of more than 600 species, and 40 biotypes have been reported worldwide, depending on host preference or the type of virus mediated. Among the various ecological types, B and Q are the most problematic. Especially, Biotype Q, which causes serious damage in the green house, mediates more than 40 kinds of viruses, including *Tomato yellow leaf cull virus* (TYLCV) is known to be the most problematic virus. Control of *B. tabaci* is based on biological control using *Encarsia formosa* and physical control using yellow sticky traps. The development of tolerance to *B. tabaci*, adverse effects on non-target organisms, and strengthened legal regulations are making chemical control difficult. Insect pathogenic fungi can be a good alternative to this situation. Fungi were evaluated to control the B biotype in the 1990s, and *Lecanicillium* and *Aschersonia* species have been used to control whitefly and related insects in greenhouses in Europe and Canada. In this study, 72 strains were tested for pathogenicity by dipping *B. tabaci* larvae into spore suspension, and a strain library was constructed. 10 strains of *Beauveria bassiana* showed high pathogenicity to *B. tabaci*.

[Supported by grant from Cooperative Research Program for Agriculture Science & Technology Development, Rural Development Administration, Republic of Korea]

Keywords: *Bemisia tabaci*, Biotype, Entomopathogenic fungi, Fungal library

F055

Genomic Analysis of the Insect-killing Fungus *Beauveria bassiana* JEF-007 as a Biopesticide

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Insect-killing fungi have high potential in pest management. A deeper insight into the fungal genes at the whole genome level is necessary to understand the inter-species or intra-species genetic diversity of fungal genes, and to select excellent isolates. In this work, we conducted a whole genome sequencing of *Beauveria bassiana* (*Bb*) JEF-007 and characterized pathogenesis-related features and compared with other isolates including *Bb* ARSEF2860. A large number of *Bb* JEF-007 genes showed high identity with *Bb* ARSEF2860, but some genes showed moderate or low identity. The two *Bb* isolates showed a significant difference in vegetative growth, antibiotic-susceptibility, and virulence against *Tenebrio molitor* larvae. When highly identical genes between the two *Bb* isolates were subjected to real-time PCR, their transcription levels were different, particularly in *heat shock protein 30* (*hsp30*) gene which is related to conidial thermo-tolerance. In several *B. bassiana* isolates, *chitinases* and *trypsin-like protease* genes involved in pathogenesis were highly conserved, but other genes showed noticeable sequence variation within the same species. This genetic approach could support the development of excellent biopesticides with intellectual property protection.

[Supported by grant from the Strategic Initiative for Microbiomes in Agriculture and Food, Ministry of Agriculture, Food, and Rural Affairs and Korea Forest Research Institute, Republic of Korea.]

Keywords: Entomopathogenic fungi, *B. bassiana* JEF-007, ARSEF2860, *Tenebrio molitor*, Thermotolerance, Sequence variation

F056

Screening of Insect Killing Fungi to Manage Melon Thrips, *Thrips palmi* and Fungal Library Construction

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Melon thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae) is one of the serious insect pests in many economic crops, and the management of this pest mainly on chemical insecticides. However, the overuse of chemicals caused insect resistance and environmental residual issues, and now the thrips management needs additional solutions. We need to consider alternative strategies which are less harmful to the environment and working on different target points. Here in this work, we isolated entomopathogenic fungi from soil, and identified with morphological and molecular biological methods, followed by a preliminary virulence assay against *Tenebrio molitor* larvae. Selected fungal isolates were adjusted to 1×10^7 conidia/ml for an indoor virulence assay against *T. palmi* adults and highly virulent isolates were added to a thrip-pathogenic fungal library. Biological characteristics of an efficacious isolate were investigated by comparing with a previously commercialized fungal isolate *Beauveria bassiana* ERL836. This entomopathogenic fungal library could be used as a valuable resource for developing effective strains to control *T. palmi* in agricultural fields.

[Supported by grant from Cooperative Research Program for Agriculture Science & Technology Development, Rural Development Administration, Republic of Korea]

Keywords: Biological control, Entomopathogenic fungi, Library, *Thrips palmi*

F057

Regulation of *Tannerella forsythia*-induced IL-24

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Interleukin-24 (IL-24) is a member of Interleukin-10 family, which can induce immune responses via JAK/STAT pathway activated by binding to IL-20 receptors (IL-20R1/IL-20R2 and IL-22R1/IL-20R2). Unlike other IL-10 family members, IL-24 must be glycosylated to be secreted from cells and only glycosylated IL-24 has bioavailability. The aim of this study is to investigate the regulation of IL-24 by *Tannerella forsythia* and the signaling pathway of IL-24 expression. HOK-16B cells and human gingival fibroblasts (HGFs) were treated with *T. forsythia* and *Streptococcus oralis* at various MOIs for 12 h – 48 h. IL-24 was secreted into the supernatants as a glycosylated form and increased in a MOI-dependent manner by *T. forsythia*, when analyzed by Western blotting. On the contrary, IL-24 was not secreted in the cells treated with *S. oralis*, one of major commensals in oral cavity. *T. forsythia* activates phosphorylation of Erk and p38 in HOK-16B cells and inhibition of Erk and p38 reduced IL-24 expression and secretion of its glycosylated form. Our results show that *T. forsythia* regulates expression of bioavailable IL-24 via MAPKs.

Keywords: IL-24, *Tannerella forsythia*, Periodontitis

F058

Tannerella forsythia Activates AIM2 Inflammasome in Human Gingival Fibroblasts

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Tannerella forsythia is one of the major periodontal pathogens which can cause excessive immune responses. Inflammasome is crucial for host defense against pathogens. The AIM2 (absent in melanoma 2) inflammasome does not have LRR which is a sensor of PAMPs. The AIM2 protein directly detects cytosolic double-stranded DNA (dsDNA) via its C-terminal HIN-200 domain and activates the inflammasome pathway, activating caspase-1. Active caspase-1 induces maturation of pro-inflammatory cytokines and induces pyroptosis. The aim of this study is to investigate whether *T. forsythia* activates AIM2 inflammasome in human gingival fibroblasts (HGFs), contributing to chronic inflammatory disease such as periodontitis. HGFs were stimulated with various MOIs of *T. forsythia*. The expression of AIM2, caspase-1/4 and IL-1α was determined by immunoblotting. The IL-1α level was also assessed by ELISA. HGFs were transfected with siRNA specific for AIM2 or irrelevant control siRNA. HGFs were pre-treated with caspase-1 inhibitors (Z-YVAD-FMK) for 30 min before infection with *T. forsythia.* Cell death was measured by LDH cytotoxicity assay kit. we found that *T. forsythia* activated caspase-1/4, induced AIM2 expression, IL-1α release, and Pyroptotic cell death. It also induced AIM2 release, which was inhibited by a caspase-1 inhibitor. AIM2 knockdown reduced *T. forsythia*-induced inflammasome activation and IL-1α secretion, which may contribute to the inflammatory response in periodontitis.

Keywords: *Tannerella forsythia*, AIM2 inflammasome, Periodontitis

F059

Differences of Soft Contact Lens Material between Etafilcon A and Hilafilcon B on Biofilm Formation of *Staphylococcus epidermidis*

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Staphylococcus epidermidis is the most common cause of eye infections and one of the most studied virulence factors in *S. epidermidis* is the biofilm formation. So, in this study, we investigated differences in biofilm formation between etafilcon A and hilafilcon B of soft contact lens of *S. epidermis*. Also, for making an environment similar to the human condition, the formation of biofilm was examined by deposition of tear protein. The XTT assay and dry weight measurement were used to compare the amounts of biofilm formation. Reverse transcription PCR and real time PCR were used to compare the expression level of genes related to biofilm formation. The adhered *S. epidermidis* was observed by scanning electron microscope. The biofilm formation was higher on the surface of etafilcon A than on the surface of hilafilcon B when measured by the XTT assay and by dry weight measurement. In the comparison of gene expressions of icaA, icaB, icaC, icaD and arcA was higher on etafilcon A than on hilafilcon B. In these experiments, under the deposition of tear protein, biofilm formation was less than when not deposited. When observed with SEM, it was confirmed that *S. epidermidis* was more prolific and formed thicker layers on etafilcon A than on hilafilcon B. This suggests that *S. epidermidis* is more active in the formation of biofilm than etafilcon A than hilafilcon B, which may affect the pathogenicity of soft lens related eye disease.

Keywords: Etafilcon A, Hilafilcon B, *Staphylococcus epidermidis*, Biofilm, Soft contact lens

F060

Transcriptome Analyses of VZV Infection in Mouse Dorsal Root Ganglia

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Varicella Zoster Virus (VZV) responsible for herpes zoster (shingles) causes chronic pain in older generation of adults. The reactivation of VZV is associated with host pain responses, however the mechanism of how reactivation of virus correlates to nerve damage and nociception is poorly understood. In this study, using previously demonstrated capability of VZV to infect and induce nociception in mouse Dorsal Root Ganglion (DRG); we performed transcriptome analysis by Next Generation Sequencing (NGS) to delineate expression profile of mouse DRG against VZV infection. Using publicly available Kyoto Encyclopedia of Genes Genomes (KEGG) pathways, functional get set enrichment test revealed high enrichment of inflammatory, apoptotic, metabolic signaling pathways. Cancer and immune related pathways also showed high enrichment. Neurotransmission pathways were also examined and found enhanced dopaminergic and glutamatergic pathways that play crucial roles in perception of pain. Gene Ontology (GO) enrichment database was searched for genes that matched for keywords nociception and nociceptor then used to sort Differentially Expressed Genes (DEG) showing significant fold change |FC|>=2. Well accepted pain related genes such as Aqp1, Ntrk1 and Ret emerged significantly changed in VZV infected DRG cells. These results show important implications for many host responses to VZV infection in neuronal cells which may help us further delineate molecular mechanisms of VZV induced nociception.

Keywords: Varicella zoster virus, Dorsal root ganglion, Transcriptome ANALYSIS, KEGG, Nociception

F061

Detection of Novel Polyomavirus, Related to Japanese Eel Endothelial Cells-infecting Virus, in *Anguilla anguilla*

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Recently a severe pathogenic DNA virus, polyomavirus sized 75 nm, was isolated from diseased Japanese eel *Anguilla japonica,* and was designated as Japanese eel endothelial cells-infecting virus (JEECV). The present study was conducted to carry out a risk-based random sampling targeted survey which involves screening of *A. anguilla* (DNA extracted from gill tissue) for JEECV virus using polymerase chain reaction (PCR) method by using specific primer set A (targeting polyomavirus large T like protein (LTLG) region) and set C (targeting other putative open reading frame, ORF) and also by histopathological observations. The PCR results came positive for the target product (270 bp) by primer set A in several *A. anguilla* samples but negative for primer set C. The sequence analysis of the positive amplified products (for set A) showed maximum homology with the previously reported large T antigen of JEECV. Histopathological examination revealed fat tissues replaces large area of the liver and kidney along with the previously reported symptoms of congestion in the central venous sinuses of the gill, liver and intestine. Thus the study suggested that the detected virus from *A. anguilla* was very similar but not identical to the JEECV infecting *A. japonica*. Further, the study may be helpful for the eel aquaculture industry to be aware of the importance of the VECNE disease and to develop preventive strategies against the virus.

F062

Prevalence and Characterization of Linezolid Resistant *Staphylococcus aureus* from Animal Carcasses in Korea

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Linezolid is one of the only few antimicrobial agents currently available for treatment of multidrug-resistant Gram-positive bacteria. The aims of this study were to determine the occurrence of linezolid resistance in *Staphylococcus aureus* (*S. aureus*) and characterize the linezolid resistant (LR) *S. aureus* from animal carcasses in Korea. We collected a total 2,547 *S. aureus* from 2010 to 2017. A total of 25 (1.1%) of *S. aureus* were resistant to linezolid from pig carcasses. All LR *S. aureus* isolates showed high-level ($\geq$64) chloramphenicol resistance and carried the *cfr* gene. In addition, mutations at *rplC* (G121A) and *rplD* (C353T) genes were identified in one and seven LR *S. aureus* isolates, respectively. However, mutation of domain V of 23S ribosomal RNA was not detected in any of the strains analyzed. Six different MLST types were identified in 25 LR *S. aureus* isolates. Seven isolates belonged to ST541-t034 (*spa* type) or ST398-t034, among them six isolates carried *mecA* gene. This is the first report of *cfr*-mediate resistance to linezolid in *S. aureus* from animal carcasses in Korea. LR *S. aureus* could be potential reservoir of important resistance gene for human, therefore required continuous monitoring.
[Supported by a grant from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food, and Rural affairs, Republic of Korea (N-1543081-2015-24-01)]

Keywords: Linezolid, *S. aureus*, Animal carcasses

F063

Approaches for Bacterial Isolation from Dogs Infected by *Brucella canis* for Unequivocal Diagnosis

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Animal and Plant Quarantine Agency (APQA)

For canine brucellosis caused by *Brucella canis*, isolation of organism is the only available test as a diagnostic golden standard. In this study, the main aim is to compare efficiency of the *B. canis* isolation methods between using tissue, whole blood and buffy coat. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood of dogs and cultured for 4 days. All *Brucella*-suspected colonies were confirmed as *B. canis* by differential multiplex PCR and classical biotyping assay. Of 21 dogs, *B. canis* were isolated from canine PBMCs in 12 dogs (57.14%) whole blood in 13 dogs (61.90%) and tissues in 17 dogs (80.95%). In comparison with *B. canis* isolation from tissues, canine PBMCs moderately correlated with tissues [agreements(A) 76.19%; kappa(K), 0.478]. The results from whole blood also had a fair correlation with that from tissues [A 71.43%; K 0.330). These results indicated *B. canis* isolation method from canine PBMCs could be as efficient as whole blood method in isolation rates. In conclusion, due to non-specific responses in serological tests, unequivocal diagnosis should be confirmed by isolating bacteria from infected dogs. Thus, we highlighted the implication of modified diagnosis using cultured PBMCs, comparing with direct culture of whole blood, on canine brucellosis. Indeed, we suggest cell-based *B. canis* isolation method for supporting the golden standard diagnosis of *B. canis* infection.
[This study was supported by grants by APQA(B-1543081-2017-18-02)]

Keywords: *Brucella canis*, Dogs, Bacterial isolation, Diagnosis

F064

Development of Zika Virus Envelope Protein-specific Monoclonal Antibody Using Phage Antibody Display Technique

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Zika virus (ZIKV), an enveloped virus included in the family *Flaviviridae*, has to be diagnosed rapidly to prevent from diseases such as Guillain-Barre syndrome and microcephaly from ZIKV infection. In this study, we developed anti-ZIKV monoclonal antibody (mAb) using inactivated whole ZIKV and recombinant ZIKV envelope protein for antigen, and single chain variable fragment (scFv)-displayed phage library for antibodies. The phage library was screened four times with the antigens, and antigen-specific B9-5 phage clone was selected by following indirect enzyme-linked immunosorbent assay (ELISA) and Western blot(WB). Then the B9-5 scFv nucleotide sequence from phagemid was isolated and transformed into human immunoglobulin G (hIgG) form (IgG B9-5) using molecular cloning. Subsequently, IgG B9-5 was analyzed by ELISA and WB to check its antigen-binding activity and finally investigated its neutralizing activity by *in vitro* neutralization assay.

Keywords: Zika virus, Monoclonal antibody, Phage antibody display, ELISA

F065

Effectiveness of Silver Ionic Water (SIW) to Antagonize the Vacuum Inoculated Bacterial Fruit Blotch (BFB) Causing Pathogen, *Acidovorax citrulli* in Watermelon Seeds

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Kangwon National University

Silver have been known to have antimicrobial properties. Silver Ionic Water (SIW) has broad range of suppression efficacy in several plant pathogens by altering their cell membrane structure and functions. In this study, we have used SIW to screen its potentiality to inhibit the colony of vacuum inoculated BFB causing pathogen, *A. citrullii* and germination ability in watermelon seeds. 15 ppm concentration of SIW has been used to sterilize the BFB vacuum inoculated watermelon seeds. 100 vacuum inoculated watermelon seeds were dipped 8 h in 15 ppm SIW. BFB in the concentration of 1×10^7 colony forming units (CFU/ml) was used for the vacuum inoculation. In BFB vacuum inoculated seeds, large number of BFB colonies were observed in seed coat and seed endosperm. Seeds dipped in 15 ppm of SIW after vacuum inoculation, the infestation level in and outside of the seeds were observed lower as compared to control. Moreover, germination rate was found 67% in SIW treated seeds after BFB vacuum inoculation than in non-treated seeds. However, normal seeds without vacuum inoculation showed 72% germination. According to the results, BFB vacuum inoculation seeds and only treated with sterilized distilled water (SDW) showed only 46 % of germination.

Keywords: Germination, Potentiality, Silver ionic water, Sterilization

F066

In vitro Antagonistic Efficacy of Rhizobacteria against Gray Mold Causing Fungi *Botrytis cinera* in Pepper

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Kangwon National University

Gray mold is cause by the fungus, *Botrytis cinerea* (teleomorph: *Botryotinia fuckeliana*) is a serious threat to the pepper growers throughout the world. *Botrytis cinerea* is a soft rot that have an elapsed and water soaked appearance on soft fruit and leaves of pepper plants. Rhizobacteria are root colonizing bacteria that helps to suppress the various plant pathogens and promote the plant growth. In this study, 30 rhizobacterial isolates were isolated from 20 soil samples collected from the rhizospheric soil of pepper field. The bacterial isolates were named as B1-B30. Among the isolated 30 bacterial isolates, 5 of them exhibited high suppression efficacy as compared to control. B6, B7, B18, B24 and B29 were the effective bacterial isolates against the tested pathogen, *B. cinerea*. Identification of all the five bacterial isolates were done by morphologically, biochemically and molecularly. 16S rRNA sequence analysis confirmed the identification of effective 5 bacterial isolates as B6 (*Bacillus siamensis*), B7 (*Bacillus siamensis*), B18 (*Bacillus siamensis*), B24 (*Burkholderia arboris*) and B29 (*Bacillus siamensis*). Results showed that, inhibition rate were 42.86%, 71.43%, 51.72%, 57.58% and 63.64% in B6, B7, B18, B24 and B29 respectively as compared to control.

Keywords: Identification, Isolate, Rhizobacteria

F067

Antibacterial Effects of Silver Ionic Water against Bacterial Fruit Blotch Causing Pathogen (*Acidovorax citrulli*) of Cucurbitaceae

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Bacterial Fruit Blotch (BFB) affects the foliage at all growth stages and fruit of a wide range of cucurbitaceous host. BFB is caused by *Acidovorax avenae* subsp. *Citrulli*, is a serious threat to cucurbits grower around the world. Silver in ionic forms has a high antimicrobial activity. The present study was conducted to screen antibacterial effect of silver ionic water against 30 different strains of BFB causing pathogen of cucurbits. Three different concentrations (1×10^6, 1×10^5, 1×10^4) of each bacteria were treated with 15 ppm of silver ionic water for 7 diferent time interval (0, 2, 4, 6, 8, 12 and 24 h). *In vitro* petri dish assays indicated that silver ionic water had a significant inhibitory effect on the colony formation of these thirty strains of *Acidovorax citrulli*. No colony were observed when the 1×10^4 (CFU/ml) of this pathogen were treated for 2 h and 1×10^5 (CFU/ml) for 6 h with silver ionic water. However, in case of 1×10^6 (CFU/ml) of this pathogen 15 out of 30 strains showed no colony after 24 h. Our results revealed that the antibacterial effectiveness of the silver ionic water against this BFB causing pathogen of cucurbits depend upon the concentration of the BFB, time interval of treatment and bacterial strains.

Keywords: *Acidovorax citrulli*, Concentration, Silver ionic water

F068

Antimicrobial Resistance Patterns and Corresponding Molecular Characteristics of Coagulase-negative *Staphylococci* Isolated from Two Tertiary Hospitals in Two Time Periods, 2000 and 2014-2015, in Seoul, Korea

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A total of 68 coagulase-negative staphylococci (CNS) clinical isolates were obtained from two tertiary hospitals in two time periods (2000 and 2014–2015) before and 15 years after the implementation of the separation of pharmacy and clinic policy in Korea. These were identified as *Staphylococcus capitis*, *S. epidermidis, S. haemolyticus, S. hominis*, *S. saprophyticus,* and *S. warneri.* Resistance rates to gentamicin and oxacillin in the year 2000 were significantly higher than those of the 2014–2015 ($P < 0.05$). Among 68 isolates, 57 isolates were turned out to be MR-CNS and 42 isolates were multidrug resistant CNS. In the year 2000, 22 MR-CNS isolates were: six single SCC*mec* type including type II (n=1), III (n=1), and IV (n=4); 13 single SCC*mec* type plus one *ccr* gene complex including III+*ccr*5 (n=9), IV+*ccr*5 (n=2), V+*ccr*4 (n=1), and V+*ccr*1 (n=1); two single SCC*mec* type plus two *ccr* gene complexes including IV+*ccr*5+*ccr* 3 (n=1); III+*ccr* 4+*ccr* 5 (n=1). In 2014~2015, 13 isolates had a single SCC*mec* type including type I (n=1), IV (n=7), V (n=5) while 10 isolates had a single SCC*mec* type plus one *ccr* gene complex including III+*ccr*5 (n=3), IV+ *ccr* 1 (n=1), IV+ *ccr* 5 (n=2), V+ *ccr* 2 (n=1), V+ *ccr* 4 (n=2), and VIII+ *ccr* 5 (n=1). SCC*mec* type of the remaining 12 isolates could not be determined. A total of 25 STs were obtained and ST2 appeared the most frequent one in both periods.
[This work was supported by a basic research grant from Seoul Women's University (2017).]

Keywords: Coagulase-negative Staphylococci clinical isolates, MRSE, Antimicrobial susceptibility testing, Molecular typing

F069

Analysis of Specific Identification and Genetic Characteristics of *Brucella canis* Using MLST Assay

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Animal and Plant Quarantine Agency

Brucella species have a high genetic similarity, it is thus difficult to establish the differentiation point and analyze their genetic characteristics. In addition, it is difficult that we follow up the genetic features and sources of *B. canis* infection, because *B. canis* can be flowing into the internal country via a variety of routes. To analyze the genetic characteristics and relationships of *Brucella* (*B.*) *canis* isolates, therefore, we classified *B. canis* by performing multi-locus sequencing typing (MLST) assay based on single nucleotide polymorphisms (SNPs). We used the DNAs of 24 *B. canis* to compare with other strains including 134 *Brucella* strains. We selected the genes and specific SNPs using insilico analysis, and then, classify according to genetic characteristics of *B. canis* using MLST assay. As the result, we selected 4 specific SNPs from 3 genes to identify as *B. canis* and 13 specific SNPs of 5 genes to analyze genetic characteristics. In analysis of the MLST assay and phylogenetic tree drawing using all SNPs, 24 of *B. canis* strains were divided into 3 sequencing types (STs). Twelve *B. canis* isolates from South Korea were classified as ST1 displaying nationwide distribution. In conclusion, we proved MLST with 17 SNPs of *B. canis* can identify and analyze the genetic characteristics of that. Moreover, MLST assay established in this study could be applied to a rapid and accurate diagnostic technique for epidemiological trace-back of *B. canis*.

Keywords: *Brucella canis*, SNPs, MLST assay, Genetic characteristics, Epidemiological trace-back

F070

Evaluation of Multiplex Polymerase Chain Reaction Assay for the Simultaneous Detection of Sexually Transmitted Infections Using Swab Specimen

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A sexually transmitted infection (STI) is caused by the spread of various pathogens through sexual activity. STIs can be major causes of inflammation in the pelvis, ectopic pregnancy, and infertility, as well as increase HIV prevalence threefold if not diagnosed early and treated. Therefore, it is very important to diagnose sexually transmitted pathogens early. Molecular diagnostics using the multiplex polymerase chain reaction (PCR) technique can detect pathogens quickly. In the study, we evaluated multiple PCR kits that can simultaneously detect 13 different strains that cause an STI. Performance evaluation was evaluated for sensitivity, repeatability, reproducibility, and interference between bacteria. In addition, the sensitivity and specificity of the clinical evaluation were estimated through clinical samples. The results of the LOD showed 0.021 to 50.104 copies for each bacterium. In the repeatability and reproducibility tests, the positive substances were 100% positive at low concentrations, and the negative results were all negative. In the interference test between bacteria, the efficiency of amplification was not significantly deteriorated and it was confirmed that a nonspecific amplification product was not formed. The results demonstrated that there was no interference between the viruses. We also tested 322 vaginal swab samples using the multiplex PCR kit and confirmed clinical sensitivity and specificity for 100% of the pathogens. Among them, we confirmed the cases with inconsistencies through sequencing. Therefore, the multiplex PCR kit used in this study was considered to be outstanding through analytical and clinical performance tests. It should be used widely as a rapid diagnostic tool and for monitoring STIs.

Keywords: Sexually transmitted infection, Multiplex PCR, Evaluation

F071

Characterization of Hypothetical Protein ChuY from *Escherichia coli* Strain CFT073

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Urinary tract infection is a very common infectious disease in humans. Uropathogenic *Esherichia coli* CFT073 strain is a major causative agent of the urinary tract infection and has specific genes found only in pathogenic strains. The hypothetical protein ChuY is one of uropathogenic *E. coli* specific gene products and might be involved in pathogenesis. The crystal structure of ChuY protein is highly similar to that of human biliverdin IX β reductase and the *chuY* gene also belongs to heme utilization gene cluster, which strongly suggests that ChuY protein might be involved in heme metabolism. To assess the cellular function of ChuY protein, we generated *chuY* gene deleted knockout strain of *E. coli* CFT073 and found that *chuY* deleted KO strain and *chuY* complemented strain show different level of cellular heme contents. Moreover, the green and orange colored pigment accumulations were observed in *chuY* deleted and *chuY* complemented bacterial culture pellet, respectively. Now the identification of both green and orange pigments, probably biliverdin and bilirubin, is performed using mass spectrometry. Further study will elucidate the contribution of ChuY protein to the host-pathogen interactions by regulating heme homeostasis and producing a potent cellular antioxidant.

Keywords: Urinary tract infection, Uropathogenic *E. coli*, ChuY, Biliverdin β reductase, Heme homeostasis

F072

Sacbrood Virus (SBV)-infection Effect on Gut Microbiome in Adult Honeybee (*Apis cerana*) and Larvae

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Gut microbiome of honeybee is known to be supportive for host nutrition and defense against detrimental pathogens. Sacbrood virus (SBV)-infected honeybee larvae often results in failure to pupate and death, while SBV-infected adult honeybee shows little clinical signs. Therefore, it is thought that the difference of gut microbiome between adult honeybee and larvae could effect on the susceptibility of SBV infection. In this study, the bacterial communities in the guts of the adults and larvae of SBV-infected Asian honeybee (*Apis cerana*) in Korea was investigated by using 16S rRNA gene amplicon sequencing to explore correlation of gut microbiome and SBV-infection.
Overall, the numbers of operational taxonomic units (OTUs) were much lower in the SBV-infected larval guts than in the SBV-infected adult guts. For the larvae infected by SBV, the diversity was significantly less than in healthy ones with remarkable domination of the genus *Gillimella* (99.7%) of the family *Orbaceae*. Most of the adult honeybee gut bacterial 16S rRNA gene sequences were highly similar to the known honey bee-specific ones and affiliated with *Gilliamella, Lactobacillus, Apibater* and *Bifidobacterium.* The results substantiated the previous observation that SBV-susceptible larvae guts was dominated by specific bacterial group, and showed that the relative abundances of OTUs could be markedly changed depending on the developmental stage of the honeybee and larvae. The microbiomes of SBV-infected and –uninfected larvae and Asian honeybee adults were analyzed for the first time in this study. For further study, the effect of the different microbiomes on the pathogenesis of SBV will be elucidated through culture-based approach in the future.

Keywords: Gut microbiome, Honeybee, *Apis cerana*, Sacbrood virus, Bacterial community

F073

The Rapid and Accurate Whole Genome Sequencing of Dengue Virus by Nanopore Sequencing

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Serious human diseases have been occurred by RNA viruses (e.g. Dengue, Ebola, severe acute respiratory syndrome [SARS] CoV, Chikungunya, West Nile, Zika, and Influenza). To characterize these viruses, cloning and sequencing methods have traditionally been used, but they have one sequence per clone and are not efficient in terms of time and cost. In contrast, 'Nanopore' technique, one of the next generation sequencing (NGS), can analyze hundreds of thousands of long sequence data reads in real-time in a short time. So, we used this to perform whole genome sequencing (WGS) of the dengue virus and compare it with gold standard methods.

To prepare the sequencing library, dengue virus was purified from culture supernatant using an ultracentrifuge, RNA was extracted and cDNA was synthesized. Next, about 24,000 partial contigs were obtained through nanopore sequencing, and they were aligned with 'Geneious' program. The accuracy of sequencing were 99.9% compared with partial results obtained by cloning and sequencing methods.

The workflow allowed the virus to be sequenced in a 2 days rather than several days as required for cloning and sequencing methods. Our results can be applied to the rapid characterization of various viruses.

[This work was supported by a research grant from Korea Research Institute of Bioscience and Biotechnology (KGM4571811).]

Keywords: Dengue, Whole Genome Sequencing (WGS), Next Generation Sequencing (NGS), Nanopore technique

G001

Junctional Adhesion Molecules Mediate Transendothelial Migration of Dendritic Cell Vaccine in Cancer Immunotherapy

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In vitro generated dendritic cells (DCs) have been studied in cancer immunotherapy for decades. However, the detailed molecular mechanism underlying transendothelial migration (TEM) of DC vaccine across the endothelial barrier to regional lymph nodes (LNs) remains largely unknown. Here, we found that junctional adhesion molecule (JAM)-Like (JAML) is involved in the TEM of mouse bone marrow-derived DCs (BMDCs). Treatment with an anti-JAML antibody or JAML knock-down significantly reduced the TEM activity of BMDCs, leading to impairment of DC-based cancer immunotherapy. We found that the interaction of JAML of BMDCs with the coxsackie and adenovirus receptor of endothelial cells plays a crucial role in the TEM of BMDCs. On the other hand, human monocyte-derived DCs (MoDCs) did not express the JAML protein but still showed normal TEM activity. We found that MoDCs express only JAM1 and that the homophilic interaction of JAM1 is essential for MoDC TEM across a HUVEC monolayer. Our findings suggest that specific JAM family members play an important role in the TEM of *in vitro*-generated mouse and human DCs from the inoculation site to regional LNs in DC-based cancer immunotherapy.

G002

Regulation of AIM2 Inflammasome by Human Cytomegalovirus

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Absent in melanoma 2 (AIM2) recognizes cytosolic dsDNA and interacts with apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) to activate a caspase-1-activating inflammasome. Currently, the role of AIM2 inflammasome in human cytomegalovirus (HCMV) infection is unclear. In this study, a cDNA library containing HCMV-Towne ORFs was screened to determine viral genes that interferes with AIM2 inflammasome activation, and 5 viral ORFs significantly reduced AIM2 and ASC-induced NF-κB activation. Among these viral ORFs, UL47 strongly inhibited AIM2 inflammasome-induced caspase 1 activation and bound to ASC.
This study proposes the mechanism of counteraction to AIM2 inflammasome employed by HCMV.

Keywords: AIM2, HCMV

G003

M2 Macrophages are More Potent Osteoclast Precursors than M1 Macrophages

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Bone-resorbing osteoclasts are mainly differentiated from macrophages by receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Macrophages can be divided into two subtypes, M1 and M2 macrophages, based on immunological properties under environmental changes, but little is known about their osteoclastogenic potential. Here, we investigated the osteoclastogenic potential of macrophage subtypes. When the macrophages were incubated with M-CSF and RANKL, osteoclast differentiation was remarkably increased from M2 compared to M1 macrophages. In addition, robust bone resorptive capacity and giant actin rings were observed in M2, but not M1 macrophages, under the osteoclast differentiation condition. High expression of RANK and c-Fms was evident in M2 macrophages. Enhanced osteoclastogenesis of M2 macrophages exhibited high expression levels of c-Fos and NFATc1. Notably, osteoclastic potential was higher in M-CSF-exposed M1 for converting into M2 macrophages than GM-CSF-exposed M2 for converting into M1 macrophages. Silencing IRF5, responsible for the determination of M1 macrophages, increased osteoclast differentiation by activating CREB and inducing NFATc1, which was inhibited by overexpression of IRF5. Taken together, these results suggest that M2 macrophages might be more efficient osteoclast precursors than M1 macrophages because of the attenuated expression of IRF5.

Keywords: M1 macrophage, M2 macrophage, IRF5, Osteoclast differentiation

G004

Analysis of Immune Responses of Dendritic Cells to *Helicobacter pylori* Infection

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Helicobacter pylori (*H. pylori*) is a causative pathogen of chronic gastritis and is known to induce gastric cancer. Immune responses to *H. pylori* infection could be characterized by chronic inflammatory without complete clearance. In previous studies, dendritic cells (DCs) showed the increment of cell population and migration to gastric epithelial layer responding *H. pylori* infection. These results showed that DCs should have a strong influence on distinguishing immune responses in which *H. pylori* is not cleared. In this study, characteristics of immune responses of DCs to *H. pylori* infection were analyzed. To complete our aims, mouse DCs were infected with 10 MOI of *H. pylori*. RNA was extracted from infected DCs, and submitted to quantitative real-time PCR and global gene expression analysis. Expression of MHC II and lysozyme formation were also investigated using immunofluorescence. At 18 h after infection, *H. pylori*-infected DCs showed down-regulated gene expression in IFN-γ and showed no-differences in CCR7, MHC II and TGF-β compared to uninfected DCs. Immunofluorescence microscopic investigation showed down-regulation of MHC II expression and lysozyme formation in *H. pylori*-infected DCs compared to the positive control of *E. coli*-infected DCs. All results in this study demonstrated that antigen presentation activities of DCs were down-regulated after *H. pylori* infection, and this may be one of the reasons for the difficulty of *H. pylori* clearance.

Keywords: *Helicobacter pylori*, Dendritic cells

G005

Bacillus lentus (BL) from Traditional Korean Fermented Soybean Paste is a Promising Natural mTOR Inhibitor in Colorectal Cancer (CRC)

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The striking increase in colorectal cancer (CRC) has shown the great fatality in Korea for more than 10 years. The leading edge of this rising incidence rate is mainly due to the people's dietary changes in Korea. The nonpathogenic spore-forming *Bacillus* species among the fermented bacterium might have optimistic effects on colorectal disease. The drug resistance is counted as a major factor to mortality rate. The mammalian target of rapamycin (mTOR) inhibitor based therapeutic methods are available in the market, still showing poor prognosis. This study aims to explore the potential effects of BL, CRC targeted aggregative adhesive bacteria in order to overcome drug resistance and as a natural trigger to inhibit mTOR signaling pathways. The nonpathogenic spore-forming *Bacillus* species among the fermented bacterium might have optimistic effects on CRC incidence rate. It will contribute to the understanding of the tumor-microbe cross-talk and BL could be a candidate for the development of mTOR inhibiting anti-tumor agent. Taken together, BL can become a promising specific tumor targeting anti-tumor therapeutic agent in colorectal cancer cells. In this study, we investigated the growth inhibition effects of BL in CRC cell lines via molecular mechanism studies of BL inhibiting mTOR signaling pathways

Keywords: *Bacillus lentus*, Colorectal cancer, mTOR inhibitor, Rapamycin, Antimicrobial peptide

G006

Improved Fluorescent Antibody to Membrane Antigen Test Using Flow Cytometry for the Measurement of the Immunity against Varicella Zoster Virus

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Fluorescent Antibody to Membrane Antigen (FAMA) test is considered as the gold standard of measuring antibody for Varicella-Zoster Virus (VZV) with high sensitivity and specificity. However, some aspects of classic FAMA test such as time consumption, labor, and subjective interpretation by observer are obstacles for handling a large number of samples in a short period. To solve the problems in classic FAMA, flow cytometry (flow FAMA) was adopted for analyzing results and compared with classic FAMA results which were analyzed by fluorescence microscope. Also, the stability of VZV-infected MRC-5 cells (FAMA antigen) stored in liquid nitrogen for up to 6 month was investigated. Two hundred fifteens of pre- and post-varicella vaccinated children's sera and adult VZV panel plasma were used. International Standard VZV IgG Immunoglobulin (W1044, NIBSC, UK) was used as a positive control. The sensitivity, specificity, and accuracy of flow FAMA were 83.5%, 95.8%, and 90.2%, respectively. The correlation coefficient r between classic and flow FAMA was 0.961 (95% [CI], 0.931 to 0.985). Based on the above data, flow FAMA results were considerably comparable to classic FAMA, and lot-to-lot variations were reduced using stocked FAMA antigens. Therefore, the flow FAMA has a potential to evaluate the humoral immunity against VZV as an alternative method to classic FAMA. Moreover, long-term studies suggest that using stocked FAMA antigens may reduce the consumption of time, and variability.

Keywords: FAMA, VZV, Flow cytometry, Immunity

G007

Follow-up Study on Immunogenicity of Zoster Vaccine in Korean Adults by gpEIA Kit and IFN-γ ELISpot Assay

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A live attenuated zoster vaccine (ZOSTAVAX^{TM}, Merck) has been launched in Korea since 2012 to prevent zoster in persons aged 50 years or older. However, there are no sufficient follow-up studies of specific immunogenicity against zoster vaccine in Korean adults. The aim of this study was to evaluate zoster vaccine efficacy over a 5 years period (mean, 42.2 month) in Korean adults. A total of 21 healthy Korean adults aged 50 to 69 years were enrolled in Yeungnam University Hospital from Dec. 2013 to April. 2018. Two person experienced mild zoster during follow-up period, however no serious vaccine-related adverse effects occurred. Humoral and cell-mediated immunogenicity were assessed using a glycoprotein enzyme-conjugated immunoassay kit (gpEIA) and an interferon-gamma (IFN-γ) enzyme-linked ImmunoSpot (ELISpot) assay. The geometric mean antibody titer (GMT) of gpEIA were 580.4, 2654.3 and 1088.4 mIU/ml at pre-vaccination, 5 weeks and 42.2 months after vaccination, respectively. IFN-γ ELISpot Spot Forming Counts (SFCs) per 10^6 peripheral blood mononuclear cells were 89.4, 134.4 and 127.9 at pre-vaccination, 5 weeks and 42.2 months after vaccination, respectively. The cell-mediated immunity induced by the zoster vaccine remained on average for more than 3 years, but humoral immunity was decreased. This is a small scale study, so it is necessary to perform long-term follow-up studies of the incidence and immunity of shingles in a large number of adults after zoster vaccination.

Keywords: Herpes zoster, Vaccine, Immunogenicity, gpEIA, ELISpot

G008

Bacterial Endotoxin-preconditioned Periodontal Ligament Stem Cells Induce M1 Polarization of Macrophage through Extracellular Vesicles

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Periodontitis is a common disease that characterized with chronic inflammation and tissue destruction of gums. To resist pathogenic microbes, gingival epithelial cells and inflammatory cells produce various pro-inflammatory cytokines, chemokines, and enzymes. Human periodontal ligament stem cells (PDLSCs) derived from mature periodontal ligaments have stem cell properties similar to mesenchymal stem cells. PDLSCs possess not only differentiation potential to other tissues but also immunomodulatory abilities. Therefore, PDLSCs might be a vital role in the modulation of immune response. In this study, we investigated the effect of PDLSCs on the polarization of macrophages. While the conditioned media from PDLSCs in normal culture condition did not affect the polarization of macrophage, lipopolysaccharide (LPS)-preconditioned PDLSCs induce significant changes in M1 polarization of macrophages. Extracellular vesicles (EVs) isolated from the conditioned media of LPS-preconditioned PDLSCs by centrifugal filter device or differential centrifugation showed strong M1 polarization effect of macrophages. Additionally, M1 polarization was abolished by DNase I treatment on EVs. Our results demonstrated that LPS-stimulated PDLSCs induce M1 polarization of macrophage through EVs, suggesting EVs from PDLSCs might be a potential therapeutic target for the inflammation in the periodontium.
[This work was supported by the National Research Foundation of Korea (NRF-NRF-2017R1A2B4002405).]

Keywords: Periodontal ligament stem cell, Macrophage, Polarization, Extracellular vesicles

G009

Extracellular Vesicles from KSHV-infected Cells Stimulate Antiviral Immune Response through Mitochondria DNA

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Interferon-stimulated genes (ISGs) are vital to controlling virus infections. As many antiviral ISGs continue to be identified, their roles in viral pathogenesis are also being explored in more detail. Kaposi's Sarcoma-associated herpesvirus (KSHV) is the etiologic agent of Kaposi's sarcoma, which is the most common cancer in acquired immune deficiency syndrome patients. Because KSHV has many viral proteins modulate antiviral response, type 1 Interferon response has known to be strongly suppressed in KSHV-infected cells. However, antiviral effects by extracellular vesicles (EVs) during *de novo* KSHV infection has not been investigated to our best knowledge. In this study, we show that KSHV-infected cells induce ISGs response in uninfected cells using EVs. mRNA microarray indicated that ISGs and IRF activating gene were prominently activated in EVs from KSHV-infected cells (KSHV EVs)-treated human endothelial cells, which was validated by RT-qPCR. Furthermore, we found that mitochondrial DNA on the surface of KSHV EVs would be a causative factor for ISGs response through cGAS-STING pathway. In additional, KSHV EVs-educated cells showed lower infectivity for KSHV and viral replication activity than those of Mock EVs-treated cells. Our results indicated that EVs from KSHV infected cells would be an initiating factor for the innate immune response against viral infection, which is helpful to expand our understanding of the microenvironment of virus-infected cells.

Keywords: Extracellular vesicles, Exosomes, KSHV, Antiviral, Interferon stimulating genes

G010

Immunomodulation by a ppGpp-defective Enteropathogenic *Escherichia coli*

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Under nutrient-deficient environment, stringent response is triggered in bacteria, which is mediated by an alarmone ppGpp (guanosine 3',5'-bispyrophosphate). During stringent response, ppGpp is rapidly synthesized by RelA and/or SpoT and subsequently alters various cellular processes such as molecule biosynthesis and virulence. Our previous study showed that stringent response is essential for full virulence of enteropathogenic *Escherichia coli* (EPEC), a major etiological agent of diarrhea in infants and weaning pigs. To assess host immune response toward ppGpp-defective ($ppGpp^0$) mutant of EPEC E2348/69 strain, a porcine macrophage cell line, 3D4/31, was infected with the $ppGpp^0$ EPEC. Whole transcriptome analyses revealed that pro-inflammatory cytokine genes were highly induced in 3D4/31 infected with $ppGpp^0$ EPEC when compared with that of infected with the wild-type EPEC. Increased protein expression of interleukin (IL)-6 was confirmed using ELISA. Coinciding with the RNA-seq results, *in vivo* murine peritoneal challenge assays showed high increase of IL-6 and improved bacterial clearance in response to $ppGpp^0$ EPEC. These observations imply that $ppGpp^0$ EPEC elicited stronger and harmonized host immune response that was not observed in case of the wild-type EPEC. The present results provide insights to the role of ppGpp-mediated regulation during EPEC pathogenesis.
[This study was supported by a grant from National Research Foundation (NRF-2017R1A2B4013056), Republic of Korea]

Keywords: Stringent response, ppGpp, Enteropathogenic *Escherichia coli*, Interleukin 6

G011

Mycobacterium tuberculosis Protein BfrB Promote to a Th1 Polarization via DC Activation

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The attenuated vaccine *Mycobacterium bovis* BCG (Bacille Calmette Guerin) has limited protective efficacy against TB. The development of more effective TB vaccines has focused on the mycobacterial antigens that cause strong T helper 1 (Th1) responses. Mtb protein BfrB (bacterioferritin B) is known to play a crucial role in the growth of Mtb. Nonetheless, it is unclear whether BfrB can induce protective immunity against Mtb. Here, we studied the action of BfrB in maturation of dendritic cells (DCs) and its engagement in the development of T cell immunity. We found that BfrB functionally activated DCs by upregulating costimulatory molecules and increased secretion of proinflammatory cytokines. Activation of DCs by BfrB was mediated by Toll-like receptor 4 (TLR4), followed by triggering of mitogen-activated protein kinase and nuclear factor κ B signaling pathways. In addition, BfrB-matured DCs effectively proliferated and polarized Th1 immune response of naïve $CD4^+$ and $CD8^+$ T cells. Moreover, BfrB specifically caused the expansion of $CD4^+CD44^{high}CD62L^{low}$ T cells from Mtb-infected mice; besides, the T cells activated by BfrB-matured DCs inhibited intracellular mycobacterial growth. Our data suggest that BfrB induces DC maturation and protective immune responses, a finding that may provide candidate of effective TB vaccines.

Keywords: *Mycobacterium tuberculosis*, Dendritic cell, BfrB, Immune response, Th1 polarization, Effector/memory T cell

G012

A Recombinant Rv0315-Rv35xx-ESAT6 Fusion Protein Enhances Anti-mycobacterial Activity via Activation of Macrophages

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Since *Mycobacterium tuberculosis* is one of the most successful pathogens in some areas of the world, many researches delivered immunodominant mycobacterial antigens for development of new sub-unit vaccines against tuberculosis. In this study, we found that Rv35xx, a newly identified as an immunostimulatory antigen, induced pro-inflammatory cytokine production in macrophages. However, despite Rv35xx functionally activated macrophages, Rv35xx-treated macrophages couldn't fully stimulate T cells to produce Th1-cytokines. In addition, anti-mycobacterial effect of Rv35xx was insignificant. To produce more effective TB subunit vaccines, we constructed Rv0315-Rv35xx-ESAT6 fusion protein by combining Rv35xx with antigens which activated immune cells. Our data showed that Rv0315-Rv35xx-ESAT6 fusion protein increases the production of pro-inflammation cytokines and expression of costimulatory molecules than Rv35xx. Especially, our results indicated that this fusion protein activates macrophages to stimulated Th1 response and enhances the anti-mycobacterial effect than Rv35xx. Taken together, these results suggest that Rv35xx fusion protein may be used as TB subunit vaccine candidate.

Keywords: *Mycobacterium tuberculosis*, Macrophages, Host immune response

G013

Mycobacterium tuberculosis Rv07XX Induces Macrophage Apoptosis in RAW264.7 Cells

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Macrophages infected with *Mycobacterium tuberculosis* undergo increased rates of apoptosis. Important objectives are to define the microbial factors that cause apoptosis, the mechanisms involved and the impact on infection. In this study, using multidimensional fractionation, we identified mycobacterial proteins, which induced macrophage apoptosis in *Mycobacterium tuberculosis* culture filtrates. Mycobacterial proteins interact with host macrophages and modulate their functions and cytokine gene expression profile. We investigated whether the recombinant Rv07XX protein could effectively induce apoptosis in macrophages. The annexin V binding to membrane phosphatidylserine was used to measure apoptosis. The results show that Rv07XX induce apoptosis in a dose-dependent manner in macrophage cells. DNA fragmentation, caspase activation, and poly (ADP-ribose) polymerase (PARP) cleavage were observed in apoptotic macrophages treated with Rv07XX. Enhanced ROS production was essential for Rv07XX induced apoptosis, and pretreatment with N-acetylcysteine (NAC), a potent ROS scavenger, brought restoration of the viability of macrophages. The loss of $\Delta\Psi_m$, and release of cytochrome *c* from mitochondria were occurred in Rv07XX treated macrophages. Interestingly, Rv07XX induced apoptosis was significantly reduced in TLR4-deficient macrophages. In conclusion, the results suggest that Rv07XX may act as a strong pathogenic factor to cause apoptosis of macrophages.

Keywords: *Mycobacterium tuberculosis*, Apoptosis, ROS, RAW264.7 cells

G014

Periodontopathogens Suppress Biogenesis of Lysosomes in Macrophages

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Periodontopathogens are inhabitants in subgingival area and induce inflammation. Macrophages, one of the innate immune cells, engulf microbes which are degraded within the lysosomes. However, some microbes can evade the killing mechanisms of the host cells. To check the response of the macrophages to the periodontopathogens, we analyzed lysosomal activation using LysoTracker Red, a red-fluorescent dye for labeling lysosomes. Fluorescence intensity of LysoTracker increased in macrophages infected with *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, while it decreased in macrophages infected with *Treponema denticola* and *Tannerella forsythia.* The expression of LAMP-1, a lysosomal marker, was also reduced in macrophages infected with *T. denticola* and *T. forsythia*. Our results indicate that *T. denticola* and *T. forsythia* may suppress biogenesis of lysosomes in macrophages.

Keywords: Periodontopathogens, Macrophages, Lysosome

G015

Protective Effect of Heat-treated *Lactobacillus plantarum* nF1-fortified Yogurt against Influenza A Virus Infection

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Influenza A virus (IAV) infection causes respiratory diseases, ranging from mild to fatal disease making it considered as a global public health concern. Therefore, prevention and treatment of IAV infection has been important issues. In this study, we investigated a preventive effect of yogurt administration against influenza A virus (IAV) infection. Daily administration of heat-treated *Lactobacillus plantarum* nF1-fortified yogurt for 21 days before IAV infection increased survival rate of the infected mice. The yogurt administration enhanced inflammatory cytokine responses in the lung of infected mice. Furthermore, the results showed that the yogurt elevated immune response against IAV by enhancing NK cell activity leading to the preventive effect on IAV infection. These results suggest that daily administration of heat-treated *L. plantarum* nF1-fortified yogurt may provide protective effect from IAV infection.
[This study was funded by Purmil Co., Ltd. (Seoul, Korea) and the School of Life Sciences & Biotechnology of Korea University for BK21PLUS.]

Keywords: Influenza A virus, Heat-treated *Lactobacillus plantarum* nF1-fortified yogurt

G016

Therapeutic Adjuvant FlaB Conjugates Human EphA2-specific Monobody for Enhanced Bacterial Immunotherapy

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In a previous study, we developed an E1 monobody specific for the tumor biomarker hEphA2 [PLoS ONE (2015) 10(7): e0132976]. E1 showed potential as a molecular probe for *in vitro* and *in vivo* targeting of cancers overexpressing hEphA2. Also, we demonstrated to the potential of E1 conjugated to optical reporter, Renilla luciferase variant 8 *in vitro* and *in vivo* [PLos ONE (2017) 12(7): e0180786]. In present study, we constructed expression vectors for E1 conjugated to therapeutic adjuvant such as Flagellin B (flaB) and purified such recombinant proteins by affinity chromatography in *E. coli*. E1-FlaB specifically bound to hEphA2 in human prostate cancer PC3 cells but not in LNCaP cells, which express hEphA2 at high and low levels, respectively. Purified E1-FlaB and FlaB were stimulated to NF-kB-luciferase reporter activity in TLR5-surface expressed PC3 and LNCaP cells but not in normal cells.
Then, it is known that the *in vitro* stimulation of cancer cells with FlaB did not increase phosphorylation of NF-kB p65 and FlaB-mediated antitumor effects were not caused by direct action on cancer cells [Science Translational Medicine (2017):Vol. 9, Issue 376, eaak9537]. Our objective is to amplify the adjuvant effect of bacterial immunotherapy, which increases the cancer targeting efficiency of FlaB using E1. The changes of cytokines on the binding of E1-FlaB to cells will be analyzed and applied to *in vivo* experiments.

Keywords: Adjuvant, FlaB, EphA2, Monobody, Immunotherapy

H001

Membrane Engineering via *Trans*-unsaturated Fatty Acids Production Increased Succinic Acid Production in *Mannheimia succiniciproducens*

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Product toxicity often limited the production of desired bio-products with high titer, yield, and productivity using metabolically engineered microorganisms. This is also true for succinic acid (SA), which is a four carbon dicarboxylic acid of industrial importance. Acid 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: Mannheimia succiniciproducens, Succinic acid, Membrane engineering, Tolerance, *Trans*-unsaturated fatty acid

H002

Development of Microbial Succinic Acid Producer from Formic Acid Using Metabolically Engineered *Mannheimia succiniciproducens*

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Much effort has been exerted to reduce one carbon (C1) gas emission due to the climate change nowadays. As one promising way utilize C1 gas, several technologies have been developed to convert C1 gas into useful chemicals such as formic acid (FA). Here, *Mannheimia succiniciproducens*, a facultative anaerobic gram negative rumen bacteria, 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: C1 gas, Formic acid, *Mannheimia succiniciproducens*, Succinic acid

H003

Enhanced Production of Succinic Acid from *Mannheimia succiniciproducens* Using Elementary Mode Analysis with Clustering

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To systematically analyze SA production routes of *Mannheimia succiniciproducens*, a facultative anaerobic gram negative rumen bacteria, elementary modes (EMs) generated from the central carbon metabolic network of *M. succiniciproducens* were clustered. Based on the results of EMC analysis, *zwf* gene was selected as a novel overexpression target for the improved SA production. This gene was overexpressed in SA-overproducing *M. succiniciproducens* LPK7 strain. Heterologous NADPH-dependent *mdh* was selected for overexpression to synergistically improve SA production by utilizing abundant NADPH pool mediated by the overexpressed *zwf*. The LPK7 strains co-expressing *mdh* alone and both *zwf* and *mdh* genes were subjected to fed-batch fermentation to examine their SA production performances. Strategies of EMC analysis will be useful for further metabolic engineering of *M. succiniciproducens* and other microorganisms to improve production of SA and other chemicals of interest.
[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: Elementary mode analysis, *In silico* strain design, Metabolic network analysis, Succinic acid production, Systems metabolic engineering

H004

Utilizing rTHF Cycle and Reverse Glycine Cleavage Pathway in C1 Assimilation Pathway Development

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Reduction of gaseous one-carbon (C1) compounds such as CO_2 is important for solving climate change and global warming. Biological C1 assimilation is a promising way because of its advantages such as low energy requirement, while slow reaction kinetic is a limitation in biological way of C1 conversion. One of the solution is utilizing efficient C1 assimilation pathway and co-utilization with other C1 source. To solve the problem, we reconstructed the THF cycle and reductive glycine pathway, producing pyruvate from FA and CO_2. Through these pathways, 96% of proteinogenic serine was synthesized from FA and CO_2. Pyruvate forming flux from FA and CO_2 was reached up to 14.9% of the total pyruvate forming flux. The reconstructed pathway will provide efficient way of C1 assimilation and this study will be a useful guidance for the reconstruction of natural pathways.
[This work was supported by C1 Gas Refinery Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (NRF-2016M3D3A1A01913250) is appreciated]

Keywords: Formic acid, CO_2, Tetrahydrofolate cycle

H005

Microbial Synthesis of Various Nanomaterials and Their Characteristics

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We report biosynthesis of 60 different nanomaterials (NMs) by employing a recombinant *Escherichia coli* strain co-expressing metallothionein, a metal-binding protein, and phytochelatin synthase that synthesizes a metal-binding peptide phytochelatin. The periodic table is scanned to select 35 suitable elements, followed by biosynthesis of their NMs. Nine crystalline single-element NMs are synthesized, while the other elements resulting in biosynthesis of amorphous NMs or no NM synthesis. Producibility and crystallinity of the NMs are analyzed using a Pourbaix diagram that predicts the stable chemical species of each element for NM biosynthesis by varying reduction potential (Eh) and pH. This strategy is extended to biosynthesize multi-element NMs that allows biosynthesis of NMs with various characteristics, providing a novel platform for manufacturing various single- and multi-element NMs in an environmentally friendly manner.

[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-2012M1A2A-2026557).]

Keywords: Biosynthesis, Nanomaterials, *Escherichia coli*, Single element, Multi-element

H006

One-step Genome Engineering Method for Rapid Development of *Escherichia coli* Strains of High-Performance

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For rapid genome engineering in *E. coli*, integration helper plasmid pCW611 was developed to express two recombinases (Red and Cre) by using two independent (IPTG and Arabinose) inducible systems. For iterative transformation of the conventional helper plasmids and repetitive curing of plasmids are not required in this new method, the time and effort it takes for genome engineering can be significantly reduced when compared to the conventional method of using several integration helper plasmids. Therefore, by using this novel plasmid, we could delete one target gene in just 3 days. To verify the effectiveness of this novel system, gene deletion experiments were performed by knocking out four target genes individually (*adhE*, *sfcA*, *frdABCD*, and *ackA*) and two genes simultaneously for two cases (*adhE-aspA* and *sfcA-aspA*). In addition, fumaric acid producing *E. coil* strain was developed by deleting four target genes (*fumB*, *iclR*, *fumA*, and *fumC*) in as short as 10 days as a proof-of-concept study.

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

Keywords: Metabolic engineering, *Escherichia coli*, Genome engineering, Recombinase

H007

Metabolic Engineering Using Synthetic Small Regulatory RNAs for High-level Production of Chemicals in *Escherichia coli*

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KAIST

Genome engineering or gene expression manipulation using converntional methods have been laborious and time-consuming. Therefore, synthetic small regulatory RNAs were developed for targeted regulation of gene expression in *Escherichia coli.* Gene expression can be knocked down by blocking the translation initiation region (TIR) of a target gene. As a proof-of-concept study, a tyrosine overproducer *E. coli* strain was obtained by testing combinatorial knockdown of four target genes in 14 *E. coli* strains. The isolated strain was capable of producing 2 g/L of tyrosine. Also, by using a library of 130 synthetic sRNAs, we screened effective gene knockdown targets including *murE*, which increased cadaverine production by 55%.

[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the MSIT through the NRF of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557); the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) of the MSIT through the NRF of Korea; the Commercializations Promotion Agency for R&D Outcomes (COMPA) funded by the MSIT]

Keywords: Small RNA, Gene knockdown, *Escherichia coli*, Metabolic engineering

H008

Ultrasensitive Detection of Uranyl Ion Using Nanowire-based Surface-enhanced Raman Scattering Sensor

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In this research, nanowire-based surface-enhanced Raman scattering (SERS) sensor was developed for the ultrasensitive detection of uranyl ion in natural water. The SERS sensor combines with a DNAzyme-cleaved reaction. The presence of UO_2^{2+} in sample induces the cleavage of DNAzyme into enzyme strands and released strands, which include Raman-active molecules. The released strands bind with capture DNAs on the nanowire sensor and this complex provides SERS signal. The nanowire-based surface-enhanced Raman scattering sensor showed a detection limit of 1 pM with high selectivity. Using this sensor, uranyl ion was successfully detected in diverse UO_2^{2+}-contaminated natural water. Based on these results, we anticipate that the practical usefulness of this SERS sensor can be expanded to detect diverse toxic metal ions by applying various ion-specific DNA-based ligands to NW sensors.

[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: Uranyl ion, Nanowire, Sensor

H009

Corynebacterium glutamicum Chromosome Engineering with CRISPR/Cas9-coupled Recombineering Tool

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Corynebacterium glutamicum is regarded as an important industrial microorganism, especially in amino acids production. Compared with its importance, however, its genome engineering is still in primitive state. Here we present a quick genome engineering tool for iterative and scarless knockout of multiple genes in *C. glutamicum*. In this system, synthetic single-stranded oligodeoxyribonucleotides are integrated into the genome by recombinase RecT, and unedited organisms are counter-selected by CRISPR/Cas9. CRISPR/Cas9- and RecET-cloned plasmids were engineered to be curable for the generation of final strain without CRISPR/Cas9 and RecET vectors after iterative engineering. Demonstration of the rapid and iterative genome engineering system was performed with generating seven different mutants within two weeks for deletion of three different genes on the production of γ-aminobutyric acid, an industrially relevant chemical of much interest. This genome engineering tool is expected to accelerate metabolic engineering of *C. glutamicum*.

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

Keywords: CRISPR/Cas9, CoPaR, *Corynebacterium glutamicum*, Curable plasmids, Recombineering

H010

Free Heme Production Using Engineered *Escherichia coli* by Secretion

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Heme is an emerging bioproduct with its various applications, including healthcare and food supplement. Previous research showed small amount of intracellular heme production through the C4 pathway with engineered *Escherichia coli*, which requires extraction for applications. Here we present free heme production by secretion using engineered *E. coli* strains, through the C5 pathway and the optimized downstream pathway for heme biosynthesis. Furthermore, inactivation of *ldhA*, *pta* and also *yfeX*—encoding potential heme-degradiong enzyme—showed a result of 7.88 mg/L of total heme including 1.26 mg/L of extracellular heme in flask cultivation. Fed-batch fermentations of heme exporter CcmABC overexpressing strain from glucose only and glucose supplemented with L-glutamate secrete 73.4 and 151.4 mg/L of heme, respectively, which are 63.5% and 63.3% of total heme produced for each. This engineered *E. coli* strain would be valuable for free heme microbial production.

[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: Heme, Secretory production, *Escherichia coli*, C5 pathway, Heme exporter

H011

Markerless *Pseudomonas putida* Chromosome Engineering with RecET Recombineering System

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Pseudomonas putida is one of the most common workhorse for producing valuable natural products. While several gene knockout strategies for *P. putida* have been reported, heterologous gene integration to *P. putida* chromosome requires development of a more efficient method. Here we report a recombineering system using RecET recombinase for markerless integration of heterologous genes into the chromosome of *P. putida*. Demonstration of efficiency and capacity of this system were first shown by knocking out various genetic loci on the chromosome with wide range of knockout lengths from 0.6 to 101.7 kb. The system developed here allowed successful integration of gene clusters synthesizing four proof-of-concept bioproducts into the target genetic locus of *P. putida* chromosome. Combining Cre/*lox* system and developing efficient plasmid curing systems completes the recombineering system markerless and plasmid-free. This efficient gene knockout and integration system will accelerate metabolic engineering of *P. putida*, a bacterial host strain with increasing academic and industrial interest.

[This work was supported by the Novo Nordisk Foundation (CFB core funding and NNF 160C0021746) and further supported by Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (Grants NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea]

Keywords: *Pseudomonas putida*, Recombineering, RecET, Cre/lox, Gene integration

H012

PETase: The Key Enzyme for Recycling Plastic Waste to Various Polyhydroxyalkanoate

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Poly(ethylene terephthalate) (PET) are very popular plastics with their desirable characteristics. However, non-biodegradability, notable merit to plastics, causes the contamination on the earth. Therefore, the degrading waste of plastics and producing biodegradable plastics become significant. With the recently found PET-degrading bacterium, *Ideonella sakaiensis*, effective microbial degradation and/or recycling of PET get close to a reality. Here we firstly report the crystal structure of *I. sakaiensis* PETase (*Is*PETase) with high resolution. This crystal structure helps to confirm the catalytic features of *Is*PETase and predict its molecular mechanism to degrade PET. Based on these, we developed the new variant for *Is*PETase with more enhanced PET-degrading activity than its wild type. This variant makes it more efficient to recycle PET to polyhydroxyalkanoate in *Pseudomonas putida* KT2440. With the optimized method on chemical pretreatment of PET, PET can be used as a carbon source to produce various polyhydroxyalkanoate. This study gives the new perspective to manage useless PET waste as a source. It is a remarkable microbial application regarding both economical and eco-friendly aspects.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science and ICT (MSIT) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A-2026557).]

Keywords: PETase, Plastic degradation, Protein engineering, Metabolic engineering, Polyhydroxyalkanoate

H013

Structural Insight into the N-terminal Domain of *Ralstonia eutropha* Polyhydroxyalkanoate Synthase with the Proposed Structure and Mechanisms of the Whole Enzyme

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The polyhydroxyalkanoates (PHAs) are bacterial polyesters, which can be alternatives of petroleum-based plastics. To enhance the activity for polymerization for PHA synthesis, understanding the structure and the mechanism of PHA synthase(PhaC) is important. Therefore, we first demonstrate the 3D reconstructed models of PHA synthase from *Ralstonia eutropha* and its complex with PhaM, a PHA granule associated protein by small angle X-ray scattering (SAXS) analysis. The catalytic C-terminal domain of *Re*PhaC1 dimer is located at the center, and the N-terminal domain of *Re*PhaC1 is located opposite the dimerization subdomain of C-terminal domain. These studies newly identify that N-terminal domain plays crucial roles on positioning the enzyme to the PHA granules and stabilizing the growing PHA, even though it does not directly affect enzyme catalysis. The serial truncation study on N-terminal domain indicates that the predicted five a-helices (N-a3 to N-a7) are essential for proper folding and granule binding function of N-domain. This work is meaningful in that it provides in-depth research into PHA biosynthesis and basis of enzyme engineering for tailor-made bio-plastic production.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science and ICT (MSIT) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A-2026557).]

Keywords: PHA synthase, Mechanism, Structure, N-terminal domain, Polyhydroxyalkanoate

H014

Expression and Purification of Recombinant Shiga Toxin Protein

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Nowadays, the morbidity and mortality associated with gastrointestinal disease caused by Shiga toxin-producing *Escherichia coli* (STEC) have posed the threat to piglet health. Antibiotics were used as an effective way to prevent gastrointestinal disease. However, with the increase of using antibiotic caused the antibiotic-resistant strains produced. So, it is necessary to develop a new method for treating this kind of disease and vaccine as a promising candidate for defeat with gastrointestinal disease.
In our study, recombinant Shiga toxin (Stx2e) protein was designed for preparation of vaccine making. Shiga toxin contains two parts namely A subunit and B subunit is one of AB_5 toxins. Intact A subunit possesses high toxicity. It is not only unsafe to researcher also challenged to piglet. To avoid this trouble truncated A subunit without active part was a good choice. So, *E. coli* BL21(DE3) pLysS contain pStx2e-A-C domain was designed. In order to make efficient Shiga toxin complex, the pStx2e-B-N tag was also designed. They were co-expressed and purified to develop novel candidate antigens. After purified protein injection, the blood serum of piglet was collected and the antigen was detected by western blotting.
[This research was supported by Technology Development Program for Agriculture and Forestry; the Ministry for Food, Agriculture, Forestry, and Fisheries, Republic of Korea (no. 117031-3)]

Keywords: Shiga toxin, Gastrointestinal disease, Recombinant protein antigen, Protein expression

H015

Cloning and Functional Analysis of tcsK Gene Encoding Sensor Kinase of Two-component System from *Amycolatopsis mediterranei* ATCC 13685 Producing Rifamycin

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Most prokaryotes and some eukaryotes have a two-component system (TCS) as the primary means by which they can recognize and react, even when various environmental stimuli do not directly penetrate the cytoplasm. However, the TCS has not been studied well in industrially important rare actinomycetes. We confirmed fragments consisting of open reading frames (ORFs) of *tcsK* encoding sensor kinase and *tcsR* encoding response regulator in *Amycolatopsis mediterranei* ATCC 13685 producing rifamycin, ansamycin family. In this study, part of the *tcsK* gene from *A. mediterranei* was obtained by PCR using the primers that are designed based on amino acid sequence previously studied from other actinomycetes, and this PCR product showed a high similarity to the part of the putative *tcsK* gene. To examine whether the *tcsK* is related to the production of antibiotics, a high expression vector (pSET152tcsK) for the *tcsK* was constructed, and then introduced into *A. mediterranei*. As a result, rifamycin production from *A. mediterranei* was increased in exconjugant including *tcsK* high expression vector compared to wild type strain, however this exconjugant did not affect morphological differentiation. Therefore, our results have demonstrated that the *tcsK* gene might influence the rifamycin production of *A. mediterranei*.
[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: *Amycolatopsis mediterranei*, *tcsK*, Rifamycin, Functional analysis

H016

Significant Improvement for Indigo from Wild-type *Celeribacter* to Mutated FMO Recombinant *E. coli*

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Indigo is a blue dye extracted from plants and is primarily used for the production of denim for blue jeans. This dye is presently produced in a chemical process. However, the chemical synthesis of indigo provoked not only health problems to workers but also severe environmental pollution. As the interest is growing in finding environment-friendly methods for indigo production, microbiological indigo synthesis process is attracting attention. *Celeribacter* sp. TSPH2 was isolated for indigo production strain from oil-contaminated sediment. Wild-type *Celeribacter* sp. TSPH2 produced 13 mg/L indigo on optimum conditions. And, to increase indigo, recombinant *E. coli* harboring *fmo* from *Celeribacter* sp. TSPH2 was prepared, indigo produced up to 370 mg/L in 5 L jar. To further improve indigo, FMO mutated recombinant strains were produced. In the newly constructed FMO mutated vector, the threonine at position 424 was changed to alanine(T424A). In the 5 L jar culture, 633 mg/L Indigo was produced within 24 h and 934 mg/L Indigo was produced in 50 L. Indigo production sharply increased by 71.8 fold in FMO mutated recombinant *E. coli* compared to wild-type. Another important feature is that the FMO mutated strain, indigo production is possible even at 37℃.
[This work (Grants No. C0563841) was supported by Business for Cooperative R&D between Industry, Academy, and Research Institute funded Korea Small and Medium Business Administration in 2017 and Gyeongsangbuk-do R&D Program.]

Keywords: Indigo, *Celeribacter*, Flavin containing monooxygenase (FMO)

H017

A Novel *β*-agarase Producing Neoagarotetraose from *Catenovulium agarivorans* Y3

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A novel ß-agarase CaAga5 belonging to the glycoside hydrolase family 16 was identified from a novel agar-degrading bacterium *Catenovulium agarivorans* Y3. CaAga5 was composed of 1320 amino acids (142 kDa), including a 28-amino acid signal peptide. The agarase activity of purified CaAga5 was confirmed by zymogram analysis. The optimum pH and temperature for CaAga5 activity were determined to be 8.0 and 40°C, respectively. CaAga5 is a cold-tolerant ß-agarase that has a strong activity at a low temperature range from 20°C to 10°C. CaAga5 requires monovalent ions (K^+ and Na^+) for its maximum activity, and severe inhibition by several metal ions, such as Zn^{2+}, Fe^{2+}, and Cu^{2+}, was observed. Notably, thin layer chromatography and agarose-liquefying analyses revealed that CaAga5 is an endo-type ß-agarase that hydrolyzes agarose into mainly neoagarotetraose. A relative small amount of neoagarohexaose and neoagarobiose was produced. Therefore, this study shows that CaAga5 from *C. agarivorans* Y3 is a novel ß-agarase producing neoagarotetraose that may be useful for industrial applications.
[This work was supported by a grant the NRF funded by the Ministry of Science and ICT (number NRF-2018R1A1A1A05023049)].

Keywords: Agarase, *Catenovulium agarivorans*, Neoagarotetraose

H018

Production of Anti-dementia Acetylcholinesterase Inhibitors from the Wild Yeasts *Saccharomyces cerevisiae* WJSL0113 and *Wickerhamomyces anomalus* JSF0128

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The screening of potent acetylcholinesterase inhibitor - producing yeasts from wild yeasts and the optimal condition for the production of anti-dementia acetylcholinesterase inhibitors were investigated. Among one hundred seven non-pathogenic wild yeast strains from the waters and soils of three main rivers in Daejeon and midstream of Gumgang in Gonju, Korea, Sporogenous *Saccharomyces cerevisiae* WJSL0113 and asporogenous *Wickerhamomyces anomalus* JSF0128 were selected as useful strains for the production of potent acetylcholinesterase inhibitors. The acetylcholinesterase inhibitors of *Saccharomyces cerevisiae* WJSL0113 and asporogenous *Wickerhamomyces anomalus* JSF0128 had a maximum yield when they were incubated in yeast extract-peptone-dextrose media (pH 6.0 in *Saccharomyces cerevisiae* WJSL0113 and pH 5.0 in *Wickerhamomyces anomalus* JSF0128) for 18 h at 30 ℃, respectively.

Keywords: Anti-dementia, Acetylcholinesterase inhibitor, Wild yeasts, *Saccharomyces cerevisiae* WJSL0113, *Wickerhamomyces anomalus* JSF0128

H019

The Cultivation and Deammonification of Anammox Bacteria Using Synthetic Substrate

Kwanghyun Hwang, Hyungjin Park, and Hyosang Kim*

GS E&C, Environment Process Engineering Team

The Anammox (Anaerobic Ammonium Oxidation) process is a technology that uses microorganisms to reduce costs such as aeration and external carbon source supply for the nitrogen treatment process. Anammox microorganism is easy to granule and it can be applied to media, so that high concentration microorganisms can be maintained.
However, they are reported to have very slow growth rates (doubling time of 11–20 days). It is also highly sensitive to nitrite and DO concentration, and is also affected by temperature and pH. Since cultivation is very difficult, there have been no practical cases in Korea.
Cultivation and nitrogen treatment were carried out on Lab scale for the development of an energy saving type sewage / wastewater treatment system. Anammox bacteria were cultured in bio-reactor and using the seed sample from Yeungnam University. The culture conditions were 35°C and pH was maintained 7.0. The culture was performed with synthetic substrate and SBR type for 12 h HRT.
Cultivation using synthetic substrates was started at substrate loading conditions of 0.2 kg-N / m 3.day and the nitrogen removal rate of 85% was monitored at substrate loading conditions of 1.0 kg-N / m 3.day through the incubation period of about 6 months. After 4 months of acclimation period, the nitrogen removal rate was rapid. The two-stage (partial nitrite-denitrification) process for ammonia treatment showed removal rate of over 90%.
[This research is supported by GS E&C.]

Keywords: Anammox bacteria, Deammonification, Cultivation, Nitrogen treatment

H020

Simultaneous Saccharification and Bioethanol Fermentation Using Bio-capsule

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Simultaneous Saccharification and Fermentation (SSF) process needs to be developed for economically bioethanol production. Bio-capsule formation was tried for SSF process and formed bio-capsule was used practically to perform the simultaneous saccharificationa and fermentation process for bioethanol production. Various saccharifying fungal strains and fermentative yeast strains were screened in this study. Pellet formation and optimal conditions for bio-capsule formation were secondly evaluated. Lastly, optimum temperature, shaking speed and bio-capsule concentration for SSF had been found to be 33℃, 120 rpm and 5%, respectively. 4.9% and 11.1% ethanol yield was achieved using *M. purpureus* KACC 42430 bio-capsule after 2 days of incubation in fermentation medium with starch and liquefied fermentation medium, respectively. 11.1% ethanol yield was similar to that obtained after fermentation using commercial saccharification enzymes. The results provide valuable insights about the bio-capsule formation for bioethanol SSF process and practical use of bio-capsule for bioethanol SSF process.

Keywords: Bio-capsule, Simultaneous saccharification and fermentation, Bioethanol

H021

Isolation and Characterization of Functional Bacteria Form Insect Intestines for Development of Animal Feed Supplemented Microorganism Product

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Feed supplement mainly used to farm animals for help digestibility, hygiene, and intestinal health. Microorganism feed supplements mainly used lactic acid bacteria, yeast, and probiotics, those microorganisms confer several benefits for animals when used with animal's food. The aim of this study, we isolate and characterized beneficial bacteria within the insect gut focused on enzymatic activity for apply with feed supplement to farm animals. Six insect were used to isolation of beneficial bacteria such as cellulolytic and proteolytic activity and lactic acid bacteria. The result of genetic analysis based on 16S rRNA gene, total 24 bacteria were identified, such as mainly are *Bacillus* spp., *Pseudomonas* spp. and *Lactobacillus* spp., and it is identified the effective functionality of each sector. Functional microorganisms discovered in this study are expected to be useful as animal feed supplements.
[This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT)]

Keywords: Feed supplement, Functional microorganisms, Insect gut

H022

Expression and Purification of IFN-γ for Diagnostic Tool of Bovine Tuberculosis in Cattle

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Bovine tuberculosis is a threat to the meat processing industry. There are several screening methods to detect tuberculosis within cattle herd like skin test, and one of the established assays is IFN-gamma assay. Most diagnostic kits are high in price even though the market for the item is quite large, considering latent tuberculosis can be detected by the assay. In this study, we expressed and purified recombinant IFN-gamma that can be beneficial for the assay kit as standard. Cloning and expression of the peptide was conducted by using vector pET-30 MBP-tev-IFN-γ into *E. coli* BL21 (DE3). 5-L fed-batch fermentation of the recombinant *E. coli* was also performed to produce recombinant IFN-gamma in larger scale that might be suitable for commercialized production later in the future. Purification was done by two-steps purification, both steps are using affinity chromatography to obtain the highest purity of IFN-gamma peptide with high yield.

Keywords: Tuberculosis, IFN-γ, Fermentation, *E. coli*, Affinity chromatography

H023

Development of an *ex vivo* Porcine Lung Model : Possible Application for Respiratory Infection Study

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Development of drugs targeting respiratory pathogen is essential for controlling respiratory disease and enormous experiments have been tried with *in vivo* situation. However, *in vivo* experiments have economical and ethical issues. Our study was aimed to know the possibility to use *ex vivo* lung culture system and relevant culture conditions. After isolation of lungs from naïve pigs, agarose-inflated lung tissues were prepared and sliced manually, subsequently, sliced lung tissues were placed on 24-well plate. Eight different combinations of media were used to determine the optimum *ex vivo* lung culture condition. In addition, the lung tissues were infected with porcine reproductive and respiratory syndrome (PRRS) virus at the titers of 1×10^4 $TCID_{50}$/ml. Virus growth was confirmed by titration in MARC-145 cell at 2, 4, 6 days post infection. By observing lung lesion patterns and virus titer in each medium, we concluded that *ex vivo* lung culture in the normal physiological environment is not our media specific. However, in the pathological environment, it was revealed that F12K medium is suitable on PRRS virus infected lung tissues for tissue maintenance and virus infection. The present study possibly shows that porcine *ex vivo* lung model can be used for respiratory infection study.
[This work was supported by the Co-operative Research Program for Agriculture, Science and Technology Development (PJ012612012018) in the Rural Development Administration, Republic of Korea.]

Keywords: *ex vivo*, PRRS virus, F12K

H024

UV Mutagenesis for Improving Erythritol Production by *Candida sorbosivorans* JM409

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Erythritol is getting an attention as an alternative natural sweetener with no calories. A new domestic isolate of *Candida sorbosivorance* JM409 has recently reported as a promising erythritol producer. For a commercial level production of erythritol by this yeast, we performed UV mutagenesis of the strain to improve erythritol yield on glucose. Using triphenyltetrazolium chloride (TTC) solution as a indicator, we screened for mutant with a high reductase activity. Among 30 mutants we tested, one mutant had significantly higher erythritol yield with no ethanol production. By optimizing the fermentation conditions, the erythritol yield and productivity of the mutant were significantly improved. Because the selected mutant was not genetically engineered, it has a potential to be used as an industrial host.
[This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ0127982018)" Rural Development Administration, Republic of Korea.]

Keywords: Erythritol production, Yeast, UV mutagenesis

H025

Minicell-based Oral Delivery of Single Chain Insulin-57

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Type 1 and 2 Diabetes mellitus (T1DM, T2DM) are both caused by inappropriate production of insulin. The former results from the lack of ß cell, while the later results from insulin resistance and impaired function of ß cell. In order to treat T1DM, as well as severe cases of T2DM, patients should inject insulin analog multiple times a day. Because these analogues are readily degraded upon oral intake, the only method of injecting insulin is via invasive methods. We aimed to develop minicell-based insulin delivery system that can be orally administered. Minicells are achromosomal cells that do not reproduce. Overexpression of FtsZ gene in *Escherichia coli* induces abnormal cell division that produces minicells. We have engineered the minicell to produce single chain insulin associated with cell penetrating peptide that facilitates cellular intake through Gibson Assembly. The expression of insulin was assessed through Western Blot. The cells lyse in response to bile salt, which leads to targeted secretion and subsequent intake of insulin in duodenum.

Keywords: Minicell, Insulin, Diabetes, Synthetic biology

H026

Determination of Nitrate and Nitrite Concentrations by Spectrophotometer and Liquid Chromatography in Plasma Activated Water, an Emerging Disinfectant with Wide Applications

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Non-thermal plasma activated water (PAW) has been attracted to be an effective disinfectant with applications in food preservation and hygiene. It has been known that non-thermal PAW contains various oxidative species such as hydrogen peroxide, nitric oxide, ozone, hydroxyl radical and peroxynitrite etc. In addition, it has been shown that nitrate and nitric acid are also generated in PAW as major components of the products. Although the oxidized forms of nitrogen are regarded one of the components responsible for antimicrobial activities, the measurement of exact concentrations of these species and the reaction mechanism for the formation of these species in PAW have not been established. In this study, we compared the spectra obtained from UV/Vis spectrometer and liquid chromatography (HPLC), and revealed that the peak at 300 nm generated by nitrate is correlated to that of HPLC. Unlike to the previous data, a group of 5 peaks in the range of 330–390 nm, which is previously known to be originated from nitrite, is not well matched to the nitrite concentrations obtained by HPLC. Thus, the absorbance peaks in the range of 330–390 nm may represent an unidentified oxidized form of nitrogen or oxygen. In addition, the formation of the levels of nitrate and nitrite were monitored in the presence of oxidizing agents, radical scavenging agents. Our results will help understanding the mechanism of nitrogen oxides formation during plasma activation in water.
[This research was supported by the KERI Primary research program of MSIP/NST (No. 18-12-N0101-52)].

Keywords: Plasma activated water, Nitrite, Nitrate

I001

Control of *Salmonella* Typhimurium in Milk Using Bacteriophages

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Milk quality and safety has become a great concern over the processing raw milk and milk-based products. Therefore, this study aimed to evaluate the inhibitory effect of bacteriophage P22 on the growth of *Salmonella* Typhimurium. The P22 belonging to Podoviridae family has a hexagonal head and short tail. The number of *S.* Typhimurium in milk was significantly reduced by more than 3-log at early storage period at 4°C and 37°C. The noticeable change in pH was observed at the milk treated with bacteriophage P22, which was decreased from 6.7 to 6.3 after 24 h incubation at 37°C. The milk color was not changed at the control and bacteriophage treatment throughout the storage at 4°C for 12 days and 37°C for 24 h. This study provides useful information for enhancing milk safety and quality and applying bacteriophage control in food system.

Keywords: Bacteriophage, Salmonella, Milk, Food safety

I002

Co-existence of Plasmid-mediated quinolone resistance (PMQR) Genes in Extended-spectrum β-lactamase (ESBL) Producing *Escherichia coli* Strains from Retail Raw Chicken in Korea

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Fluoroquinolone and cephalosporin drugs are important for the treatment of foodborne infections. The plasmid-mediated quinolone resistance (PMQR) genes are responsible for insensitivity to fluoroquinolones, and extended spectrum β-lactamases (ESBLs) confer resistance to cephalosporin antibiotics. Since these resistance genes are encoded on plasmids, the resistance is likely to be transmitted horizontally. Despite the increasing prevalence of PMQR- and ESBL-harboring *Escherichia coli* in food, little has been studied in Korea regarding this. The aim of this study was to investigate the frequency of co-existence of ESBL and PMQR genes in *E. coli* from retail raw chicken. PCR detection of PMQR genes was performed with 80 ESBL-producing *E. coli* strains isolated from retail raw chicken in South Korea. Surprisingly, 52.5% (42/80) of the ESBL-producing *E. coli* strains harbored at least one PMQR gene. The most prevalent PMQR gene was *qnrS* (90.5%, 38/42), and *aac(6')-Ib-cr* was detected in six strains. The *oqxAB* and *aac(6')-Ib-cr* genes were detected in three (7.1%) and one (2.4%) strains, respectively. The most common ESBL type in the PMQR-positive strains was CTX-M-65 (81%, 34/42). Antimicrobial susceptibility test revealed all the strains that co-harbored PMQR and ESBL genes were resistant to multiple antibiotics belonging to different classes. Based on the findings in this study, PMQR genes are frequently located in ESBL-producing *E. coli* strains from retail chicken in Korea.

Keywords: Plasmid-mediated quinolone resistance, Extended-spectrum β-lactamase, *Escherichia coli*, Chicken, Multi-drug resistance

I003

Isolation and Characterization of Lactic Acid Bacteria from Korean Traditional Fermented Foods

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A total 107 isolates of lactic acid bacteria were isolated from Korean traditional fermented foods such as kimchi, soybean paste, red pepper paste, soy sauce. These bacteria were identified as *Lactobacillus sakei*, *Lactobacillus plantarum*, *Weissella hellenica* on the basis of morphological and 16S rDNA sequence analysis. Out of 107 isolates tested, only 18 isolates exhibited antimicrobial activity against pathogenic bacteria such as *Pseudomonas aeruginosa* (KCTC2513) and *Bacillus thuringiensis* (KCTC1507). Among them, the strain KC68 isolated from Kimchi showed the strongest antibacterial activity against both pathogenic bacteria. The strain KC68 was identified as *Lactobacillus sakei* and named *Lactobacillus sakei* JNU68. The antimicrobial activity of the strain KC68 was stable in sterilized supernatant at 100℃ for 15 min, but the activity was lost in the supernatant after adjusting pH 7. Optimal temperature for the growth of *Lactobacillus sakei* JNU68 was significantly better at 37℃ than at 30℃ and the stationary phase was reached after 18 h of inoculation. The *Lactobacillus sakei* JNU68 was also cultivated in rice flour and then tested for antimicrobial activity, and the results showed that it was reproduced as shown in the MRS medium. These results suggested that *Lactobacillus sakei* JNU68 isolates from Korean traditional fermented foods has good potential in extending the shelf-life of rice cakes.

Keywords: Lactic acid bacteria, Traditional fermented foods, Kimchi, *Lactobacillus sakei*, Rice flour

I004

Bioprospecting Potential of Bacteria Isolated from Honey and Bee-associated Products from Indonesia

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Many recent studies support the link between symbiotic and pathogenic microbial interactions in gut microbiota of honey bee. However, our knowledge of the honey microbial community still remain largely unknown. The present study was designated to evaluate the occurrence of bacteria and yeasts in honey and bee-associated products from Indonesia. A total of 115 isolates were isolated from 24 samples. The supernatants were used to screening against fungal pathogenic (*Aspergillus fumigatus* ATCC 204305) by using dual assay method; *Candida albicans* ATCC 10231 and bacteria (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923) by using well diffusion methods. More of the isolates shown antimicrobial activity against fungal pathogenic compared than bacteria. Eighty four out of 115 isolates (73.04%) exhibited enzymatic activity on at least one of the assay. Protease activity is the most exhibited activity shown by the isolates (62.61%) followed by amylase (42.61%) and lipase (41.74%). Six isolates (L30, L35, Y12, Y33, Y21, and Y23) were selected to characterize by using 16S rDNA sequencing. Molecular identification of the isolates performed that L30 and L35 were identified as *B. subtilis*; Y12 and Y33 were *B. velezensis*; and Y21 and Y23 were *B. amyloliquefaciens*. These data can be explored further to be applied as an alternative way to test the purity of honey. The results demonstrated that isolates with high antimicrobial and enzymatic activity had potential application as biopreservation agents and probiotic.

Keywords: Honey, Bee-associated products, Antimicrobial, Enzymatic activity

I005

Bacterial Community of Oncom, an Indonesian Fermented Food

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Oncom is a traditional fermented food made from tofu waste or peanut press cake. Oncom fermentation is done mostly by molds *Neurospora intermedia* var. *oncomensis*, which produces red/orange oncom or *Rhizopus oligosporus,* which produces black oncom. The bacterial community living in oncom may contribute to the degradation of macro molecules such as carbohydrate, protein, or lipid during fermentation process. However, this bacterial community is still underexplored. To study what kind of bacteria present in oncom, we used 16S metagenomics analysis. Oncom sample were purchased from local market in Jakarta and West Java province, Indonesia. Three samples of red oncom (B2, P2, AB1) and three samples of black Oncom (HAB1, HCB1, HJB1) were used in this study. Metagenomic DNA was extracted from oncom followed by sequencing of V3-V4 region of 16S-rDNA by using MiSeq System Platform (Illumina Inc, USA). Firmicutes and Proteobacteria were the predominant phyla in all samples. Interestingly, Actinobacteria phyla was only presented in black oncom samples. In order level Lactobacillales and Enterobacteriales were found dominantly in Oncom. In total, 16S metagenomic analysis of black oncom and red oncom generated 18906 and 18958 OTU, respectively, with 3934 sharing OTU. This 3934 OTU could indicate the important bacteria for oncom fermentation. This findings open the possibility to explore more about the role of bacteria during fermentation process and the source of bacteria itself.

Keywords: Fermented food, Oncom, Bacterial community, 16S-rDNA metagenomics

I006

Comparison of Physicochemical Properties of Three Bacteriocins of Genus Bacilli Isolates from Fermented Foods

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Three bacteriocins produced from *Bacillus subtilis* PARZ2, *Bacillus* sp. BJ-45 and *Bacillus* sp. NIOT had similar antibacterial spectrum. The bacteriocins showed similar pH stability and retained antibacterial activity at the pH range from 2.0 to 8.0. The bacteriocins were resistant to solvents such as acetonitrile, isopropanol, methanol, chloroform and acetone up to 50% concentration. The bacteriocins were purified via ammonium sulfate precipitation, anion exchange chromatography and reverse-phase high performance liquid chromatography. MALDI-TOF mass spectrometry revealed all bacteriocin had almost the same molecular weight of 3.4 kDa. The determination of partial N-terminal amino acid sequences showed GPIPADTLVDGEIAG and LPIPADLVDGPXGPR. The N-terminus of the bacteriocin of *Bacillus* sp. NIOT was blocked. Trypsin digestion and partial amino acid sequencing resulted in LASTLGISTAAAK. BLAST homology search indicates two bacteriocins are potentially novel ones. Bacteriocin activity was retained for more than three months at both 4°C and -20°C. The bacteriocins inhibited growth of *Listeria monocytogenes* KCCM 40037 in beef stored at 4°C for 15 days. Taken these together, the bacteriocins might have potential to be used as bio-preservatives in food products.

Keywords: Bacteriocin, Purification, Amino acid sequencing, Molecular weight

I007

Comparative Profiling of *Campylobacter jejuni* Isolates from Chicken and Duck Meats Based on Aerotolerance, Antibiotic Resistance, and Virulence Gene Prevalence

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Human infections with *Campylobacter* are primarily associated with the consumption of contaminated poultry meat. In this study, we isolated *C. jejuni* from retail raw chicken and duck meats in Korea and compared their aerotolerance, antibiotic resistance, and virulence gene prevalence. *C. jejuni* isolates from chicken dominantly belonged to multilocus sequence typing (MLST) clonal complex (CC)-21, whereas CC-45 is the common MLST sequence type in duck meat isolates. *C. jejuni* strains from both chicken and duck meat were highly tolerant to aerobic stress. Virulence genes were frequently detected in *C. jejuni* strains from chicken and duck meats. However, antibiotic resistance was higher in *C. jejuni* strains from duck meats than chicken isolates. Based on the prevalence of virulence genes and antibiotic resistance, fluoroquinolone-resistant *C. jejuni* strains harboring all tested virulence genes except *virB11* were predominant on retail poultry. The comparative profiling analysis in this study successfully demonstrated that antibiotic-resistant and pathogenic strains of *C. jejuni* are highly prevalent on retail poultry, and that duck meat is also an important vehicle that may transmit high-risk *C. jejuni* to humans.
[This research was supported by a grant (16162MFDS029) from Ministry of Food and Drug Safety in 2018.]

Keywords: *Campylobacter jejuni*, Poultry, Aerotolerance, Antibiotic resistance, Virulence gene prevalence

I008

Comparison and Optimization of Detection Methods for Norovirus in Various Foods

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Human Norovirus (HuNoV) is the major cause of outbreaks of foodborne viral disease. They are typically transmitted through the fecal-oral, person-to-person or consumption of contaminated water and food. In this study, the efficacy of different methods for detecting norovirus from vegetables, fruits and salted foods was compared and optimized using murine norovirus (MNV), a surrogate closely related human norovirus. The original method used in outbreak investigation of MFDS was modified for optimal recovery and concentration of surrogate virus. Specifically, a known amount of MNV was spiked onto the surface of food, then the virus was recovered with 0.25 M threonine-0.3 M NaCl (pH 9.5), beef extract [100 mM Tris-HCl, 50 mM glycine, 3% beef extract (pH 9.5)], 0.25 M glycine-0.3 M NaCl, Phosphate buffered saline (PBS) and Trizol reagent. The downstream viral concentration was acheived by the polyethylene glycol precipitation. The result showed that beef extract was the most efficient for the elution of MNV from all tested food groups with the recovery rate of 7–45%, suggesting the renovated methods could be applied for future surveillance of noroviruses in Korea.

Keywords: Norovirus, Vegetables, Fruits, Salted foods, Recovery rate

I009

Effects of Antioxidizing Agents on the Resuscitation of Viable-but-nonculturable *Vibrio parahaemolyticus*

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Preliminarily, it was revealed out that pathogenic bacteria can be induced into the viable-but-nonculturable (VBNC) state upon exposure to a proloned period of cold-starvation stress. VBNC bacteria could be also recovered back to the culturable state by eliminating the causative environmental condition. Now, there are restricted evidences available on understanding the physiological characteristics of VBNC organisms. Then, the aim of the present study was to examine the effects of antioxidizing agents such as catalase and sodium pyruvate on the resuscitation of VBNC *V. parahaemolyticus*. Bacterial cells were shown to enter into the VBNC state in artificial seawater (pH 6) microcosms at 4°C within 70 days. VBNC cells were harvested, inoculated in formulated resuscitation-buffers, and then incubated at 25°C for several days. TSB (pH 8) supplemented with 3% NaCl (TSBA) exhibited the higher resuscitation-availability of VBNC cells. It was also shown that TSBA containing 10,000 U/mg/protein catalase, 2% sodium pyruvate, 20 mM $MgSO_4$, 5 mM EDTA, and cell free supernatants extracted from the pure cultures of *V. parahaemolyticus* was more effective in resuscitating VBNC cells of *V. parahaemolyticus*, showing by 7.69-8.91 log_{10} CFU/ml. From these results, it was indicated that such a reversible conversion of VBNC cells to the culturable state would be significantly influenced by ROS-detoxifying agents such as catalase and sodium pyruvate.
[Supported by grants from NRF-2016R1A6A3A11932794]

Keywords: *Vibrio parahaemolyticus*, Viable-but-nonculturable, Antioxidizing agents

I010

Quality Characteristics of *Jochung* by *Nuruk*

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This study was performed to increase the taste and flavor of the Jochung by using *nuruk*. In order to evaluate the quality characteristics and yields of Jochung. The *nuruk Jochung* made by the rice and wheat *nuruk*. The pH, acidity, viscosity, reducing sugar, free sugar contents, color and sensory evaluation were measured. The rice *nuruk Jochung* yield was 7.6 percent more than the wheat *nuruk Jochung*.
The pH of rice *nuruk Jochung* and wheat *Jochung* were 6.23, 6.05 and brix were 76.75% and 76.50%, respectively. The color of L values were 42.43, 32.95, a values were 3.74, 4.62, b values were 11.96, 7.86, respectively. The sensory evaluation results of *nuruk Jochung* were generally higher than traditional methods. In addition, the overall acceptability score was the highest allowed by wheat *nuruk*. Therefore, using *nuruk* of *Jochung* process is believed to improve not only production but also the quality of the senses.

Keywords: Jochung, Nuruk

I011

Complete Genome Sequence and Comparative Genomic Analysis of Multidrug-resistant *Staphylococcus aureus* Isolated from Pork

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Staphylococcus aureus is a pathogen that causes food poisoning and community-asssociated 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: Complete genome sequence, Comparative genomic analysis, *Staphylococcus aureus*, Multidrug-resistant

I012

Development of Potential *Cronobacter sakazakii*-controlling Phage Cocktail

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Although bacteriophage is re-highlighted as a novel antimicrobial to control harmful pathogens, emergence of phage-resistance is pointed out as one of the problem of phage application. To surmount this obstacle, we tried here to develop a potential phage cocktail consisted of three different phages controlling foodborne pathogenic *Cronobacter sakazakii*. Two phages, HJC01 and HJC02, and another phage HMC01 were isolated from Hongje-stream and Han-river, respectively, Seoul, South Korea. All three phages target *C. sakazakii* only, but exhibited different characteristics. HJC01, formed clearer and larger plaques than others, belonged to the family *Siphoviridae*, and HJC02 and HMC01, formed smaller plaques, were classified as the family *Myoviridae*. Phage spotting assay with various gene-deletion mutants of *C. sakazakii* revealed that HJC01 and HJC02 use lipopolysaccharides while HMC01 uses flagella as a host receptor. Compared to the single phage treatment, a simultaneous treatment of two or more phages that use different host receptor more effectively inhibited the growth of *C. sakazakii* up to 8 hours. These results suggested that phage cocktail consisted of HJC01, HJC02, and HMC01 would be a potent biocontrol agent to prevent *C. sakazakii* infection.
[This work was supported by Basic Science Research Program (2016R1C1B-2015578) through the NRF of Korea funded by MSIT, ATC program (10076996) funded by MOTIE, and the Yonsei University Future-leading Research Initiative of 2017-22-0060.]

Keywords: *Cronobacter sakazakii*, Bacteriophage, Phage cocktail, Foodborne pathogen

I013

Physicochemical and Flavor Components of *Lycium chinense Miller* by Fermented with *Leuconostoc mesenteroides*

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Lycium chinese Miller is a health-functional material used as tea, liquor and medical materials. There is a problem that the longer the storage period of the odor of *Lycium chinese Miller* is generated. In this study, we aimed to reduce the odor and improve the quality of the functional material by fermentation. *Lycium chinese Miller* was fermented by *Leuconostoc mesenteroides* for 3 days. Samples were prepared by adding 10% honey to the *Lycium chinese Miller* (LFH) and honey free *Lycium chinese Miller* (LF). The pH, total acidity (%) and reducing lactic acid fermentation. The free sugar, free amino acid and color values relatd to taste components were analyzed. The pH level decreased while total acidity increased during lactic acid fermentation. The free sugar, free amino acid and color values related to taste components were analyzed. The content of free sugar in the LF was the highest after 1 days of fermentation. In addition, the free amino acid contents of LFH decreased during fermentation. In case LF, aspartic acid had more contents than other free amino acids and increased during fermentation. GABA was the highest after 1 days of fermentation. The content of betaine, which is a major component of *Lycium chinese Miller*, increased during fermentation in both LF and LFH. These result are indicated that *Lycium chinese Miller* by fermented imporve quality and flavor.

[Supported by National Joint Agricultural Research Project of RDA (PJ013570012018)]

Keywords: *Lycium chinese Miller*, *Leuconostoc mesenteroides*, Flavor, Fermentation

I014

Effect of Acetic Acid and Their Salt Forms Combined with NaCl on Inactivating *Salmonella* Typhimurium

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Combination of NaCl and organic acid is commonly used for preserving the quality of food products. This study aimed at examining the effect of acetic acid (AA), sodium acetate (SA), calcium acetate (CA) or potassium acetate (PA) in a combination with NaCl for inactivating *Salmonella* Typhimurium in laboratory broth. *S.* Typhimurium was inoculated into Luria-bertani broth without NaCl (LB) and LB with 3% NaCl (LBS) containing 0.1 M AA, 0.05 M CA, 0.1 M SA or 0.1 M PA and then incubated at 25°C for 15 days. The number of *S.* Typhimurium was enumerated on tryptic soy agar (TSA) and *xylose lysine deoxycholate* agar (XLD). It was shown that treatments with AA, CA, SA or PA alone were effective in reducing *S.* Typhimurium in LB broth, showing by 3.08–4.37 $\log_{10}$ CFU/ml reduction in TSA after 3 days, whereas, when NaCl was added to the same treatments, effectiveness of treatments were significantly reduced and lower reductions (1.82–2.41 $\log_{10}$ CFU/ml) were observed compared than acid single treatments. However, there was no significant difference on reduction levels of *S.* Typhimuriumn by acid form during treatments. Therefore, increased resistances of *S.* Typhimurium against acetic acid and their salt forms were observed in the presence of NaCl in LB broth. Therefore, further studies need to be performed better to understand the mechanisms associated with the antagonism of organic acid and their salt forms combined with NaCl against *S.* Typhimurium.

[Supported by grants from NRF and ICT.]

Keywords: *Salmonella* Typhimurium, Combined effect, Acetic acid, Salt, Acetate

I015

Analysis of Microbiota on Pork Meat Collected from Large-scale Retail Distribution in South Korea

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Pork meat is one of popular primary food materials throughout the world. Meat is a complex niche with various physical and chemical properties, thus various microbes can colonize in meat materials. Therefore, the ingestion of pork meat can cause food poisoning due to the pathogenic bacteria during cooking processes. In this study, we analyzed the microbiota of pork meat obtained from large-scale retail distribution in different regions and seasons. Metagenomic DNA was extracted from each sample, and the microbiota was analyzed by Illumina MiSeq system based on 16S rRNA genes. The presence of pathogens was detected by pathogen-specific primers, and the bacterial amounts were quantified by real-time PCR. Different composition of microbiota was detected between sampling sites and seasons. In addition, we analyzed the effects of pathogen infection on the indigenous microbiota of pork meat by artificial infection experiments. The shift of microbiota composition and functional genes after pathogen infection was compared with non-infected samples. This study can help our understanding of the microbiota in pork meat and the risk of foodborne illness by ingestion of pork meat products.

[This research was supported by a grant (14162MFDS972) from Ministry of Food and Drug Safety in 2018.]

Keywords: Microbiota, Pork meat, Foodborne illness

I016

Characterization of Fermented Soybean Natto Inoculated with Biogenic Amine-reducing Bacteria

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Fermented foods such as natto, cheonggukjang and doenjang produce biogenic amines during fermentation process. Biogenic amines such as tyramine, histamine, putrescine and cadaverine may be detrimental to consumers' health and should be managed as harmful substances in food. The inhibitory effect of cell-free solution (CFS) of 6 isolates on the production of biogenic amines by amine-positive food pathogens were investigated using high performance liquid chromatography (HPLC). As a result, two different CFS concentrations, which were 25% [2.5 ml CFS + 7.5 ml amino acid decarboxylase broth (ADB)] and 50% (5 ml CFS + 5 ml ADB), tended to reduce the biogenic amine production by *Escherichia coli* up to 88% compared with the control without CFS. Natto was prepared by inoculating the supernatant of 5 strains. After inoculation with *Bacillus* sp. 9094 and *Bacillus* sp. 9170, biogenic amine contents were reduced by 38% and 34% as compared to control, respectively, and natto products were determined to have better thrombolytic activity and antioxidant activity. Therefore, it is suggested that *Bacillus* sp. BCNU 9170 and *Bacillus* sp. BCNU 9094 can be used as a starter for natto to prevent accumulation of large amounts of biogenic amines in fermented natto.

Keywords: *Bacillus*, Biogenic amine, Fermented food, Natto

I017

Characterization of Fermented Cheonggukjang Inoculated with Biogenic Amine-reducing Bacteria

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Cheonggukjang is one of the Korean traditional fermented soybean products. Some biogenic amines are created from free amino acids in fermented cheonggukjang like another fermented soybean products such as doenjang during fermentation process. Biogenic amines such as tyramine, histamine, putrescine and cadaverine may be detrimental to consumers' health and should be managed as harmful substances in food. Biogenic amines are reduced essentially by human metabolic precess but some non-degraded amines or remnants can cause a lot of side effects especially in the over-intake of biogenic amines. Therefore, we tried to reduce the biogenic amines contents in soybean fermentation food. Four *Bacillus* spp. strains were firstly isolated from Meju and Doenjang, and then characterized. They were inoculated into seven Cheonggukjang subgroups in concentration of 10^7–10^8. In cheonggukjang samples after 72 h fermentation, the contents of tryptamine, β-phenylethylamine, putrescine and tyramine were lower than those in the previously published studies. The maximum reducted level of biogenic amines was also observed in *Bacillus* sp. BCNU 9170 cheonggukjang subgroup. Therefore, *Bacillus* sp. isolates were considered to have potential as a biogenic amine reducing starter for cheonggukjang fermentation.

Keywords: *Bacillus*, Biogenic amine, Cheonggukjang, Soybean fermented food

I018

Kimchi by Inoculated with Biogenic Amine-reducing Strains

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Kimchi is a Korean traditional fermented side dish. Kimchi produce small amount of biogenic amines during fermentation process. Biogenic amines such as cadaverine, histamine, putrescine, spermine, tryptamine and tyramine are also found in many fermented food and may be detrimental to consumers' health and should therefore be managed as harmful substances in food. In this work, 17 kinds of Kimchi were fermented with different types of lactic acid bacterial starter cultures and then production and reduction of biogenic amines in kimchi was investigated using high performance liquid chromatograpy (HPLC). As a result, biogenic amines tended to be reduced in Kimchi containing two kinds of bacteria more than kimchi containing one kind of bacteria. In addition, the sensory evaluation results showed no significant difference in all kimchi. Therefore, these lactic acid bacterial isolates can be used as starters for Kimchi to prevent accumulation of large amounts of biogenic amines during Kimchi fermentation.

Keywords: Biogenic amine, Fermented food, Kimchi, Lactic acid bacteria

I019

Solar Salt Concentration Effects on the Bacterial and Fungal Communities and Metabolites of Korean Traditional Doenjang during Fermentation

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Chung-Ang University

Four doenjang samples with different salt concentration (9, 12, 15, and 18%) were prepared in triplicate and their microbial communities and metabolites during the entire fermentation period were analyzed to compare their fermentation features using Illumina MiSeq, 1H-NMR, and SPME-GC/MS approaches, respectively. The pH values in doenjang with 9% salt were a little lower during the early and middle fermentation periods, but they increased to the higher pH values than those in doenjang with 12%, 15%, and 18% salt during the late fermentation period. Bacterial community analysis revealed that Bacillus and Staphylococcus were identified more abundantly in high salt doenjang, while Weissella, Tetragenococcus, Lactobacillus, and Clostridium were identified more abundantly in low salt doenjang. Fungal community analysis revealed Mucor was identified more abundantly in 12% and 15% salt doenjang. Debaryomyces and Aspergillus were present more abundantly in low salt doenjang. Microascaceae and Penicillium were identified more abundantly in high salt. The metabolic analysis revealed that organic acids were more produced in low salt doenjang. These results revealed the fermentation properties of the Korean traditional doenjang with different salt concentrations during entire fermentation period, contributing to the production of high-quality doenjang.
[This work was supported by the National Research Foundation (2017M3C1B5019250) of the Ministry of Science and ICT, Republic of Korea.]

Keywords: Doenjang, Salt concentration, Bacteria, Fungi, Metabolite

I020

Molecular Characterization of Erythromycin and Tetracycline Resistant *Enterococcus faecalis* Isolated from Retail Chicken Meats

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Enterococcus faecalis (*E. faecalis*) is a ubiquitous intestinal bacterium in human and animals, that can easily acquire antimicrobial resistance, which allows it to play the role of an antimicrobial resistance indicator. One hundred and forty-nine among 335 *E. faecalis* isolates from 7 integrated broiler operations showed the simultaneous resistance to erythromycin and tetracycline, and more than 50% among 149 isolates showed multidrug resistance. The most common resistance genes were *ermB* (96.0%, 143 isolates), and *tet*(M) (95.3%, 142 isolates) and *tet*(L) (89.3%, 133 isolates). Furthermore, 140 (93.9%) isolates simultaneously possessed *ermB*, and *tet*(L) and/or *tet*(M) genes. Eight isolates with of transposon of the Tn*916/1545*-like were detected, which also carried *ermB* and *tet*(M) genes. The most prevalent of virulence genes were *gelE* (142 isolates, 95.3%) and *ace* (137 isolates, 91.9%). Five *E. faecalis* isolates successfully transferred antimicrobial and virulence genes to *E. faecalis* FA2-2. The antimicrobial resistant *E. faecalis* isolates as well as their corresponding genes and mobile genetic elements, such as transposons may be disseminated nationwide by broiler operation system in Korea.
[Supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Research Center Support Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (716002-7).]

Keywords: Chicken meat, *Enterococcus faecalis*, Antimicrobial resistance, Virulence genes, Transposons

I021

A Trend in Resistance to Cephalosporins among *Salmonella* Isolates from Poultry in Korea, 2010-2017

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Resistance to cephalosporins in *Salmonella* spp. has become a serious public health concern worldwide. 141 *Salmonella* isolates were collected from various poultry industry sources, and 38 (27.0%) among them were resistant to cephalosporins. Cephalosporin resistance has significantly increased over time: cephalothin (from 5.0% to 29.2%), cephalexin, cefuroxime, cefotaxime, and ceftazidime (from 0% to 25.0%), and cefepime (from 0% to 12.5%). Twelve isolates carried a β-lactamase gene. A non-ESBL/pAmpC gene, bla_{TEM-1}, was found in 3 isolates in 2010–2011 and 2012–2013, respectively. The $bla_{CTX-M-79}$ (n=4) and $bla_{CTX-M-15}$ (n=1) for ESBL genes and bla_{CMY-2} (n=1) for pAmpC genes were only present in 2016–2017. All ESBL/pAmpC-positive isolates had high MIC for most cephalosporins and showed multi-drug resistance. In a conjugation experiment, the transfer of ESBL/pAmpC genes was confirmed in transconjugants. This demonstrates that ESBL/pAmpC-producing *Salmonella* isolates might be transmitted to humans through contaminated poultry products. This results suggest the need for the development of monitoring program and the guidelines for prudent use of antimicrobial agents in the poultry industry.

[Supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Research Center Support Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (716002-7).]

Keywords: *Salmonella*, Poultry, Cephalosporin, Antimicrobial resistance

I022

Characteristics of Third-generation Cephalosporin-resistant *Salmonella* from Retail Chicken Meat Produced by Integrated Broiler Operations

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Integrated broiler operations, which control and operate vertically through all phases of the chicken industry, have adopted different antibiotic applications. We aimed to compare the prevalence and antimicrobial resistance profiles of *Salmonella* isolates from 6 different integrated broiler operations and to analyze the characteristics of ESBL- and pAmpC-producing *Salmonella* isolates. Although 57 of 336 samples were positive for *Salmonella,* the prevalence varied from 6.8% to 45.8% indicating variations in *Salmonella* occurrence among the operations. Among 14 third-generation cephalosporin-resistant isolates, 9 (64.3%) ESBL/pAmpC-producing isolates were only obtained from two operations: $bla_{CTX-M-15}$ (n = 7), $bla_{CTX-M-79}$ (n = 1), and bla_{CMY-2} (n = 1). All ESBL/pAmpC-positive isolates exhibited a high MIC ($\geq$ 128 μg/ml) for most cephalosporins and showed multidrug resistance. The transfer of ESBL/pAmpC genes was confirmed in transconjugants. Our findings suggest the need for the development of monitoring and prevention programs in integrated operations because third-generation cephalosporin-resistant *Salmonella* can now be found in association with integrated broiler operations.

[Supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Research Center Support Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (716002-7).]

Keywords: *Salmonella*, Broiler operation, Cephalosporin, β-lactamase, Antimicrobial resistance

I023

Modified Listeria Enrichment Broth to Reduce Enrichment Time of *Listeria monocytogenes*

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Currently, the standard detection method presented in FDA takes at least five days to identify *Listeria monocytogenes.* In this procedure, the most time consuming is enrichment. Thus, a method to reduce enrichment time is necessary. The aim of this study was to develop an enrichment broth to improve *L. monocytogenes* enrichment. Carbohydrate sources, nitrogen source, and mineral and micronutrients sources were added into Listeria enrichment broth (LEB). A mixture of five *L. monocytogenes* strains isolated from environmental samples was inoculated in LEB and modified LEB to obtain 2 Log CFU/ml, and the samples were incubated at 30°C. The cell counts of *L. monocytogenes* were enumerated on tryptic soy agar with 0.6% yeast extract (TSAYE) at 0, 3, and 6 h after incubation. The most effective component was $MgCl_2$, and $MgCl_2$-treated broth had 3.3±0.5 Log CFU/ml of *L. monocytogenes* after 6 h of incubation, but *L. monocytogenes* cell counts enriched in LEB were 2.8±0.4 Log CFU/ml. Followed by this component, 0.15% sucrose with LEB enriched 2.6±0.1 log CFU/ml after 6 h incubation, but *L. monocytogenes* in LEB enriched 2.4±0.1 log CFU/ml. The other components were no noticeable difference, compared to LEB. This result shows adding $MgCl_2$ into LEB can reduce enrichment time for *L. monocytogenes.*

[Supported by Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ013536).]

Keywords: *Listeria monocytogenes*, Detection, Listeria enrichment broth, Enrichment

I024

Antimicrobial Resistance and Genetic Characteristics of *Escherichia coli* Isolates from Edible Offal

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Edible offal including all parts of a live animal that are not part of the dressed carcass contribute to the bottom line of the meat industry. However, many edible offal can easily become spoiled because of their functional characteristics and lead to serious contamination by fecal bacteria such as *E. coli* during slaughtering, handling, processing and storage. This study aimed to investigate prevalence and characteristics of antimicrobial resistant *E. coli* isolates from edible offal in Korea. A total of 118 *E. coli* isolates were collected from edible offal produced at 8 chicken, 9 pig and 7 cattle slaughterhouses during 2017. In antimicrobial resistance genes of 69 (58.5%) multidrug-resistant (MDR) *E. coli*, bla_{TEM-1} (97.1%) and *tetA* (76.6%) were most prevalent. Class 1 and class 2 integrons were detected in 82.6% and 2.9% of the MDR isolates, respectively. Seven virulence genes (*eaeA, escV, astA, fimH, papC, sfa/focDE,* and *iucC*), and IncF group plasmids were predominant among the MDR isolates. The results of this study suggest that edible offal can become relevant reservoir of *E. coli* strains carrying various antimicrobial resistance and virulence genes.

[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Research Center Support Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (716002-7).]

Keywords: Edible offal, *Escherichia coli*, Antimicrobial resistance, Virulence, Plasmid replicon

I025

Prevalence and Characterization of β-lactamases Genes and Class 1 Integrons in Multidrug-resistant *Escherichia coli* Isolates from Chicken Meat in Korea

Kwang Won Seo and Young Ju Lee*

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Antimicrobial resistance has become a serious public health threat throughout the world, and therapeutic options for several infectious diseases are currently limited by the presence of multidrug-resistance (MDR) bacteria. This study was designed to examine the drug resistance patterns, the prevalence of the β-lactamases and class 1 integrons in MDR *E. coli* isolates from chicken meat. Among 200 chicken meat samples, 57 were identified as MDR *E. coli*. The $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}14}$ and $bla_{TEM\text{-}1}$, were identified in 2, 4, and 16 *E. coli* strains, respectively; only 1 *E. coli* strain had both, $bla_{TEM\text{-}1}$ and $bla_{CTX\text{-}M\text{-}1}$ genes. Twenty-one of the 57 MDR *E. coli* isolates also carried class 1 integrons, and 5 different gene cassette arrangements were found in 14 of the 21 class 1 integron-positive isolates. The β-lactamase-producing *E. coli* and integron-positive *E. coli* had significantly higher resistance to 16 antimicrobial drugs than the non-β-lactamase-producing *E. coli* and the integron-negative *E. coli* ($P<0.05$). Our findings suggest that β-lactamase and class 1 integrons are widely distributed in *E. coli* isolates from chicken meat, and directly contribute to resistance to diverse antimicrobial agents.
[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Research Center Support Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (716002-7).]

Keywords: *Escherichia coli*, Antimicrobial resistance, Commercial layer, Extended-spectrum β-lactamase, Plasmid-mediated AmpC β-lactamase

I026

Characteristics of *Salmonella* Bareilly Isolated from Commercial Layer Farms and Raw Shell Eggs in Korea

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Salmonella enterica serovar Bareilly (*S.* Bareilly) has been among the top 20 most frequently isolated serovars in clinical cases of salmonellosis in the United States and observed recently in layer flocks in Korea. Forty-five *S.* Bareilly isolates were obtained from feces, dust, egg belts and eggshells at 5 commercial layer farms and from raw shell eggs at 9 retail markets in Korea. The isolates were tested for the antimicrobial susceptibility and analyzed by using pulsed-field gel electrophoresis (PFGE). Among the 45 isolates, four PFGE patterns were observed: patterns A, B, C, and D, with pattern B being the predominant and comprising 67% of the 45 isolates. The most common antimicrobial agents to which the isolates were resistant were streptomycin (24.4%), cephalothin (6.7%), and nalidixic acid (2.2%). This is the first report describing epidemiological characteristics of *S.* Bareilly, including geographical variation in Korea. Although *S.* Bareilly dose not account for the highest proportion of foodborne salmonellosis cases in Korea, continuous monitoring and investigation of salmonellosis characteristics are required because *S.* Bareilly is present in Korean layer farms and retail markets.
[Supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Research Center Support Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (716002-7).]

Keywords: *Salmonella* Bareilly, Antimicrobial resistance, PFGE

I027

Genetic Diversity and Antimicrobial Resistance of *Salmonella* Serotypes Recovered from Edible Pork Offal

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Department of Public Health, College of Veterinary Medicine, Kyungpook National University

The edible offal is widely used in many countries worldwide in different traditional dishes, and grilled and steamed intestine slices, blood and head meat, and internal organs are easy to be found in markets in Korea. In this study, a total of 52 *Salmonella* isolates from small (n=28) and large (n=24) intestine of pork were characterize to genetic diversity and antimicrobial resistance profile for the first time in Korea. Forty-two (80.8%) of these *Salmonella* isolates were resistant to at least one antimicrobial agent, and 33 (63.5%) and 30 (57.7%) of isolates showed the highest resistance to tetracycline and ampicillin, respectively. In 24 multidrug-resistant *Salmonella* isolates, bla_{TEM} and *ant(2'')-I* were identified in 12 (50.0%) and 11 (45.8%) isolates, respectively, and 18 (75.0%) isolates harbored a class 1 integrase (*intI1*). In beta-lactam resistance determinants and plasmid replicon, each 10 (41.7%) Salmonella isolates had *dfrA12-aadA2* and IncB/O, respectively. *Salmonella* are considered as one of the most common zoonotic foodborne pathogens causing gastroenteritis in humans worldwide. Therefore, more refined hygienic standard regulations for edible offal are necessary in Korea.
[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Research Center Support Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (716002-7).]

Keywords: Edible offal, *Salmonella*, Antimicrobial resistance

I028

Characterization of Plasmid Mediated Quinolone Resistance Determinants in Ciprofloxacin Resistant-*Escherichia coli* from Chicken Meat Produced by Integrated Broiler Operations in Korea

Kwang Won Seo and Young Ju Lee*

College of Veterinary Medicine, Kyungpook National University

Vertical integration of the broiler industry allows producers to combine different biosecurity and sanitation practices, housing technologies, and feeding regimens to improve food safety. This study was to determine the genetic characterization of ciprofloxacin resistant-*Escherichia coli* recovered from 7 different integrated broiler operations in Korea. Among the 157 *E. coli*, 75 (47.8%) were variation observed in various broiler operations as ciprofloxacin resistant-*E. coli*. Among the 75 ciprofloxacin resistant-*E. coli* isolates, 10 isolates showed plasmid-mediated quinolone resistance (PMQR) gene (*aac(6')-Ib-cr*, *qnrS1* and *qnrB4*) and a double amino-acid exchange in both *gyr*A and *par*C with ciprofloxacin minimum inhibitory concentrations of ≥ 16 μg/ml. Four transconjugants (40.0%) expressed similar antimicrobial resistance patterns and revealed the presence of PMQR/β-lactamase genes. Our findings suggest that *E. coli* with resistance to ciprofloxacin can now be found in association with integrated broiler operations, thus highlighting the need for monitoring and prevention programs in integrated operations.
[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Research Center Support Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (716002-7).]

Keywords: *Escherichia coli*, Integrated operation system, Fluoroquinolones, Plasmid-mediated quinolone resistance, Chicken meat

I029

Characterization of Multidrug-resistant of High-level Aminoglycoside Resistant *Enterococcus faecalis* and *Enterococcus faecium* Isolated from Retail Chicken Meat

Yeong Bin Kim and Young Ju Lee*

Department of Public Health, College of Veterinary Medicine, Kyungpook National University

Enterococci are considered as opportunistic pathogens mainly responsible for nosocomial infection in humans and many types of infection in animals such as mastitis in cattle, diarrhea in swine and cattle, and septicemic diseases in poultry. This study was to investigate the genetic characterization of high-level aminoglycoside resistant (HLAR) 28 *E. faecalis* and 1 *E. faecium*. Among 345 *Enterococcus* spp. from 200 chicken meat samples, 136 (39.4%) isolates showed multidrug resistance, that also involved 29 HLAR isolates. All HLAR *Enterococcus* spp. harbored the *aac*(6′)-*Ie-aph*(2″)-*Ia* including IS 256 flanking pattern A (3 isolates) or D (10 isolates), and/or *ant*(6)-*Ia*. The efflux pump genes, *efr*(A), *efr*(B), *emeA* and *lsa*, were also detected in all HLAR *E. faecalis* isolates. The most prevalent of resistance genes were *tet*(M) (69.0%) and *ermB* (65.5%), and *ace* (86.2%), *efaA* (86.2%) and *gelE* (82.8%) in virulence genes. Although a considerable amount of information regarding HLAR enterococci from chicken industry have not exist, these results revealed that HLAR enterococci showed the multidrug resistance and various genetic characteristics related with antimicrobial resistance.
[This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agriculture, Food and Rural Affairs Research Center Support Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (716002-7).]

Keywords: *Enterococcus faecalis*, *Enterococcus faecium*, Chicken meat, High-level aminoglycoside, Antimicrobial resistance

I030

Taxonomic Study of *Monascus* Species in Korea

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Monascus species are filamentous fungi that have been used for fermented foods such as red fermented rice for thousand years in Eastern Asia. They are known as producers of various secondary metabolites, useful bioactive compounds such as monacolin K, GABA, pigments and the mycotoxin such as citrinin. In this study, we have collected 15 strains of *Monascus* from various sources such as Meju, soybean paste and red fermented rice. And we also introduced 37 reference strains from CBS in the Netherlands and KCCM, KCTC in Korea. RDNA-ITS, β-tubulin, calmodulin, LSU and RPB2 genes were used for phylogenetic analysis, and almost Korean *Monascus* strains were identified as *Monascus ruber*. One strain that have been used for producing red fermented rice in Korea was identified as *Monascus purpureus*. We are also analysing production of their secondary metabolites such as monacolin K, GABA, pigments and citrinin.

Keywords: Filamentous fungi, *Monascus*, Red fermented rice, Monacolin K, Citrinin

I031

Determining the Probiotic Potential of Cholesterol-reducing *Lactobacillus* and *Weissella* Strains Isolated from Korean Traditional Fermented Food

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Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Recommended properties for a probiotic microbe include survival of the gut, persistence in the host, and proven safety for human consumption. Lactic acid bacteria (LAB) are beneficial microorganisms for the gut health in human as well as animals when consumed. LABs are frequently found in the human intestinal tract. We isolated to evaluate the probiotic potential of LAB isolated from Korean traditional fermented food. A total of 153 LAB strains were isolated, among which five were identified as *Lactobacillus* spp. and one as *Weissella* spp. The strains were screened for their probiotic potential. Isolation of *Weissella koreensis* BSS10 from fermented kimchi was carried out in order to study *Weissella koreensis* BSS10 as probiotics. All isolated *Lactobacillus* and *Weissella* strains were capable of surviving under low pH and bile salt conditions. *Weissella koreensis* BSS10 and *Lactobacillus plantarum* HS729 were able to survive at pH 3.0 and 0.50% bile salt for 3 h without losing their viability. Furthermore, *Weissella koreensis* BSS10 and *Lactobacillus plantarum* HS729 exhibited maximum cholesterol reduction with bile salts.
[Supported by grants from Ministry of Science and ICT (grant No. 2018-JB-RD-0009-01-101)]

Keywords: *Weissella koreensis* BSS10, *Lactobacillus plantarum* HS729, Cholesterol-reducing bacteria, Probiotics

I032

Antimicrobial Resistance of *Enterococcus faecalis* and *faecium* Isolated from Cattle Farm, Slaughterhouse Samples and Beef in Markets in South Korea

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Infection caused by multi-drug resistant bacteria is a serious problem. Infectious bacteria from food system also followed up fast the drug resistance associated community, healthcare centers and livestock. This is countinous problem. Epidemiological study for unveiling the route for spreading antimicrobial resistant bacteria and developing prevention methods are necessary. In this study, we collected a total of 1,611 farm environment samples from South Korean cattle farm, slautherhouses and the beef from commercial market. 294 *Enterococcus faecalis,* 50 *Enterococcus faecium* were isolated from the samples. Disk diffusion test and E-test strip were performed for evaluating the antimicrobial resistance. Resistance of *Enterococcus faecalis* and *faecium* was the highest in tetracycline (40.1%). There was no vancomycin, teicoplanin, linezolid and tigecycline resistance. For evaluating the epidemiological relationship, we divided our isolates as three groups, livestock-, environment-, and human-origin. the resistant rate of human-origin group were highest than other groups. Our results offered useful data for evaluation the expected risk to Korean desease control authority for public health care.

Keywords: Antimicrobial resistance, *Enterococcus faecalis*, *Enterococcus faecium*, Beef, Cattle

I033

Characterization of a Bacteriocin from *Lactococcus lactis* Presenting Antimicrobial Activity against *Stapylococcus aureus*

Jung-Mo Yang and Gi-Seong Moon*

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A *Lactococcus lactis* was isolated from kimchi and identified by 16S rRNA gene sequencing. The crude bacteriocin showed antimicrobial activity against several *Staphylococcus aureus* strains. The bacteriocin was acidic and the activity was abolished by a protease treatment. Its stability was maintained under pH 2.0–10.0 and 100°C for 30 min. The stability also maintained under various organic solvent treatments such as methanol, ethanol, acetone, acetonitrile, chloroform. Finally the bacteriocin showed bactericidal action mode where 200 AU of the bacteriocin decreased the viable cell number by 2 log scale. At the moment the crude bacteriocin is subjected to purification process and the biochemical characterization would be performed.
[This research (grant no. 117060031HD040) was supported by High alue-added Food Technology Development Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea]

Keywords: Bacteriocin, *Lactococcus lactis*, *Staphylococcus aureus*, Kimchi, Bactericidal

I034

Characterization and Application of *Leuconostoc mesenteroides* Isolated from Breast Milk

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A *Leuconostoc mesenteroides* was isolated from breast milk and identified by 16S rRNA gene sequencing. The strain was further confirmed to be *Leu. mesenteroides* via production of dextran using sucrose. The cells were resistant to pH 4.0 until 4 h but resistant to pH 3.0 for only 2 h. In addition, the cells were resistant to 0.5% bile salt in MRS broth. It presented antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* which are reside in skin epidermal surface when the cell cultures were used. The strain was applied to several hot-water extracts of Annual Wormwood, Carrot, Corn silk, Green tea, etc. and we optimized the fermentations to produce dextran. In near future, these fermentations would be tested for feasibility as cosmetic biomaterials.
[This was supported by Korea National University of Transportation in 2018]

Keywords: *Leuconostoc mesenteroides*, Breast milk, Dextran, Hot-water extract, Natural ingredients

I035

Construction of Efficient Database for Antimicrobial Resistance Genes Detection and WGS Analysis

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At the beginning of the 20th century, the development of antibiotics was a turning point in modern medicine. By using antibiotics, "simple infection", which took the lives of people at the time, was easily cured. The ability of antibiotics to slow the infection has made it possible to perform a variety of surgical procedures as well as "chemotherapy" for organ transplantation and cancer treatment. However, the effects of antibiotics are decreasing. In recent years, infections with strains resistant to antibiotics have become very common, and in recent years, such Antibiotic resistant strains in Korea have caused various problems such as the death of newborn babies. All these cases have a warning message about Antibiotic resistant strains. There is also a prospect that by 2050 more deaths will come from cancer. Therefore, it is very important to prevent the spread of Antibiotic Resistant genes.
Our study has established a major database that can detect variant genes such as VRSA, VRE, and MRSA. We have constructed a database that can detect more Antibiotic resistance genes by combining the five major variants of WGS obtained from NCBI and the existing database. Through these WGS studies, it is possible to detect rapidly spreading Antibiotic resistant strains and to analyze the pathogenicity and AMR of existing microorganisms. Single Nucleotide Polymorphism analysis is also possible based on this database.

Keywords: Antibiotic resistance, MRSA/VRSA, MRE/VRE, Pathogenic bacteria, Database construction, Single Nucleotide Polymorphism analysis, Vancomycin, Methicillin

I036

Development of Multiplex Detection Method for Methicilin & Vancomycin Antibiotics Resistance Genes Using Real-Time PCR in Genus *Staphylococcus* and *Enterococcus*

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Increasing antibiotic resistance in pathogenic bacteria is a serious public health concern, notably in industrializing countries. Therefore, a technique for controlling Antibiotic Resistance in pathogenic bacteria is needed. In this research, we have developed a multiple diagnostic kit that can simultaneously detect various antibiotic resistance genes using SYBR green real-time PCR assay. A SYBR green real-time PCR assay was designed by targeting vancomycin and methicillin resistance genes. An evaluation of this assay was performed using reference strains of 46 pathogenic bacteria. Out of them, 8 strains were reported to be resistance to vancomycin and 7 strains were reported to be resistance to methicillin. We report here the development of a particular method composed of the set of real-time PCR for the simultaneously rapid detection of the most frequently encountered Vancomycin & Methicillin Resistance.

Keywords: Antibiotic resistance, MRSA/VRSA, MRE/VRE, SYBR green real-time PCR assay, Pathogenic bacteria, Multiple diagnostic kit, Vancomycin, Methicillin

I037

A Comparison of Enrichment Methods of Enterohemorrhagic *E. coli*

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Enterohaemorrhagic *Escherichia coli* (EHEC) is a pathogen of worldwide importance causing foodborne infections in human. There are various culture methods commonly used for optimal growth of EHEC. This study evaluated different enrichment methods provided by several reliable resources to compare their enrichment efficacy for EHEC growth.

In this study, four culture conditions such as TSB at 35℃ and 37℃, BHI at 37℃ and TP broth at 42℃ were selected and incubated for 9 h. The strain used in the experiment is NCCP-15739 EHEC. The EHEC was spread on PCA plate using easySpiral® Dilute (Interscience, France) and incubated at 37℃ for 24 h. Bacterial cell counting was performed using Scan 4000 (Interscience, France) and the CFU was automatically calculated.

When the initial number of cell was about 2.5 log CFU/ml, the number of cells could be increased up to 5 log CFU/ml after 4 h of incubation. After 8 h of incubation, the cell number reached upto 8 log CFU/ml, which can be thought to have been fully grown. The fastest culture time to 8 log CFU/ml was shown in culture of TP broth at 42℃. However, there was no difference in growth rate between TSB at 35 and 37℃.

Therefore, it is confirmed that the clear detection of EHEC could be found in initial 4 h of incubation. Thus, the cultivation on TP broth at 42℃ is the fastest for rapid detection of EHEC.

Keywords: Enterohemorrhagic *E. coli*, EHEC, Enrichment methods

J001

Establishment of Anti-venom National Reference Standard for Potency Test of Freeze-dried Gloydius (Salmusa) Antivenom

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In 2017, the first candidate for the NRS of Gloydius snake anti-venom was newly produced. This study is to establish the potencies of the candidate material as the first NRS in Korea. The potencies of the candidate were determined by measuring the anti-lethal titers and anti-hemorrhagic titers calibrated against the RWRS (anti-lethal titer: 32,000 U/vial, anti-hemorrhagic titer: 36,000 U/vial) for anti-venom using the test methods described in the previous report for the establishment of RWRS of Gloydius snake anti-venom at the NIFDS. The anti-lethal titer expressed in terms of the number of 50% effective doses (ED50) was calculated using the Reed-Muench method. The anti-hemorrhagic titer was expressed in terms of the size of the hemorrhagic spots (average cross-diameter of 10 mm). The results were determined relative to that of the standard anti-venom (RWRS). The general common potencies of the anti-lethal and anti-hemorrhagic titers were obtained from the test results of 10 times each performed at the NIFDS. The results are expressed in units (U) with a 95% confidence interval. The anti-lethal titer was 2,909 U (95% confidence interval: 2,844-2,973) and anti-hemorrhagic titer was 2,765 U (2,632-2,897). After verifying the quality and stability, the stated potencies of the candidates for using as the NRS shall be determined with further collaborative study with at least 2 participants including the NIFDS. [This research was supported by a grant from 18201MFDS201 in 2018.]

Keywords: Anti-venom, Potency, Anti-lethal titer, Anti-hemorrhagic titer

J002

Influence of Cell Surface Hydrophobicity on Adhesion and Biofilm Formation in *Candida albicans* and Several Bacterial Species

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The purpose of this study is to investigate the correlation of cell surface hydrophobicity (CSH) and biofilm formation or adhesion in *Candida albicans* and several pathogenic bacteria. All of *Candida albicans* (n=82) and 7 bacterial species (*Escherichia coli*, n=25; *Klebsiella pneumoniae*, n=33; *Morganella morganii,* n=21; *Proteus mirabilis,* n=33; *Proteus vulgaris,* n=12; *Pseudomonas aeruginosa,* n=31; *Staphylococcus aureus,* n=31) were isolated clinically. CSH was quantified with microbial adhesion to hydrocarbons. Biofilm formation was determined by tetrazolium salt reduction assay. Adhesion assay was performed by counting colonies after culture the microbes adhered to HeLa cells. Although high CSH-expressing bacterial species showed greater adherence to HeLa cells and larger amounts of biofilm formation on polystyrene, the significant relationships within same species were not shown. In *C. albicans*, however, strong positive correlations were observed between CSH and biofilm formation ($r=0.708$; $p < 0.05$) or cell adhesion ($r=0.509$; $p < 0.05$). These results suggest that hydrophobic force of bacteria may play a minor role in adhesion and biofilm formation, but CSH of *C. albicans* may be an important factor for adherence on surface and biofilm forming process.

Keywords: Cell surface hydrophobicity, Biofilm formation, Cell adhesion, *Candida albicans*

J003

Difference of Biofilm Formation Activity between Hydrophilic Isolates and Hydrophobic Isolates in Several Microorganisms

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Cell surface hydrophobicity (CSH) of microorganisms plays a crucial role in the attachment to surface and CSH positively correlate biofilm formation. To examine the correlation of between CSH and biofilm formation in several microorganisms, the differences of biofilm formation activity between hydrophilic isolates and hydrophobic isolates were investigated. The *Candida albicans* (n=20) and 6 bacterial species (*Escherichia coli, Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*; n=10, respectively) were isolated clinically. CSH was evaluated by the microbial adhesion to hydrocarbon and the amount of biofilm formation was measured by XTT reduction assay. Hydrophilic and hydrophobic isolates obtained from aqueous phase and hydrocarbon phase, respectively. Biofilm formation activities of isolates from hydrophilic or hydrophobic phase were similar. So the significant correlation of between CSH and biofilm formation activity of isolates was not observed.

Keywords: Cell surface hydrophobicity, Biofilm formation

J004

The Relationship between Dyslipidemia and Health Variables of Korean Postmenopausal Women as Investigated with the 2016 National Health and Nutrition Examination Survey

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This study investigated demographic, anthropometric, and hematologic of postmenopausal women suffering from dyslipidemia, to identify the factors that increase the risk of developing the disease. The principle data were collected from the results of a health questionnarie administered in 2016 to 604 middle-aged and 609 elderly postmenopausal women. A number of factors were important to the incidence of postmenopausal dyslipidemia, as follows: obesity, hypertension, diabetes mellitus, subjective health status and depression. Middle-aged women suffering dyslipidemia were associated with hypertension, diabetes mellitus, and subjective health status; dyslipidemia in elderly women was linked to education level, marital status, obesity, hypertension, and depression. In addition, results showed that specific factors known to effect the incidence of dyslipidemia were related to the occurrence of the disease in the postmenopausal women, and included waist circumference, body mass index, and glucose level. The mean waist circumference was evidenced to be significantly higher in the middle-aged than the elderly women, BMI was significantly higher in the elderly than the middle-aged, whereas fasting glucose was significantly related to the incidence of dyslipidemia in both the elderly and middle-aged groups. Therefore, there is a necessity to reduce dyslipidemia risk in postmenopausal women by treating the aforementioned factors before and after menopause.

Keywords: Postmenopausal, Dyslipidemia, Middle-aged, Elderly

J005

Masks Usage and Awareness Regarding Wearing Masks in General Population

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This study was conducted to emphasize the needs of using masks as a preventive measure of acute respiratory infections by examining the overall use of masks and investigating public awareness about the masks. We used the data of 862 questionnaires, collected from November 2016 to February 2017, targeting students of elementary schools and universities, office workers, members of churches and subway passengers from Seoul and Gyeonggi-do province. For data analysis, descriptive statistics and x^2-test analysis were used. We found out that 16.5% of the respondents had experiences spreading common cold by coughing and contacting people with colds on public transportation or places. Also we found out that 50.9% of the respondents recognized the needs for wearing masks to avoid catching a cold. However, only 20.9% of the respondents used the masks when they were sick. We discovered that people who did not wear masks properly had the discomfort, such as 'suffocation' (36.6%) and low risk perception of acute respiratory infections including 'laziness' (26.0%) and 'it would be okay not wearing masks' (23.0%). In case of having respiratory symptoms, the public should be well aware of using masks in crowded places to reduce societal and economic losses caused by acute respiratory viruses.

Keywords: Acute respiratory infectious disease, Common cold, Preventive adhere, Risk awareness, Wearing masks

J006

Resistome Analysis of Carbapeneme-resistant *Klebsiella pneumoniae* Using Whole-genome Sequencing

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Carbapenem-resistant Enterobacteriaceae is great concern to public health. Rapid diagnosis of antibiotic resistance is essential for successful treatment of bacterial infection. With the advance of affordable whole-genome sequencing (WGS), researchers have tried phenotype prediction without labor-intensive test such as disk diffusion and broth dilution method using WGS. In this study, we aimed to identify the whole antibiotic resistance genes and evaluate accordance between phenotype and genotype in 19 carbapenem-resistant *Klebsiella pneumonia* isolates. Resistome analysis using Resfinder 3.0 revealed that all isolates had various resistance genes related with beta-lactams, carbapenems, aminoglycosides, fluoroquinolones, and phenicols. Minimum inhibitory concentrations were determined using sensititer and microscan panels with 36 antibiotics. Most of resistance genes detected were corresponding with their phenotypes. However, there were the discrepancies between genotype and phenotype of the three antibiotics in two isolates. Further study should be followed to investigate these discordances between antibiotic resistance phenotype and genotype. Elucidation on discordances allow to establish reliable resistant phenotype prediction model using WGS
[This study was supported by a grant of the Korea Centers for Disease Control and Prevention (2018-NI005-00).]

Keywords: Resistome, Carbapenem resistance, *Klebsiella pneumonia*

J007

A Rapid System for a Fungal Promoter Analysis Using the Phosphopantetheinyl Transferase Gene *npgA* in *Aspergillus nidulans*

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To develop a convenient promoter analysis system for fungi, we utilized a null-pigment mutant (NPG) of *Aspergillus nidulans* with 4'-phosphopantetheinyl transferase (PPTase) gene *npgA,* which restores no pigment phenotype in *A. nidulans,* as a new reporter gene. The functional organization of serial deleted promoter regions of *A. nidulans trpC* gene and *Cryphonectria parasitica Crp* gene in filamentous fungi were representatively investigated for the establishment of a novel fungal promoter assay system, depending on color complementation of the NPG mutant with the PPTase *npgA* gene. Several promoter regions in each of two *trpC* and *Crp* genes were fused to the *npgA* gene, which contained the 1,034 bp open reading frame and the 966 bp 3' downstream region from the TAA, and the constructed fusions were introduced into the NPG mutant in *A. nidulans* to evaluate the color recovery due to transcriptional activity of sequence elements. Serial deletion of the *trpC* and *Crp* promoter regions in this PPTase reporter assay system reaffirmed results in previous reports by fungal transformation step at once without more laborious verification process. This approach represents more rapid and convenient system than the conventional analyses in fungal gene expression studies.

Keywords: *Aspergillus nidulans*, npgA, Promoter assay

J008

Cultural and Morphological Characteristics of a New White Button Mushroom Cultivar 'Dodam'

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The button mushroom, *Agaricus bisporus*, was one of the most widely cultivated mushrooms. The domestic production of cultivated mushrooms was almost 10,173 tons with a total value of 59 billion won in 2016. Currently, 10 cultivars have been developed, but farmers continue to demand new cultivars of mushrooms of improved quality. The newly developed 'Dodam' cultivars are crossed with s1225-16 and s1246-46. These homokaryons are selected by SSR45 marker. After breeding 'Dodam', the cultivar was confirmed productivity and stability through cultivation in mushroom farm. Mycelium of 'Dodam' on CDA grew well at 15, 20 and 25℃ when it was compared with that of 'Saedo'. This variety can be started fruiting and grew well at 13–18℃. The diameter and thick of mature cap and thick of stipe was longer than control, 'Saedo'. The fruiting body was harder than the control as 4.22±0.2 in cap and 4.4±0.4 in stipe. The yield of mushroom per surface unit (box) increased by 4 times than the control. The button mushroom reached 44.2% of the domestic distribution rate in 2017. The spread of domestic mushroom cultivars will be further expanded by early distribution of new cultivar 'Dodam'.
[Supported by grants from the international cooperation research project 'Development of the breeding technology and molecular markers based on mushroom genome' (PJ0120562018), RDA, Republic of Korea.]

Keywords: Breeding, Button mushroom, New cultivar

J009

Availability of Natural Extracts as Oral Health Food Additives

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The purpose of this study was to determine if natural extracts could be used as an additive in oral health food made with *Weissella cibaria* CMU (oraCMU). Green tea, propolis, licorice, and mulberry leaf, which are reported to have antimicrobial activities, were selected and used in this study. The minimum inhibitory concentrations (MIC) of extracts on periodontal pathogens *Fusobacterium nucleatum* and *Porphyromonas gingivalis* as well as their synergy effects with oraCMU by the fractional inhibitory concentrations methods were measured. As a result, all the extracts showed no effect on the growth of oraCMU. Green tea extract showed the best MIC of 1.8 mg/ml against both *F. nucleatum* and *P. gingivalis*. In addition, green tea extract had a synergistic effect with oraCMU on *F. nucleatum* not on *P. gingivalis*. Therefore, these results suggest that green tea extract is available as an oral health food additive.
[Supported by the Technology development Program(C0564353) funded by the Ministry of SMEs and Startups (MSS, Korea)]

Keywords: *Weissella cibaria*, Probiotics, Antimicrobial activity, Oral care, Oral pathogens

J010

Evaluation of ELISA for Cross-sectional Antibody Kinetics of Patients with MERS-CoV Infection

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Middle East respiratory syndrome coronavirus (MERS-CoV) spreads to humans and causes severe acute respiratory disease. Most recently, MERS-CoV outbreak occurred in South Korea in 2015 resulted in 186 patients including 38 deaths.
Of the various serological methods used for the detection of antibodies against MERS-CoV, indirect ELISA against recombinant MERS-CoV nucleocapsid (N) and spike 1 (S1) proteins were commonly used to detect the antibodies specific to MERS-CoV as screening tests. We conducted indirect ELISA followed by confirmatory serum neutralization test to evaluate serological evidence of MERS-CoV infection.
In this study, we developed an indirect ELISA for emerging infectious disease like MERS using cell -free protein expression system as this system has an advantage of rapid protein expression as compared to cell system. We optimized the method using the interactions between recombinant proteins and divalent cations to ELISA. The performance of the ELISA was validated using 108 MERS-CoV negative and 92 positive human sera by plaque reduction neutralization test (PRNT). Both MERS-CoV N and S1 ELISA assays showed high correlation and had higher sensitivity for patient samples compared to commercial MERS-CoV ELISA kits and serum neutralization tests. The ELISA assays using recombinant cell-free expression system-producing MERS-CoV N and S1 proteins have been developed and validated for the rapid serological diagnosis of MERS-CoV.
[Supported by grants from KNIH]

Keywords: MERS-CoV, N protein, S1 protein, Cell free protein expression, ELISA

J011

The Bacterial Predator *Bdellovibrio bacteriovorus* is Very Sensitive to Detergents

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Bdellovibrio bacteriovorus is a predatory bacterium which can attack and predate a wide range of Gram-negative bacteria, including many human pathogens. In a previous study, we demonstrated that predatory bacteria are not harmful to human cells and that they actually protect them from pathogens. However, certain chemicals, such as indole, negatively impact predation. Here, we studied the impact of different detergents on the viability and activity of *B. bacteriovorus* HD100. For this, several different detergents were selected based upon their chemical nature, *i.e.*, cationic, anionic, zwitterionic and neutral, including Triton X-100, SDS and sodium deoxycholate (SDC). We found all of these detergents were more toxic for the predatory strain, compared to its prey *i.e.*, *E. coli* MG1655/pUCDK. For instance, we found *E. coli* was still viable in 16% SDS for up to 3 hours, while a concentration of only 0.02% SDS was sufficient to inhibit predation. Similar results were also obtained with Triton X-100, with the predatory strain being much more sensitive to the detergent than its prey. These results suggest that detergents can be used to selectively control, and potentially even halt, bacterial predation as it occurs.
[This study was supported by the General Research grant as funded under the National Research Foundation of Korea and under the Space Core Technology Development Project]

Keywords: Detergent, Predatory bacteria, Predation

J012

Functional Role of CDC11/AspA Ortholog, Septin Gene, *CpSep1* in *Cryphonectria parasitica*

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We identified a protein spot showing down-regulation in the presence of Cryphonectria hypovirus 1 (CHV1) and tannic acid supplementation as a septin subunit with the highest homology to the *Aspergillus nidulans aspA* gene, an ortholog of the *Saccharomyces cerevisiae Cdc11* gene. To analyze the functional role of this septin gene (*CpSep1*), we constructed its null mutant. All *CpSep1*-null mutants showed retarded growth with less aerial mycelia and intense pigmentation on PDAmb plate. Conidia production of the *CpSep1*-null mutants was significantly at least ten-fold more increased. Interestingly, conidial morphology of the *CpSep1*-null mutants changed to be circular in comparison to the rod-shaped spores of the wild-type. We visualized the actin cytoskeleton from a spore to young hyphae using a specific antibody. In the wilde type strain, apical fluorescent patches were observed at the tips of the actively growing young hyphae. However, in the CpSep1-null mutants, the location of actin patches were observed subapical, instead of apical. Virulence assays using excised chestnut bark and on apple, and stromal formation on chestnut stems indicated that the *CpSep1* gene plays an important role in pathogenicity. This study indicated that the *CpSep1* gene is required for an appropriate mycelial growth and pigmentation, spore morphology, actin patches location and fungal growth in the host plant and virulence.
[Supported by NRF]

Keywords: cdc11, Septin, Hypovirulence, *Cryphonectria parasitica*

J013

A Novel Bipartite Double-stranded RNA Mycovirus from *Trichoderma atroviride*: Molecular Characteristics of a Novel Partitivirus of *Trichoderma atroviride* NFCF394

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Increasing number of novel mycoviruses have been described in fungi. Here we report on the molecular characteristics of a novel bisegmented double-stranded RNA (dsRNA) virus encompassing dsRNA-1 (2,023 bp) and dsRNA-2 (1,816 bp), which was isolated from the fungus *Trichoderma atroviride* NFCF394, designated *Trichoderma atroviride* partitivirus 1 (TaPV1). The larger segment (dsRNA1) of the TaPV1 genome comprised 2,023 bp and contained a single open reading frame (ORF) encoding a RNA-dependent RNA polymerase (RdRp). The smaller segment (dsRNA2) consisted of 1,816 bp with a single ORF encoding capsid protein (CP). The 5'- and 3'-untranslated regions in each dsRNA segment contained sequences that were strictly conserved at the termini of *Alphapartitivirus*. Evaluation of the deduced amino acid sequence and phylogenetic analysis indicated that TaPV1 is a new member of the genus *Alphapartitivirus* in the family *Partitiviridae*. Curing of virus infection by single-sporing generated three virus-free single-spore clones. To our knowledge, this is the first report of an *Alphapartitivirus* in *T. atroviride*.

Keywords: Mycovirus, *Trichoderma atroviride*, Alphapartitivirus, Partitiviridae

J014

Heterologous Expression of *Trichoderma atroviride chit36* Gene for the Expression of Chitinase in *Saccharomyces cerevisiae*

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Since the genetic manipulation of useful fungus as *Lentinula edodes*, which have high industrial value, is an untapped field in domestic and foreign countries, it is necessary to improve a protoplasting yield for a successful transformation. However, it is difficult to decompose cell wall of filamentous fungus and produce transgenic cells. Therefore, we studied to increase the efficiency of the protoplast of the fungus through heterologous expression of the degrading enzyme, chitinase, for solution of the gene manipulation in fungi, and to utilize the heterologous expressed chitinase enzyme in other industrial fields such as microbiological control. Therefore, the present study investigated the heterologous expression of the functional chtinase36 using a yeast *Saccharomyces cerevisiae,* which is harmless, environmentally friendly, and manipulated. The *chit36* gene of filamentous fungus *Trichoderma atroviride* was obtained using RT-PCR method, and finally inserted into pYEGPD-TER, a yeast transformation episomal vector for mass production of external protein. The *chit36* gene was fused with the rice amyl signal peptide gene, which is known to secrete protein secretion from the yeast into the secretion signal. The CHIT36 transformants were verified by yeast colony PCR. In addition, we confirmed the successful heterologous expression of chitinase by real-time PCR and chitinase enzymes assay methods.

Keywords: *Trichoderma atroviride chit36*, Heterologous expression, GRAS, *Saccharomyces cerevisiae*

J015

Bacterial Community Composition in the Gastrointestinal Tracts of Elk (*Cervus canadensis*)

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National Institute of Animal Science, Rural Development Administration

Ruminants have a large and complex gastrointestinal tract (GIT) and derive most of their energy requirements through breakdown of structural carbohydrates by fibrolytic bacteria. Most microbial community studies of deer gut have focused on rumen or feces, while data about the bacteria present in the different intestinal compartments of elks are sparse. In this study, high throughput sequencing was used to characterize and compare bacterial profiles from different intestinal compartments of four elks (*Cervus canadensis*). Eight distinct GIT segments including the stomach (rumen, omasum, and abomasum), small intestine (duodenum and jejunum), and large intestine (cecum, colon, and rectum) obtained from four elks were examined. We found that bacterial richness and diversity were higher in the stomach and large intestine than in the small intestine ($P < 0.05$). The study observed 733 genera belonging to 26 phyla distributed throughout the elk GITs, with the Firmicutes, Bacteroidetes and Proteobacteria predominating. In addition, data revealed significant spatial heterogeneity in composition, diversity and species abundance distributions of GIT microbiota ($P < 0.0001$). To the best of our knowledge, this is the first study to characterize bacterial communities from eight GIT regions of elk by 16S rRNA pyrosequencing.
[This work was supported by the Cooperative Research Program for Agriculture Science and Technology Development (Project no. PJ0119930).]

Keywords: Gastrointestinal tract, Elk (*Cervus canadensis*), 16S rRNA pyrosequencing, Bacterial community

J016

Selection of Entomopathogenic Fungi for Microbial Control of Plant Disease and Pest

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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 ecofriendly. This study aims to select isolate have insecticidal and fungicidal activity against pathogens causing anthracnose and thrips. Among 34 strains of entomopathogenic fungi (isolated from soil using insect-bait method) FT333 showed high inhibition activity against *Colletotrichum acutatum*. The study showed insecticidal activity of strain FT333 with mortalities of 76.7% against thrips respectively. The strain having strong insecticidal and fungicidal activity can be considered as potential candidates for biological control of red pepper enemies.

Keywords: Entomopathogenic fungi, Phytophthora blight, Anthracnose disease, Dual culture, Thrips

J017

Anti-microbial Lactic Acid Bacteria from Kimchi, and Its Application

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Nowadays, the effect of probiotic lactic acid bacteria (LAB) on maintenance of human health is increasingly reported, in addition to its usage for the human being, some LAB could be used for the improvement of the quality of food ingredients. Especially the LAB possessing anti-pathogenic bacterial activity and/or anti-fungal activities are prominent candidates for prevention of food. In this study homemade Kimchi samples, not fermented over seven days after mixing of each ingredient, were collected and the liquid part of each sample was serially diluted with sterile saline and spreader on BCP plated. After cultivation of the plate, acid-producing bacterial colonies on the plate were selected as bacterial isolates from Kimchi. The isolated LAB were confirmed its anti-pathogenic microbial activity by observing the formation of in inhibition zone. Among isolates from Kimchi, five isolates were represented anti-pathogenic bacterial activity and inhibited the growth of *E. coli* and *Ralstonia solanacearum*, a pathogen causing bacterial wilt of ginger. Among tested fungi, the growth of *Sclerotium rolfsii*, *Aspergillus* sp. and *Penicillium* sp. were inhibited by a few Kimchi LAB. The isolated LAB was identified with 16S rRNA gene sequence analysis as *Lactobacillus* species.

[Supported by IPET through Agri-Bio industry Technology Development Program, funded by MAFRA, 117118-01]

Keywords: Anti-fungal activity, Anti-pathogenic bacterial activity, Kimchi, Fermented food, Food preservation

J018

Comparison of Peaks for MALDI-TOF Mass Spectrometry According to Various Commercial Blood Agar Plates for Beta-hemolytic *Staphylococcus aureus*

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MADLI-TOF MS allowed easy and rapid analysis for the identification of *Staphylococcus aureus* in clinical laboratory. Distinguishing various phenotypes by MALDI-TOF MS is helpful for pathogenesis evaluation. We investigated the MALDI-TOF peaks of *S. aureus* in the different commercial blood agar plate (BAP) to find out whether specific MALDI-TOF peaks of *S. aureus* are better distinguished according to different nutrients. *S. aureus* ATCC 43300 was cultivated on the three kinds of commercial media with different nutrient source such as heart muscle, tryptone and yeast extract (media A)/ tryptone and soybean meal (B)/ beef extract and tryptose (C). After 18 h of incubation, colonies were analyzed by MALDI-TOF MS and peak lists were investigated using the Mass-Up software. Three individual groups are analyzed 10 replicates. The 33 peaks were to be found common peaks. Three individual group showed characteristic peaks of 1 peak in media A, 8 peaks in media B, and 5 peaks in media C, respectively. Also, the hemolysis-related peaks appeared more prominently in the rich tryptone and soybean meal containing compositions. We demonstrated that characteristic peaks of beta-hemolytic *S. aureus* cultured on tryptone and soybean meal rich conditions are distinguished against the other media. Culture conditions for MALDI-TOF MS analysis should be adjusted to investigate individual peaks for hemolytic phenotype of *S. aureus*.

[Supported by grant from KHIDI (HI17C2067)]

Keywords: *S. aureus*, MALDI-TOF MS, Blood agar plate

J019

Evaluation *in vitro* Probiotics Properties of Lactic Acid Bacteria Isolated from Piglet

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Probiotics are known to control the balance of gut microbiota and beneficial to host animal health in multiple ways. They are considered to be the efficient feed additives to stimulate local immune responses in pig and have been used as feed additives for replacement low dose in-feed antibiotics. Lactic acid bacteria are well known for their probiotic potential. In the present study, the probiotic function such as acid resistant and bile resistant ability of lactic acid bacteria, *Lactobacillus mucosae* KP-5 and *L. reuteri* KP-2 isolated from piglet feces were investigated. Antibiotics, ampicillin, chloramphenicol, gentamicin, kanamycin and tetracycline susceptibility test were carried out with these isolates. In addition, antimicrobial activity of *L. mucosae* KP-5 and *L. reuteri* KP-2 were tested against livestock pathogens, *Escherichia coli*, *Salmonella* spp. *Yersinia* spp. and *Candida* spp. Our finding demonstrate that *L. mucosae* KP-5 and *L. reuteri* KP-2, intestinal *Lactobacillus* species associated with piglet health, possesses the probiotic properties and are worth investigation as feed additives.

[This work was supported by a grant from the Next-Generation BioGreen 21 Program (Project No. PJ01322304), Rural Development Administration, Republic of Korea.]

Keywords: Piglet, Probiotics, *Lactobacillus*, Feed additives, Acid resistance

J020

In vitro Validation of Efficacy of Microorganisms for Feed Additives

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Probiotics/prebiotics are considered as replacement of antibiotics for better performance parameter like body weight gain in livestock. Probiotics are known as viable microorganisms which lead to beneficial effects for the host animal by modifying the gut microbiota. Therefore the probiotics microorganism must have the properties like as acid resistance and bile resistance. In this study, *in vitro* efficacy test was carried out for microorganisms as feed additives. Survival of the probiotic test was conducted in condition under pH 2.5 and 0.3% oxgal with various strains obtained from feed additives production companies in Korea. Enzyme activities such as amylase and protease activities were also conducted with those strains. Antimicrobial activity of those strains were tested against livestock pathogens, *Escherichia coli*, *Salmonella* spp. *Yersinia* spp. and *Candida* spp. In addition, livestock order reduction test were carried out in small scale. Bacterial strain DS_8 reduced NH_3, H_2S, CH_2NH_2 gas in pig sludge by 43.33%, 74.76%, 44.23% respectively. Our study shows that *in vitro* efficacy testing of microorganisms helpful in the investigation of probiotics as feed additives.

[This work was supported by a grant from the Next-Generation BioGreen 21 Program (Project No. PJ01322304), Rural Development Administration, Republic of Korea.]

Keywords: Efficacy, Microorganisms, Probiotics, Feed additives

J021

Genomic Characteristics of a Potential Probiotic Bacterium *Bifidobacterium* sp. CIA01 Isolated from a Dog Feces

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Bifidobacterium genus are common healthy gut microbiota in the gastrointestinal tract of mammals and widely acknowledged as probiotics with health-promoting properties. *Bifidobacterium* strain CIA01 was isolated from a canis lupus familiaris (poodle dog) and its potential probiotic properties were characterized by comparative and functional genome analysis. The complete genome of strain CIA01 containing 2.1 Mb, with a G + C content of 64.2 mol% possessed the factors beneficial to mammal health when consumed as feed, proteins related to mucosal surface adhesion, acid and bile acid-associated genes. These results add to our comprehensive understanding of *Bifidobacterium,* and suggest that this strain has potential industrial applications.

[This study was supported by the Strategic Initiative for Microbiomes in Agriculture and Food funded by Ministry of Agriculture, Food and Rural Affairs (918002-4).]

Keywords: *Bifidobacterium*, Dog feces, Probiotics

J022

Identification of the Physiological Characteristics and Selection of Mesophilic Strains of *Volvariella volvacea*

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Straw mushroom (*Volvariella volvacea*) is a popular edible mushroom in South-East Asia. It is distributed thoroughout the world, mainly in subtropical regions because it grows well in high temperature. We studied the physiological characteristics of genetic resources of *V. volvacea* and selected the mesophilic strains of that to develop a new variety that can grow well in the environment of Korean mushroom farms. We collected 11 genetic resources and analyzed the ITS sequences. As a result of ITS sequences analysis, 7 strains were indentified as *V. volvacea*. The mycelium of *V. volvacea* grew well on agar medium extracted from cotton seed hull, compost and post-harvest cotton. And the optimum temperature of mycelium growth was 30℃. We cultivated 7 strains to evaluate the morphological characteristics and collect spores from fruiting bodies. We selected total 76 spores from some parental strains. 76 strains were inoculated on PDA (Potato Dextrose Agar) medium and incubated at 25℃ to select mesophilic strains. 32 of 76 strains were selected because the mycelium of them grew well at 25℃. The wheat spawns of 32 strains were inoculated on pasteurized cotton waste medium and incubated at 25℃ to identify the morphological characteristics of them.

[Supported by grants from RDA, PJ012057]

Keywords: *Volvariella volvacea*, Straw mushroom, Mesophilic strain, Breeding

J023

Control Effect of Endophytic Bacteria on Sclerotinia Rot Caused by *Sclerotinia minor* on *Aster yomena*

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Sclerotinia rot of *Aster yomena* was occurred with *Sclerotinia minor* in Gurye in 2016. Incidence of the disease was as high as 20–80% in the *Aster yomena* fields. For a prospective endophytic bacteria selected for biological control of Sclerotinia rot caused by Sclerotinia minor in *Aster yomena*, the endophytic bacteria were isolated from the roots of Aster yomena. Among the 175 bacteria, the ten isolates inhibited the mycelial growth of *Sclerotinia minor*. When 10-fold diluted cultured liquid of the isolates was treated with 3 times at 7 day interval, control efficacy on Sclerotinia rot of Aster yomena was 61.3–80.9% in the 50 hole tray pot test. Also theses isolates were increased the germination of seed and plant growth of *Aster yomena*. The isolates that controlled effectively on Sclerotinia rot of *Aster yomena* are *Pseudomonas* spp., *Rhizobium* sp. and *Bacillus* sp.

Keywords: Control effect, Endophytic bacteria, Sclerotinia rot, *Sclerotinia mino*, *Aster yomena*

J024

Checking the Hygiene of the Hand-dryer

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Paper towels and warm air hand dryers are the most frequently used means of hand drying in washrooms. The purpose of this study is to investigate the hygiene of hand dryers in the public washroom and to investigate the changes of total microorganism when using a paper towel or a hand dryer and desiccation the hands and the change of airborne bacteria in the toilet air by using a hand dryer. The hand dryers in washroom were used to assess the bacterial contamination. The warm air hand dryer was evaluated by exposing air flows to petri dishes containing nutrient agar medium. Identification of bacterial isolates was performed using conventional methods. Seven volunteers participated in the experiment to observe changes in bacterial colonies according to the drying method. After hand drying, hand were pressed onto agar media and transfer of residual bacteria. There was significant difference between the after CFU. Bacteria were found to be relatively numerous in the air flows. Bacterially contaminated air was found to be emitted whenever a warm air dryer was running, even when not being used for hand drying. Hot air from hand dryers can deposit pathogenic bacteria onto the hands and body of users. The risk of this bacteria to the general public not entirely clear, but they can be a risk factor for patients such as immuno-deficiency. It is recommended therefore that the use of hand-dryers should be carefully considered on health grounds

Keywords: Hand dryers, Hygiene, Bacteria

J025

Isolation and Characterization of Lactic Acid Bacteria from Edible Insect and the Feed

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Lactic acid bacteria (LAB) were isolated from edible insect (*Protaetia brevitarsis*) and fermetated saw dust. The culture supernatants were screened for antibicrobial and antioxidant activity against LAB. Thirteen bacterial strains showing promising antimicrobial and antioxidant activity wer further characterized by molecular methods and various enzyme producing. These isolated were assigned to the genera *Lactobacillus*, *Pedicoccus*, *Lactococcus*, and *Weissella* on the basis of their 16S rRNA gene and bacteriocin sequences. The study showed that the LABs produce bacteriocin that inhibits a wide variety of pathogenic microbes which suggested that it can be used as an laternative type of antibiotic.

Keywords: LAB, Antimicrobial, Antioxidant, Edible insect

J026

Korea Mushroom Resource Bank

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

J027

Comparison of the MERS eS770 Spike Antigen Immunogenicity according to Adjuvant

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Middle East respiratory syndrome coronavirus (MERS-CoV) has emerged as a new pathogen that can transmit between humans as well as animals to humans. Despite high mortality rates in human and outbreaking in Korea, no licensed MERS vaccine or drug is available. In this study, we expressed the MERS spike protein (eS770-His) in a baculovirus/insect cell expression system and tested the immunogenicity of the MERS antigen according to the adjuvant difference. Adjuvants were compared using Alum adjuvant and MF59 adjuvant. Purified eS770-His MERS antigen was intramuscularly administered to mice at a dose of 1 μg with or without adjuvant. Vaccination was done twice with 2 - weeks interval. Serum samples were obtained after vaccination, and specific antibodies and neutralizing antibodies were measured using MERS-CoV pseudovirus. Spike protein specific antibodies showed antibody titers similar to Alum adjuvant and MF59 adjuvant. However, the MF59 group showed twice higher neutralizing antibody against the MERS-CoV pseudovirus compared to the Alum group. It concluded that the combination of the eS770-His antigen with the MF59 showed better adjuvant effect than that of the alum.
[Supported by grants from KHIDI (No. HI15C2842)]

Keywords: MERS, Adjuvant, Vaccine, Neutralizing antibody

J028

Suppression Effect of *Variovorax gossypii* GJ6-4 on Phytophthora Root Rot in Gom-chwi

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Gom-chwi (*Ligularia fischeri*) is severely infected by *Phytophthora drechsleri*, which is causing the Phytophthora root rot. 78 antifungal bacteria were evaluated for their ability to suppress *P. drechsleri* by seed plate assay *in vitro*. As a result, GJ6-4 isolate showed the greatest inhibitory ability and was identified as *Variovorax gossypii* based on 16S rRNA gene sequences analysis. Sequentially, for the effectively application of *V. gossypii* GJ6-4, the control efficacy of *P. drechsleri* by seed dipping treatment were evaluated in pot test. When gom-chwi seeds were dipped in cultural liquid of GJ6-4 (1×10^7/ml) for 3 hours, *V. gossypii* GJ6-4 significantly reduced Phytophthora root rot in gom-chwi seedlings by 75.0%.
[Supported by grants from National Institute of Agricultural Sciences]

Keywords: Biocontrol, Gom-chwi, *Ligularia fischeri*, *Phytophthora drechsleri*, *Variovorax gossypii* GJ6-4

J029

Inhibitory Effect of *Poncirus trifoliate* Seed Extract on Influenza Virus

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

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

J030

Developement of Pseudotyped Nipah and Hendra Virus for Vaccines and Diagnostic Studies

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Nipah virus (NiV) and Hendra virus (HeV) are recently emerged zoonotic paramyxoviruses and can infect a broad range of hosts causing severe respiratory distress syndrome with high mortality. Because of their highly pathogenic nature, a neutralizing assay with these viruses requires a biosafety level-4 (BSL-4) containment. The pseudotyped virus has advantages not only non-biohazard to test at BSL2 but also rapid test. We constructed NiV and HeV pseudotyped viruses (PVs) using fusion (F) and attachment (G) glycoproteins. To investigate the properties of constructed NiV and HeV PV, their viral titers and infectivity were measured in several mammalian cells. It is really difficult to obtain BSL4-grade Henipavirus and their serum. To validate a neutralization assay system using PVs, monoclonal and polyclonal antibodies were prepared from mice immunized with the NiV and HeV nucleocapsid (N), F and G proteins. NiV and HeV PVs would be essential tools for vaccines and diagnostic studies.
[Supported by grants from Korea Centers for Disease Control & Prevention]

Keywords: Henipavirus, Nipah virus, Hendra virus, Pseudotyped virus

J031

Development of Silicon Nanowire Field-effect Transistor Biosensors for Avian Influenza Virus Diagnosis

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For highly pathogenic viruses, sensitive and rapid diagnose is important for preventing the fast spread of the disease. We suggest silicon nanowire field-effect transistor biosensors for the fast and sensitive detection of avian influenza virus. Top down approach and CMOS standard fabrication technology was used to fabricated the silicon nanowire field-effect transistors. Fabricated device showed reasonable gate voltage shift to the positive direction due to the negative charge of the avian influenza virus. The limit of detection was determined to be 2^{-14} of the stock virus, which is 4 times lower than that of the commercial rapid kit for avian influenza. With the result described, silicon nanowire field-effect transistor biosensors are suitable for the sensitive and rapid detection of avian influenza and other highly pathogenic viruses.

Keywords: Avian influenza virus diagnosis, Silicon nanowire

J032

A Study of Vaccine Development Using Recombinant Baculoviral System against Foot-And-Mouth Disease

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Foot-and-mouth disease (FMD) is one of the most contagious and economically devastating diseases that affect cloven-hoofed livestock. Although several types of inactivated vaccines have been commonly used at livestock farms, outbreaks of FMD have continued and there is a doubt about the efficacy of current inactivated vaccines. As well as the efficacy, some safety issues like escaping of the live virus from the inactivated vaccines have raised. Therefore, a novel strategy of FMD vaccine is urgently needed. To overcome the current issues, we engineered a recombinant baculovirus vectored FMDV Type O vaccine delivering FMDV capsid and capsid-processing proteins. Since baculovirus does not replicate in any mammalian cells but insect cells, recombinant baculovirus has been widely used in order to express foreign proteins in mammalian cells without toxic effects to the cells. Also, the recombinant, replication-defective baculovirus could stimulate systemic and humoral immune responses through intramuscular or intraperitoneal administrations. Mice vaccinated with recombinant baculovirus vaccines developed an FMDV-specific systemic and humoral response and showed comparable efficacy to the current inactivated vaccine. These results indicate that recombinant baculovirus vectored FMD vaccine can be an effective and safe vaccine candidate that could potentially be used preventively and in outbreak situations.

Keywords: FMDV, Vaccine, Recombinant Baculovirus, Immunogenicity

J033

Physicochemical Properties of Antibodies Modulated by Interspecies (Mouse-chicken) Isotype Switching

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Although it is known that intraspecies isotype switching and interspecies isotype switching between mouse and human antibodies (Abs) frequently modulate antigen-binding affinity, little is known about the consequence of interspecies isotype switching between mammalian and avian (mouse and chicken) Abs. At present study, we aimed to address how mouse-chicken interspecies isotype switching affects physicochemical properties compared to mouse-human interspecies isotype switching. Two types of chimeric Abs (mouse-human chimeric IgG1/κ and mouse-chicken chimeric IgY/λ) of two different Ab clones were prepared by the processes of grafting of the genes encoding variable (V) regions of mouse antibodies into expression vectors with human IgG1/κ and chicken IgY/λ backbones, protein expression using HEK293f cells culture, and purification by affinity chromatography using Protein L column. We analyzed their antigen-binding affinity, structural and functional stability under thermal stress. Our results indicate that mouse-chicken interspecies isotype switching is acceptable to modulate the physicochemical properties of Abs, giving rise to different properties from mouse-human interspecies isotype switching. [Supported by grants from NICS]

Keywords: Isotype switch, Interspecies, Mouse-chicken antibody, Mouse-human antibody, Chimeric antibody

J034

Development of a T7 RNA Polymerase-driven Minigenome Rescue System for Severe Fever with Thrombocytopenia Syndrome Virus, KAGWT Strain

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SFTS is an emerging tick-borne viral disease that is endemic to China, Japan, and South Korea, which is caused by the SFTSV, a novel phlebovirus. Minigenome rescue systems have been reported as useful tools for the effective study of transcription and replication of negative strand RNA virus. For SFTSV, there is one report of a minigenome system for Chinese SFTSV HB29 strain.

In this study, we constructed two helper plasmids expressing the NP or RdRp of a tick-derived Korean SFTSV KAGWT strain under the control of T7 promotor and EMCV IRES. The S and M segment-based minigenomes consisting of the NSs and Gn/Gc ORFs of KAGWT replaced by reporter genes (NLuc or EGFP) were constructed to evaluate the efficacy of the each plasmid in viral replication and transcription. After co-transfection into BHK/T7-9 cells with the minigenome constructs and two helper plasmids, minigenome rescue was examined by reporter gene activity. In the T7 polymerase-driven minigenome rescue system, expression of reporter genes were detected from both the NLuc and EGFP-based minigenomes. Our results showed that the two helper plasmids are expressed and could be assembled into a functional RNP complex, which in turn could rescue the expression of reporter genes.

We have established a SFTSV minigenome system enabling employment of the generation of infectious clone with the full-length genome of SFTSV KAGWT strain. This system could be used as an effective platform in the field of SFTSV research.

Keywords: SFTSV, Minigenome system, T7 RNA polymerase

J035

Comparison of Non-catalytic Integrase Inhibitor Efficacy Assays for Anti-HIV Drug Development

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Since this TZM-bl luciferase system is the easiest assay method, it is used for primary antiviral screening. For analysis, Sup T1-CCR5 and TZM-bl cell lines were infected with M tropic HIV-1 strains. Before the infection, each anti-HIV drug candidates were serially diluted by 3-fold from 50 μM to 0.01 nM and then treated to cells. p24 ELISA was performed with culture media after five days post infection. TZM-bl luciferase assay was performed at two days after infection. Strangely, commercial CINI and NCINI showed a high p24 reduction at the SupT1-CCR5 cell, but NCINI did not show antiviral activity at TZM-bl luciferase system. We have confirmed that CINI and NCINI have different inhibition mechanism. This is presumably due to the characteristic of NCINI, which working at the second round of HIV infection. If people do not want to miss NCINI in the anti-HIV drug screening process, they have to do other (p24, RT, etc.) analysis methods rather than TZM-bl luciferase system.

Keywords: HIV-1, Non-catalytic integrase inhibitor, Efficiency test, Assay system

J036

Development of MIC Based Real-Time PCR Assay for *Bacillus anthracis*, *Brucella suis*, *Francisella tularensis* and *Yersinia pestis*

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Water Quality Research Center, K-water

Biological weapons are microorganisms or toxins that are used for the purpose of disabling or killing the enemy troops, and are often referred to as bacterial weapons. *Bacillus anthracis*, *Yersinia pestis*, *Brucells suis*, and *Francisella tularensis* are four major biological weapons. The dissemination of these agents is a severe public health concern. In this study, therefore, species-specific primers for these four bacteria were designed based on the target genes presented in the protocols from the Korea Centers for Disease Control and Prevention and specificities in each primer were verified. The design of these target primers was purposed to implement different applications, such as real-time PCR, conventional PCR, multiplex PCR, and etc., according to differences in amplicon sizes. In order to detect four kinds of biological weapons, a method based on MIC real-time PCR was established. Primers for detecting *B. anthracis* were targeted to *sspE* gene and *capC* gene. Toxin gene and *inv* gene in case of *Y. pestis*, BCSP gene and 16S rRNA gene in case of *B. suis*, and 16S rRNA gene and fopA gene in case of *F. tularensis* were targeted for each primer. In the results of performing MIC real-time PCR by fabricating the target specific primer for each bacteria as a 4X primer mixture, species-specific amplifications were verified and its detection limits were determined by about 0.1–10 ng genomic DNA/reaction.

Keywords: *Bacillus anthracis*, *Yersinia pestis*, *Brucells suis*, *Francisella tularensis*, Biodefense

J037

Antimicrobial Activities of Strain *Curtobacterium* sp. S3W-11 and Optimization of Culture Conditions

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In this study, we isolated and identified bacteria from freshwater and soil collected from Osipcheon River, to screen antimicrobial bacteria against various pathogenic bacteria. 44 strains were isolated and based on 16S rRNA gene sequence analysis. Among them, strain S3W-11 showed a good growth inhibition against methicillin-resistant *Staphylococcus aureus* subsp. *aureus* strains and *Bacillus cereus*. As a result of the 16S rRNA gene sequence analysis, strain S3W-11 show the high similarity with *Curtobacterium plantarum* CIP108988^T, *P. vagans* LMG24199^T, *F. acidificum* LMG8364^T 99.79%, 99.78%, 99.35%, respectively. We investigated cell growth and antimicrobial activity according to commercial culture medium, temperature, pH for culture optimization of strain SW3-11. Optimal conditions for growth and antimicrobial activity in strain S3W-11 were found to be: YPD medium, 25℃ and pH 6.5. When the strain was cultured in LB, NB, TSB, R2A media at 20℃ and 25℃, the antimicrobial activity did not show. Culture filtrate of strain S3W-11 showed antimicrobial activity against MRSA strains and *Bacillus cereus* with inhibition zones from 4 to 6 mm. Optimal reaction time was 48 h in YPD medium, 100 rpm and 0.3 vvm in 2 L-scale fed-batch fermentation process for antimicrobial activity. Culture optimization of strain S3W-11 can be improved on antimicrobial activity. Therefore, the antimicrobial activity of *Curtobacterium* sp. S3W-11 had potential as a novel antibiotics for pathogens including MRSA.

Keywords: MRSA, Antimicrobial bacteria

J038

Yeast Associated with Wild Fleabane and Characterization of Biosurfactant-producer

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Investigating yeast biodiversity that have potential applications in biotechnology is important. In this study, we analyzed the yeast biodiversity associated with wild fleabane (*Erigeron annus* (L.) Pers.). We compared the efficacy of different yeast media for the isolation of yeasts associated with wild fleabane, and the media included antibiotics and fungistatic agents for the suppression of fungi. We isolated yeast species from flowers, leaves, and stems of the plant because these niches have not yet been used for determining the yeast biodiversity. Yeast isolates were identified by phylogenetic analysis based on internal transcribed spacer region sequencing. Yeasts produce biosurfactants (BSs), which are important amphiphilic compounds that are used in the agricultural industry as well as cosmetic and pharmaceutical industries because of their low toxicity, biodegradability, and both commercial and academic interests. Using these yeast isolates, we developed rapid and simple screening methods for BS-producing yeast (BSPY) to design processes for the characterization of high-value yeast BSs and production of eco-friendly BSs. We screened *Aureobasidium pullulans* A57 from flower of wild fleabane, and developed fermentation processes for BS production. Determination of the chemical structure of these compounds by mass spectrometry and NMR (nuclear magnetic resonance) revealed novel glycolipid biosurfactants.

Keywords: Yeast, Biosurfactants, *Aureobasidium pullulans*, Wild fleabane

J039

Development of PCR Primers for Detection of *Didymella bryoniae*, Causal Agent of Gummy Stem Blight of Cucurbits

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Didymella bryoniae, which causes gummy stem blight of cucurbits such as cucumber, pumpkin, squash and watermelon, is a major disease limiting production of cucurbit crops. The disease on fruit is referred to as black rot. Symptoms include seedling death, leaf spots stem cankers, rapid blighting, and fruit rot. Traditionally, fungal plant pathogens were characterized based on morphology and pathogenicity. However, correct identification of fungal pathogens by conventional methods typically requires several days. In this work, we developed RAPD based SCAR marker for the detection of *Didymella bryoniae* by PCR. The specific RAPD band of *D. bryoniae* was excised from gel, cloned into vector and sequenced. Based on the sequenced RAPD amplicon a pair of SCAR primers, Dbv-3F and Dbv-3R, which could amplify 738 bp of genomic DNA of *D. bryoniae* was designed. The specific bands with expected size were amplified in five *D. bryoniae* strains but not in 15 other plant pathogenic fungal species.

Keywords: *Didymella bryoniae*, Gummy stem blight, Cucurbits, PCR detection

J040

Diversity and Antifungal Activity of Endophytes from *Spiranthes sinensis* in China

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This study investigated the diversity of endophytes (fungi and bacteria) and their antifungal activity of *Spiranthes sinensis*, an important traditional medical plant. The endophytes were isolated from the leaves, stems, flowers and roots of *S. sinensis* from Chongqing, China. A total of 263 strains were obtained by tissue culture method. Based on morphological features, 57 endophytic fungi and 48 endophytic bacteria were identified by phylogenetic analyses based on ITS rDNA and 16S rRNA respectively. The results indicated that endophytic fungi were assigned to 25 genera and endophytic bacteria were 11 genera. The most frequently isolated fungi were *Fusarium*, *Alternaria* and *Penicillium*. The dominant bacteria were *Burkholderia*, *Bacillus* and *Pseudomonas*. Antifungal activity of all fungal and bacterial strains (n=105) were evaluated against three major agricultural fungal pathogens (*Fusarium oxysporum*, *Botrytis cineria, Alternaria solani*). Among them, 9 of fungi and 8 of bacteria showed great antifungal activity against at least one of the three pathogens. Especially, the bacterial strains of *Bacillus velezensis* and *B. siamensis* exhibited the best inhibitory activity against all tested fungi. Further studies will pay attention to the mechanism of antifungal activity and their plant growth promoting.

Keywords: Edophytic fungi, Endophytic bacteria, ITS, 16S, *Spiranthes sinensis*

J041

Development of Loop-mediated Isothermal Amplification Primer Set for the Rapid and Highly Sensitive Detection of Human Enteric Adenovirus 41

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Human enteric *Adenovirus* (HuEAdV) contain subgroup F (serotype 40, 41) and G (52) which classified genus *Mastadenovirus*, family *Adenoviridae*. HuEAdV are mainly causes of acute gastroenteritis infection in human and serotype 41 is more report than 40 and 51 in worldwide. In this study, we designed three loop-mediated isothermal amplification (LAMP) primer sets, and select one LAMP primer set based on specificity and sensitivity tests. In addition, for verifying of positive LAMP reaction, developed device using the restriction enzyme *Taq* I, restriction fragment length polymorphism (RFLP). Developed LAMP primer set in this study expected to contribute rapid and highly sensitive detection of HuEAdV-41.
[This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT).]

Keywords: Adenovirus 41, Human enteric Adenovirus, Loop-mediated isothermal amplification

J042

Development of Loop-mediated Isothermal Amplification for Detection of Human *Rotavirus* A from Water Sample

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Rotavirus is a genus of family *Reoviridae* in the group III double-stranded RNA viruses, and there are nine subgroups, referred to as A to I. *Rotavirus* A causes most rotavirus infections mainly among infants and young children in humans. Transmission of RV-A is fecal-oral route and has been associated with waterborne outbreaks. This study was aimed to develop specific LAMP primer sets for simple and rapid detection of RV-A. Among the four sets of LAMP primer sets examined using the Primer Explorer software. One LAMP primer set select based on test specificity and sensitivity, and which is can detection up to one copy from pure RV-A RNA. In addition, we develop to validation of LAMP reaction using a restriction fragment length polymorphism (RFLP) assay. Amplification of 206 base pair using the LAMP outer primer set of polymerase chain reaction from RV-A RNA, and restriction enzyme Mbo I can be used to generate two bands. The successful LAMP primer set newly developed in this study will be useful for detection of RV-A from various water sample.
[This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT)]

Keywords: Loop-mediated isothermal amplification, *Rotavirus* A, Validation of LAMP reaction

J043

Establishing Adaptive Colistin Resistance Models and Investigating Synergism of Colistin and GT-01

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Colistin is a last resort antibiotic for treating multidrug resistance Gram-negative bacteria (MDR-GNB), which are often found in hospitals. However, the use of colistin has been limited due to renal toxicity and neurotoxicity. therefore, because of the lack of new antibiotics for treating MDR-GNB and the toxicity of colistin, the combination of colistin with other antibiotics is used to lower the concentration of colistin, reducing side effects. Therefore, in this research, the synergistic effects of two antibiotic compound; colistin and GT-01, a novel β-lactam antibiotic, were tested in adaptive model of colistin resistant bacteria. Two colistin adaptive resistant
strains, *A. baumannii* HGUABM-01 and *K. pneumoniae* HGUKPM-01, were verified by the minimum inhibitory concentration (MIC) test. In the fractional inhibitory concentration (FIC) test, combination of colistin and GT-01 showed synergistic effects. In the time kill assay, combination of the two compounds showed bactericidal effects in x2 MIC, x4 MIC and x8 MIC concentration. In the adapted models, the synergistic effects of colistin and GT-01 was significant, suggesting that the combined treatment of these two compounds would be an important method to defeat the MDR-GNB that are confronting humanity with its rising ability of resistance.
[This research was supported by grants from Handong Global University]

Keywords: Colistin, MDR-GNB, MIC, FIC, Time kill assay

J044

Antifungal Activities of *Paenibacillus* sp. IS404 against Plant Fungal Pathogenes

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For bioligical control of plant fungal pathogens, bacteria were isolated from soil and rhizosphere. *Paenibacillus* sp. IS404 head the antifungal activity on Cham-chwi`s leaf spot caused by *Septoria* sp. Teh isolate was tested antifungal effect against eleven plant fungal pathogens. *Paenibacillus* sp. IS404 showed the high antagonistic effect against *Altanaria* sp. and *Scleotium cepivorum*.
[Supported by grants from National Institute of Agricultural Science]

Keywords: Biologoical control, *Paenibacillus*

J045

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 betacoronavirus that has been emerging infectious disease in human. In 2015, the MERS-CoV outbreak occurred with 186 cases in the Republic of Korea. To aid preventive strategies and control of MERS-CoV outbreak in future, we have developed a MERS-CoV DNA Vaccine using the baculoviral delivery system. For enhancing cellular delivery, we constructed a non-replicating recombinant baculovirus coated with human endogenous retrovirus envelope (AcHERV). First, we constructed a recombinant baculovirus encoding each of S, S1, RBD genes under the control of the AcHERV system. We confirmed MERS-CoV S, S1 and RBD genes expression levels by western blot in Huh7 cell. To investigate the efficacy of the vaccine, we immunized with each of recombinant baculoviruses in Balb/c mice. We found that all three recombinant baculoviruses delivering each of MERS-CoV S, S1 and RBD genes elicited a high level of IgG, neutralizing antibody, and IFN-r. S1 showed the highest humoral and cellular immune response. In conclusions, AcHERV baculovirus could be a potential prophylactic vaccine against MERS-CoV

Keywords: MERS-CoV, MERS vaccine, Baculovirus

J046

Total Metabolome Profile Analysis of Teosinte in the Flooding Condition

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Teosinte, generally knows as the progenitor of maize, has been cultivated for a long time and discovered from central america regions such as Mexico, Guatemala, Honduras and Nicaragua that are known to receive frequent rainfall. Such teosinte collected from regions that are known to receive frequent heavy rainfall in some central america regions may provide a excellent genetic maize resource for the development of flooding tolerant maize. Recent studies have shown that teosinte has the ability to form adventitious roots, to develop aerenchyma tissues of teosinte, and the resistance to toxic substances under flooding soil condition. Therefore, this studies was carried to evaluate possibility of utilization for flooding resistance in teosintes. A comprehensive profiling data about primary metabolism after the flooding conditions was obtained by gas chromatography-mass spectrometry (GC/MS). Total detected metabolites were 296–356. Among them, 112 metabolites were identified and metabolite libraries were prepared. As a result of statistical analysis of sugar, amino acid, acid, sugar, alcohol, fatty acid and amine in the samples treated with flooding and non-flooding samples, the highest content of sugar was 45–46%. But after the flooding treatment, sugar decreased to 30–32%, while amine increased from 2–3% to 32–33%.
[This work was carried out with the support of the 'Cooperative Research Program for Agriculture Science & Technology Development in Rural Development Administration (Project No. PJ0124382018) Republic of Korea.]

Keywords: Teosinte, Flooding, Metabolomic, Profile

J047

First Report of *Colletotrichum gigasporum* Causing Anthracnose on Chilli Pepper in Korea

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To investigate the diversity of *Colletotrichum* species associated with anthracnose of chilli peppers in Korea, 120 isolates were collected from in 2016–2017. Out of these, unusually sized conidia were observed in two isolates. The isolates, C01049 and C01111 were obtained from Gochang and Cheongyang, respectively. Two isolates were identified as *C. gigasporum* species complex based on morphological characteristics. Conidia of the isolates were single celled, hyaline, cylindrical with an obtuse apex, and size of conidia was 17.8–40.5 × 6.0–10.0 µm. The isolates were confirmed as *C. gigasporum* based on MLST using glutamine synthase (GS), beta-tubulin (TUB2), actin (ACT), and ITS/5.8S rDNA region. The isolate clustered with the reference strain of *C. gigasporum*. Pathogenicity test was carried out on wounded or unwounded chilli pepper fruits of four cultivars using spore suspension of isolates. The appearance of disease symptoms were observed on only wounded fruits of three cultivars within 7 days after inoculation. To our knowledge, this is the first report of the association of *C. gigasporum* in causing chilli pepper anthracnose in Korea as well as worldwide.

Keywords: *Colletotrichum*, Chilli pepper, Diversity

J048

Isolation and Characterization of Halophilic *Oceanobacillus iheyensis* KMU78 from a Radish Kimchi

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Kimchi is a traditional Korean fermented food made by pickling salted cabbage or radish in a sauce of red pepper powder, salted seafood (Jeotgal), green onion, garlic, and other ingredients. The ingredients of Kimchi exhibit a wide variety of characteristics depending on personal taste and local characteristics. In particular, Radish Kimchi (Seokbakji) is a Jeot-gal kimchi that mainly uses salted radish and fermented seafood. Jeot-gal is one of the three major fermented foods in Korea, along with fermented pickling vegetables and paste. It includes various marine halophilic microorganisms because it a fermented seafood. Previous studies have focused on the lactic acid bacteria of Kimchi, but we focused on the strongly halophilic *Oceanobacillus* strains derived from Jeot-gal. The isolated strain was genetically identified by 16S rRNA sequencing and named *Oceanobacillus iheyensis* KMU78. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain KMU78 belongs to the genus *Oceanobacillus* and is closely related phylogenetically to the type strain *O. iheyensis* HTE831.

[This study was supported by 2018 research funding from Kookmin University, Korea.]

Keywords: Kimchi, Halophilic bacteria, Salted seafood (Jeotgal)

J049

A Gamma-Glutamyl Transpeptidase of *Bacillus polyfermenticus* Isolated from Fermented Korean Foods : Purification and Characterization

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γ-Glutamyltranspeptidase (GGT) catalyzes the cleavage of γ-glutamyl compounds and the transfer of γ-glutamyl moiety to either water or amino acid/peptide acceptors. GGT can be utilized to synthesize γ-glutamyl peptides, which are used as food taste enhancers. The halophilic microorganism from Korean fermented foods was screened to produce a halostable enzyme. The Strain was selected at ≥10% salt concentration. The selected strain produced halostable GGT. The strain was identified by homology search of 16S rRNA sequences using BLAST, and genome comparisons were performed by using a whole genome sequencing and assembly approach. The extracellular GGT from a *Bacillus polyfermenticus* was purified from the culture supernatant and properties of GGT was investigated.

[This study was supported by 2018 research funding from Kookmin University, Korea.]

[This work is supported by SMEs Partnership Project for strengthening tech-competitiveness.]

Keywords: *Bacillus polyfermenticus*, Halostable enzeme, γ-Glutamyltranspeptidase (GGT)

J050

Preparation and Characterization of Poly-γ-Glutamate Hydrogels

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Poly-γ-glutamate (γ-PGA) is an edible biodegradable microbial amino acid biopolymer that has an anionic polypeptide that was produced by *Bacillus* species. γ-PGA have various applications in food, cosmetics, and medicine. Studies have been carried out on hydrogels produced by γ-irradiation, but these are unstable in various solutions. This study investigates using UV to make the γ-PGA hydrogel maintain a stable form in various solutions. We prepared both γ-PGA hydrogels from γ-PGA mixtures and their characteristics were examined. The γ-PGA mixture for UV-irradiation was prepared by mixing γ-PGA and a cross-linker. We investigated the effects of the γ-PGA concentration and the molar ratio of cross-linker with γ-PGA on gelation. γ-PGA hydrogels were more cross-linked at higher γ-PGA concentrations. We examined the stability of γ-PGA hydrogels in various solutions for 10 weeks and the viscoelasticity of hydrogels. The UV-irradiated γ-PGA hydrogel maintained its shape for nine weeks, but the γ-irradiated hydrogel dissolved immediately after just one week. The obtained γ-PGA hydrogels can be used as functional cosmetic materials.

[This study was supported by 2018 research funding from Kookmin University, Korea.]

Keywords: Poly-γ-glutamate, γ-PGA hydrogel, Cross-linked hydrogel

J051

Development of Rapid LAMP Assay for Identification for the Quarantine Prohibited Pathogen, *Synchytrium endobioticum* on Potato

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Synchytrium endobioticum, the causal agent of potato wart disease, is regarded as one of the important quarantine diseases (EPPO A2 List) as well as the most destructive potato pathogen that causes yield losses with a worldwide distribution. Also, it is difficult to determine the presence or absence of wart disease on potato due to easily confused species that show similar morphological characteristic such as powdery scab, bud proliferation and potato smut in potato fields. For this reason, this study was conducted to develop a specific and sensitive diagnosis method based on loop-mediated isothermal amplification (LAMP) that is available for immediate detection of *S. endobioticum* from soils or plant materials. As according to results, 13 different fungal strains such as *Phoma exigua*, *Chytridium confervae*, *Spongospora subterranea*, *Verticillium dahliae*, 5 *Rhizoctonia solani*, 2 *Phytophthora infestans*, *Phytophthora fragariae and Synchytrium endobioticum* were screened by visual inspection using LAMP for the specificity. Among these strains, *S. endobioticum* (Pathotype 6) only showed a change in the color as a sky blue by LAMP reaction, while 12 other fungal species did not show the color, and we proved that the limit of LAMP assay to detect *S. endobioticum* was indicated from 1 ng/μl to 10 pg/μl of genomic DNA per reaction. Consequently, we developed the visual detection for *S. endobioticum* with LAMP that was very efficient and useful for non-trained staffs for the rapid identification of potato wart in quarantine fields.

Keywords: Quarantine prohibited pathogen, *Synchytrium endobioticum*, Potato wart disease Loop-mediated isothermal amplification (LAMP), Rapid identification

J052

Efficiency of a Rapid Detection Method by Polymerase Chain Reaction for the Quarantine Pathogen, *Xylella fastidiosa*

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Xylella fastidiosa, the causal agent of Pierce's disease (PD) in grapevine, is regarded as one of the most important quarantine bacterial diseases (EPPO A2 List) as well as the most destructive grape pathogen that causes yield losses. Also, it is difficult to determine the presence or absence of PD on grapevine due to easily confused that show similar symptom such fungal disease on grapevines caused by *Pseudopezicula tracheiphila*. For this reason, this study was conducted to select the most specific and sensitive diagnosis method based on polymerase chain reaction (PCR) that is available for immediate detection of *X. fastidiosa* such as prohibited pathogen from various importing plants. Consequently, 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 this study. In addition, we also confirmed that the limit of RST31/RST33 primer set on PCR assay to detect *X. fastidiosa* highly indicated from 1 pmol/μl to 10^{-4} pmol/μl of genomic DNA per reaction. Consequently, we proved this PCR assay to detect the *X. fastidiosa* was very efficient and useful for the rapid identification of PD in grapevine for plant quarantine purpose.

Keywords: Quarantine pathogen, *Xylella fastidiosa,* Pierce's disease in grapevine, PCR assay, RST31/RST33

J053

Culture Collection of Antimicrobial Resistant Microbes

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Antimicrobial was one of the great inventions of modern era. However, the abuse of antimicrobial both in human and animals has led to a high rate of occurrence of antimicrobial resistant microbes. Disease treatment caused by antimicrobial resistant microbes including super-bacteria has emerged as critical issue worldwide. Communication and cooperation among researchers in diverse fields are needed to solve the resistance to antimicrobials. Since September 1999, Culture Collection of Antimicrobial Resistant Microbes (CCARM) has been working on bio-resource supported by Ministry of Science and ICT of Korea with the main functions of establishing a biological resource center and its management system. To date, CCARM has a collection of over 26,000 strains of bacteria and yeast from clinical, agricultural animals and products, and environmental fields. CCARM is performing the roles of collection, deposit, preservation, distribution, service, and consulting designated Biological Resource Center by Organization for Economic Co-operation and Development (OECD). During the ten years, a total 250 research papers including 202 SCI paper were published and we expect more publications from the ongoing researches. CCARM plans to continue offer not only providing resource but also free research and educational information to help with better research. CCARM has been members of Clinical Laboratory Standards Institute (CLSI) since 2000, World Federation for Culture Collection & World Data Center for Microorganisms (WFCC-WDCM) since 2003, ISBER (International Society of Biological and Environmental Repositories) since 2007, Korea National Research Resource Center (KNRRC) since 2008, and Biological Repositories since 2009.

J054

Korea Bank for Pathogenic Viruses

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Korea Bank for Pathogenic Viruses (KBPV) has been established in 2005 as a repository agent for the collection, management and distribution of the various pathogenic viruses that are essential to use for researches in biomedical sciences. The Institution operates in collaboration with The Institute for Viral Disease at Department of Microbiology, College of Medicine, Korea University, founded in 1973.
The bank has unique viral collections such as Hantaan, Seoul, Muju, Soochong, and imjin the etiologic agents of hemorrhagic fever with renal syndrome. To date, total of more than 43,000 materials (~100,000 vials) from human and animal have been collected and maintained.
We have provided a highly collaborative environment for researchers in various fields by providing valuable viral resources including consulting service. We also provide the educational program related to pathogenic viruses including biosafety training.
Requestors of such agents are required to register with KBPV and to supply details of their laboratory facilities and safety management. More details about KBPV can be found at ; http://kbpv.knrrc.or.kr

J055

Korea Environmental Microorganisms Bank

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Korea Environmental Microorganisms Bank (KEMB) has been established as a microbial and genetic resource center for environmental industries. The KEMB plays an essential role as follows: (1) the collection and conservation of native environmental microorganisms and genetic resources, (2) the construction of systematic management system for effective conservation and application of microbiological resources for environmental industries, (3) the provision of microbial materials for the study of life science and environmental engineering, and (4) the provision of basic resources for the development of the BT/ET industry leading the global economy in 21C.
There are about 19,000 strains of bacteria collected from environments, at this time. These collections are classified in accordance with scientific and functional characteristics, respectively.
It is considered to promote academic and industrial activities by supplying basic materials for research and industrial applications, which accomplish the ecological recovery through constructing eco-friendly bioremediation system by supplying basic microbial resources.

J056

Korea National Center for Fungal Genetic Resources (CFGR): Housing Plant Pathogenic Fungi for Educational and Research Purposes

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Fungi are eukaryotic organisms of ecological and industrial significance. Ecologically, they colonise a wide range of habitats and play essential roles in the ecosystem, particularly in the decomposition of organic matter. They have been used as a food source and agents of fermentation and for the production of various antibiotics and enzymes that are used in research, industry and medicine. In contrary, the impact of many pathogenic fungi on animals and plants is economically and socially detrimental. For example, *Magnaporthe oryzae* causes one of the most destructive crop diseases, the rice blast. Annual yield loss of caused by rice blast is equivalent to the amount that could feed about 60 million people. The Center for Fungal Genetic Resources (CFGR) was established to collect, maintain and distribute genetic resources mainly from plant pathogenic fungi. These efforts are important for both educational and research purposes. Primarily, the center will be a valuable resource to assist the development of new strategies for management of crop diseases and other research in applied science. CFGR harbours a large collection of important fungal species; a total of 42,000 isolates from 59 species of fungi including 20,902 T-DNA transformants of the rice blast fungus and the anthracnose fungus. In addition to the biological materials, CFGR has developed user-friendly databases to maintain genetic information of fungal stocks and help to solve questions about fungal pathogenicity, population genetics, development, and evolution. Also, CFGR seeks strategies for sustainable and scientific plant quarantine to better protect our ecosystem from invasive microorganisms.

J057

Korean Collection for Oral Microbiology

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It has been known that about 700 species of oral bacteria inhabit the human oral cavity. Of them, 350 species have been cultured. The oral bacteria are the major causative agents of systemic diseases such as cardiovascular diseases as well as oral diseases, periodontitis and dental caries. However, the causative bacterial species for oral diseases have not been known because the dental diseases are occurred by the multiple infections. In addition, the prevalence of the oral bacterial species is different by the geographic location of the host and individual. It is very important to obtain the oral bacteria from Koreans for pathogenesis studies related to oral infectious diseases. The purpose of Korean Collection for Oral Microbiology is to obtain the oral clinical strains and their genetic resources, such as 16S rDNA, species-specific PCR or qRT-PCR primers, and genome sequences, for offering them to the researchers.

J058

Lichen as a Novel Bioresources in Korea

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Lichens are symbiotic organisms composed of a fungus (mycobiont) and an alga (photobiont). They produce characteristic secondary metabolites, lichen substances, which seldom occur in other organisms. Lichen and their metabolites have many biological activities. In spite of the wide spectrum of biological activities shown by the lichens, they have long been neglected by mycologists and overlooked by agrochemical industry because of its slow growth in nature and difficulties in the artificial cultivation of organisms. Use of lichen-forming fungi can overcome the disadvantage of natural lichen extracts for industrialization of their metabolites because of their much faster growth and larger production of the metabolites in culture than the natural thalli. Korean Lichen and Allied Bioresources Center focuses on isolation, maintenance and distribution of lichen bioresources to research groups in universities, national institutes and industrial sectors. It also screens their biological activities, and investigates cultural conditions for large production of lichen substances. Chemical library of some lichen extracts is also available from the center.

J059

Microbial Carbohydrate Resource Bank

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

J060

Plant Virus GenBank (PVGB)

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Plant Virus GenBank (PVGB) was established in Seoul Women's University in 1999 and is searching and acquiring the virus genetic resources isolated from various plants in South Korea and developing research resources. PVGB is conducting major works such as isolation, identification, collection, and preservation of plant viruses from plant genetic resources in the world and developing recombinant clones of plant virus genes. In addition, PVGB is conducting the mass production of Antisera against virus and development of primers and probes for detecting the virus. PVGB currently has 20,733 accessions containing plant viruses (1,707 accessions), viral antibodies (84 accessions), viral cDNA clones (5,207 accessions), viral cDNA library (9,643 accessions), full-length clones (300 accessions), mutant virus clones (301 accessions), and viral primers (3,436 accessions). PVGB is providing the preserved resources to the researchers for domestic and international researches. We hope many researchers will use PVGB more often and have many good research achievements using the plant viruses offered in South Korea.

J061

The Bacteriophage Bank of Korea

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Bacteriophages are viruses growing on bacterial hosts. They are found in sea, air, land and even foods. It is assumed that 10^{30} to 10^{32} phages exist on earth and they play a role in maintenance of biological balance. Recently, more and more new applications of phages are reported. As they are a part of useful biological resources, there are increasing demands for securing these resources. In response to these demands, the Bacteriophage Bank of Korea was established in 2010. The bank collects phages from environments as well as from working groups worldwide. Currently, 1200 different phages are stocked. The host bacteria include *Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa, Acinetobacter baumannii, Cronobacter sakazakii, Enterococcus faecalis, Enterococcus faecium, Campylobacter jejuni, Bacillus cereus, Serratia marcescens,* and *Staphylococcus aureus*. The number of stock is growing. The bank also serves as a distributor for the collected phages (www.phagebank.or.kr).