Selective isolation of Actinobacteria and other bacteria showing antibacterial activity against Paenibacillus larvae from soil samples collected in South Korea

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The previous research showed that Actinobacteria are virtually unlimited sources of novel compounds for many therapeutic applications and hold a prominent position due to their diversity. There are more than 22,000 known bioactive metabolites, 70% of which are produced by Actinobacteria, as also considered economically important. Actinobacteria were isolated from soil samples collected in the areas of South Korea by the membrane-filter method, using the media named HV, R2A and ISP4. From the total of 812 colonies, 94 (11.58%) strains showed antibacterial activity against Paenibacillus larvae, a pathogen of honey bee causing American foulbrood disease. Based on 16S rRNA gene sequence analysis, those were species in 10 genera: Streptomyces spp., Bacillus spp., Pseudomonas spp., Massilia spp., Chitinophaga spp., Kitasatospora spp., Actinomadura spp., Pseudosporangium spp., Actinoplanes spp., and Micromonospora spp. Among them, 15 strains were possible new species, which may produce new antibacterial compounds.

*Keywords: Actinobacteria, antibacterial activity, Paenibacillus larvae

Isolation and Identification of Novel Bacteria from Soil around Gyeonggi Science High School

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Numerous experiments and researches have been implemented in a lab of Gyeonggi Science High School (GS11S). So, the soil around GS11S would be likely to be polluted and there would exist novel bacteria. So, in this study, the novel bacterial strain, SBG1 (Soil Bacteria from GS11S), and other 9 strains were isolated from the soil around a lab of GS11S by using a transwell plate. The strain of SBG1 was similar to Bacillus megaterium (97.19%) and Bacillus aryabhattai (97.12%) and identified as genus Bacillus by 16s rRNA sequencing. The morphological, physiological, and biochemical characteristics were investigated by using API kits and other tests.

*Keywords: Bacillus, Soil Bacteria, Novel Bacteria

Prevalence of New marine Verrucomicrobia subgroups at the ocean surface in the late summer

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The phylum Verrucomicrobia, one of the primary lineages within the domain Bacteria, inhabits diverse habitats including marine environments. Here we report the isolation and characterization of 66 novel marine Verrucomicrobia strains which were obtained by dilution-to-extinction culture of surface seawater samples collected from a station in the East Sea of Korea. The majority of the strains (56 of 66) were isolated in August and September when the abundance of the Verrucomicrobia reached its highest levels (1.7-11.0%) as revealed by pyrosequencing of 16S rRNA gene amplicons of community DNA. Notably, 40.1% of pyrosequencing reads classified as the Verrucomicrobia showed more than 99% similarities to 16S rRNA gene sequences of the isolated strains, demonstrating that abundant groups of the Verrucomicrobia were successfully isolated in this study. Phylogenetic analyses showed that most of the isolated strains formed 3 distinct groups within the Puniceicoccales: MPG (Marine Puniceicoccaceae Group-1 (30 strains), MPG-2 (14 strains), and MPG-3 (14 strains). Physiological characters including colony-forming abilities and fatty acids compositions revealed clear differences among the 3 MPG groups.

*Keywords: Verrucomicrobia, pyrosequencing, dilution-to-extinction

Antibiotics Resistance Characterization of Escherichia coli from Water and Sediment in Yeong-San River basin

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To investigate the extent of the fecal contamination of Yeong-San riverine ecosystem which belongs to the five major water in South Korea and the level of the antibiotics resistance, isolation of *Escherichia coli* and their characterization of antibiotics resistance was carried out at 5 sites (YS1-YS4, GJ1) in May, August, and November 2012. Furthermore, we analyzed change value of antibiotics resistance based on specific locations and seasons. As a result, *E. coli* showed a high resistance especially to carbenicillin, ampicillin, and sulfamethoxazole among 15 antibiotics (Amp, Cfx, Gen, Tet, Tob, Str, ClZ, Tri, Pip, Cip, Sul, Cfd, Chl, Kan, Car). The resistances to ceftazidime, cefoxitin, and tobramycin, however, resulted in low level. Isolates from surface water showed a resistance of approximately 1.5 times higher than that from sediment. The antibiotics resistance analysis revealed that isolates in August were most resistant compared to that in May and November (Aug > May > Nov). No significant regional change of antibiotics resistance was detected. Nevertheless, isolates in the upper stream (YS4 site) influenced by agricultural land have the highest resistance level, however, isolates from YS3 site affected by both agricultural and urban area show the lowest resistance level.

*Keywords:*

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Identification of Bacterial Populations in Four Different *Prunus* Species by Metagenomics

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Peach, cherry, apricot, and plum are popular stone fruits in the world and belong to the genus *Prunus*. In this study, we performed a metagenomic analysis to identify bacterial populations in four different *Prunus* species. For that, leaves of individual tree were harvested. Using the 16S rDNA specific primers, we performed PCR and constructed libraries were sequenced by FLX 454 Plus system. The obtained sequences were blasted against the Silva rRNA database. Based on the blast results, bacteria taxonomy was determined. A total of 159 bacteria species belonging to 76 families were identified. Plum (101 species) displayed the highest bacterial diversity whereas peach (58 species) exhibited the lowest bacterial diversity. They could be further grouped in 31 orders, nine classes, and seven phylums. The most dominant bacteria species in all four trees were *Sphingomonas* and *Methylobacterium*. Moreover, we identified several tree specific bacteria such as 8 (peach), 12 (cherry), 16 (apricot), and 40 (plum) species. Furthermore, the range of uncultured bacteria was from 56% (peach) to 64.8% (plum). In summary, we provide comprehensive information on bacterial populations present in four stone fruit trees which include both cultured and uncultured bacteria.

*Keywords: Prunus, Bacteria, Metagenomics*

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Horizontal Distribution of Bacterial Assemblages on Surface Sediments in the Arctic Ocean

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The expedition for the R/V ARAON to the Arctic Ocean was progressed during summer season from 2011 to 2012. Total 22 sampling sites were occupied to monitor benthic bacterial assemblages in the Arctic Ocean. From these sampling sites, 22 sediment cores were collected by the box corer or multicorer equipped in the ARAON, and their surface layers were respectively transferred into sterile tubes for the further analyses of pyrosequencing and sediment properties. In this study, pyrosequencing approach using a 454 GS FLX Titanium Sequencing System (Roche) is applied for bacterial diversity, taxonomic classification and phylogenetic analysis. The bacterial assemblage structure of 22 samples was determined using a pyrosequencing approach, and categorized into five habitats (Group I to V). The findings indicated that Arctic marine bacteria on surface sediments have biogeography patterns. Bacterial assemblages in the samples were mostly represented by Proteobacteria (47.9%), Actinobacteria (12.0%), and Acidobacteria (9.3%), and their relative proportion seemed to be changed along biogeography patterns from continental shelf to deep sub-floor.

*Keywords: Arctic Ocean, Seafloor, Pyrosequencing*

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*Paenibacillus taehanensis* KUDC4121 sp. nov., Isolated from Rhizosphere of a Native Plant of Ulleungdo
†Island, *Acer okamotoanum*

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Strain KUDC4121(=DSMZ 24950, KCTC 13870), one of those isolates was considered as a novel species of the genus *Paenibacillus*. Strain KUDC 4121 comprised Gram positive, motile, rods and is capable of growth from 18–37 ºC and from pH 6.0–7.5 with optimal growth conditions of 30 ºC and pH 7.0. It grows on tryptic soy agar media containing up to 0.5% NaCl (w/v). Cell length ranges from 2.0 to 2.5 μm. This strain is oxidase- and catalase-positive, and does not hydrolyze starch and casein. The G + C content of genomic DNA was 48.28 mol%. The major fatty acids were anteiso-C15:0 (62.53%) and iso-C16:0 (11.57%). The DNA-DNA relatedness between strain KUDC4121T and the reference strain *P. chondroitinus* DSM 5051T was 30.1% followed by *P. pocheonesis* Gsoil 1138T(20.3%), *P. pectinilyticus* RCB-08T (14.2%), *P. alginolyticus* DSM5050T (12.2%), and *P. aestuarii* CJ25T (11.9%). Phylogenetic analysis based on 16s rRNA gene sequence showed KUDC4121 belonging to genus *Paenibacillus*. The closest type strain was *P. chondroitinus* DSM 5051T with 97.8% similarity followed by *P. alginolyticus* DSM5050T (97.6%), *P. pocheonesis* Gsoil 1138T (97.5%), *P. pectinilyticus* RCB-08T (97.1%), and *P. aestuarii* CJ25T (97.1%).

*Keywords: novel species,Paenibacillus*

Genetically distinct lineages of *Vibrio vulnificus* revealed by multilocus sequence analysis

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The genetic diversity and population structure of *Vibrio vulnificus* were investigated using MLST. rep-PCR fingerprinting resulted in a total of 52 unique genotypes out of 84 *V. vulnificus* isolates originated from environmental and clinical sources, including three genome-sequenced strains. The rep-PCR genotypes were subjected to a MLST analysis, which analyzed internal fragments of six housekeeping genes and one virulence-related gene. All seven genes showed very low dn/ds ratios of <0.02, indicating that no strong positive selective pressure is present. The mean number of alleles at each locus was 26.6, ranging from 21 (*glnA*) to 35 (*recA*). Phylogenetic analyses of the concatenated sequences from the genotypes identified 44 STs, and the STs formed two distinct monophyletic groups. Each lineage showed star-shaped topology, implying recent clonal expansions, but the genetic distance between the two lineages far exceeded intra-lineage genetic distances. In addition, Sawyer's test showed evidences of inter-lineage and intra-lineage recombination events in the *glnA*, *glp*, *gyrB*, and *pyrC* (p <0.05). Further studies on the genetic and phenotypic differences between the intra-species lineages can help us understand the evolution and diversification of *V. vulnificus*.

*Keywords: Vibrio vulnificus, diversity*

Analysis of Fixed Parameters Causing Shifts in Products of Multiple Displacement Amplification

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In a typical multiple displacement amplification (MDA), random hexamers are used to prime DNA replication, and products of such MDA are believed to be stochastically determined by random factors. In this study, we examined factors involved with stochastic behavior of MDA by varying a few fixed parameters of MDA, including primers. Using genomes of *Escherichia coli* and *Plasmodium vivax* as target genomes, primers targeting specific loci of the genomes with different melting temperature and self-complementariness were employed to perform MDA. Using four unrelated primers targeting different loci on different genomes, MDA products were generated. Subsequently, target-specific PCR was performed with serially diluted MDA products. MDA with C41,MS3 primers, generated template independent products, while MS16,K_16S primer did not produce MDA products of detectable concentration. MDA with C41 primer showed MDA products that could be amplified by PCR. C41 had high self-complementariness which was regarded as a cause of template independent products. Therefore, success/failure of amplification of a specific locus by MDA was not solely modulated by primer characteristics. In conclusion, experimental conditions other than primer also play crucial roles in causing stochastic behavior of MDA.

*Keywords: Multiple displacement amplification,self-complementariness,Melting temperature
Culture-Dependent Analysis of Microorganisms Isolated from Polychaete (Perinereis aibuhitensis) and Polychaete Habitats in Suncheon Bay

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This study aimed at investigating bacterial community occurred in polychaete and polychaete habitats of Suncheon Bay, which was known as one of the best cleaner in nearshore wetland. A total of 17 genera, 39 species were isolated from polychaete habitats, which included Bacillus spp., Gaetbulibacter spp., Halobacillus sp., Idiomarina sp., Lysobacter sp., Marinobacter spp., Marinobacterium spp., Marinomonas sp., Oceanisphaera sp., Pseudomonas spp., Rheinheimera sp., Rhodobacter sp. Shewanella sp., Sphingopyxis sp., Streptomyces sp., Tenacibaculum sp., and Vibrio sp. Bacillus spp. were occurred dominantly at a frequency of 30%, Marinobacter spp. at 12.8%, Vibrio spp. at 10.3%, and Pseudomonas spp. at 7.6%. Furthermore, 7 genera, 19 species were isolated from polychaete, which included Bacillus spp., Micrococcus sp., Pantoaea sp., Pseudoalteromonas sp., Rheinheimera sp., Shewanella spp., and Vibrio sp. Interestingly, Bacillus spp. were also occurred most dominantly at a frequency of 63.2%, which was consistent in major bacteria with the results obtained from the work on bacteria in polychaete habitats. Five Bacillus strains, designated CBW3, CBW4, CBW9, CBW14, and EBW10 which were capable of aerobic and anaerobic growth were characterized and comparatively analyzed.

*Keywords: Microorganisms from nearshore wetland, Polychaete (Perinereis aibuhitensis), Suncheon Bay

Identification for a novel strain belonging to the genus Pseudomonas isolated from the intestine of Caranx sexfasciatus

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A Gram-negative aerobic strain named as JG-7T was isolated from the intestine of Caranx sexfasciatus, a fish caught near south eastern coast of Busan, Korea. 16s rRNA sequence of JG-7T (NCBI JX624259) has 98% to 96% of similarity with other Pseudomoanas species and most closely related to type strain AL15-21T of Pseudomoanas alcaliphila. JG-7T forms yellow slimy colonies on Tryptic Soy Agar and Nutrient Agar. It grows at around 10-40℃, pH5 to pH10 and 0% to 5% of NaCl concentration at pH7 in nutrient broth. Its single-celled rod body with polar flagella was found under transmission electron microscopy. As a Gram-negative strain, JG-7T also has resistances to some antibiotics, such as penicillin and cephalothin that contain β-lactam group. The cellular fatty acids mostly consist of C12:0, C16:1 and C18:1n9c. Quinone characteristics, the strain JG-7T is proposed as a novel species of the genus Pseudomonas and we suggest to name on this type strain as Pseudomonas pukyongensis sp. nov. type strain KCTC32263T.

*Keywords:

Diversity of Soil Humic Substances-degrading Culturable Bacteria from Polar Regions

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Soil humic substances (HS), composed mainly of humic acids (HA), are widely distributed in cold natural environments and are known as an important fraction of soil organic carbon. Although bacteria dominate the soil environment, their ability to degrade and/or biotransform the stable HS has not been reported well. To study the diversity of bacterial degraders of HS in bi-polar areas, a total of cold-adapted 281 bacterial strains were isolated on minimal medium containing HA from various soil samples: 217 strains around the King Sejong Station, Antarctica (January, 2012) and 64 strains around the Dasan Station, Arctica (August, 2012). Among them, 73 good degraders were selected and identified based on 16S rRNA sequence similarity using EzTaxon program, and then grouped as follows: Phylum Proteobacteria (76.7%), Actinobacteria (19.2%), and Bacteroidetes (4.1%). They were further affiliated with Genus Pseudomonas (54 strains), Rhodococcus (10 strains), and the others (9 strains). All the strains were able to degrade HA at 15-25℃, but not at over 30℃. To characterize their HA-degradation pathway, study on the diversity and distribution of laccase-like multicopper oxidase genes is in progress.
Diversity of Lactic Acid Bacteria on Raw Meat Decaying in highly Alkaline Soil
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Lactic acid fermentation can be useful in removal of organic materials in anaerobic soil. In this study, we examined lactic acid bacteria (LAB) involved with degradation of raw meat in soils treated with quicklime (CaO). We mixed a soil sample and pork at the ratio of 3:2 and added 1% and 3% (w/w) of quicklime. The mixed sample was cultured for 7 days at 16°C and fermentative bacteria were isolated on MRS Agar media with pH 6 and pH 8. Isolates were identified by sequence of 16S rRNA gene. Samples not treated with quicklime were neutral with pH values about 7.5 while pH values of treated samples were highly alkaline with pH 10.0 for samples with 1% quicklime and pH 12.5 for samples with 3% quicklime. The number of viable cells in the samples with 1% and 3% quicklime were $3 \times 10^3$ and $3 \times 10^2$ CFU/g, respectively, implying 10 times more cells survived under 1% quicklime. Isolates were identified as *Leuconostoc gasicomitatum*, *Carnobacterium maltaromaticum*, *Brochothrix thermosphacta* and *Serratia grimesii*. *B. thermosphacta* and *C. divergens* were isolated from pH 8 MRS medium with 3% quicklime samples. Those two bacterial species were known to be tolerant to high salt and high pH. We suggest that *B. thermosphacta* and *C. divergens* are major decomposers in highly alkaline soils.

*Keywords: Lactic acid bacteria, Alkaline soil, Degradation of raw meat

Development of self-fertilizing monokaryotic strains for characterization of mating mechanisms in *Pleurotus eryngii*
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*Pleurotus eryngii* (king oyster mushroom) belongs to basidiomycetes group with a tetrapolar incompatibility system comprising of two mating type loci $A$ and $B$. RAPD and BSA were performed to screen markers linked to the specific mating types. The mating type $B3$ locus in *P. eryngii* KNR2312 P6 was identified based on a SCAR marker typing $AxB3$ mating type. We also identified 4 putative pheromone receptor genes and 4 pheromone precursors genes using Fgenesh and manual curation. In addition, gene expression of 8 putative genes in P6 by real-time RT-PCR based on the calculation of their relative expression suggested that the fungal haploid status requires more mate specific pheromone and receptor genes. Using the *Agrobacterium*-mediated transformation system, we developed several independent transgenic haploids of *P. eryngii* with the pheromone genes. Transgenes were confirmed by PCR analysis and an observation of a clamp connection after it crossed with monokaryon carrying the same $B$ mating type locus and a different $A$ mating type locus. By the additional studies, this system would be useful for characterization of mating mechanisms in *P. eryngii*. [Supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ0082162013), Rural Development Administration, Republic of Korea.]

*Keywords: Pleurotus eryngii, mating type, transformation

*Ulvibacter marinus* sp. nov., isolated from the seawater
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A Gram reaction-negative, chemoheterotrophic, yellow-pigmented, non-motile, flexirubin-positive, designated strain IMCC12008², was isolated off the coast of the Yellow Sea of Korea. Optimal growth of strain IMCC12008² was observed at 25°C, at pH 6.5-7.5 and with 2% (w/v) NaCl. Phylogenetic analysis based on the 16S rRNA gene sequences showed that strain IMCC12008² belonged to the genus *Ulvibacter*, and was closely related to the type strains of *Ulvibacter litoralis* KMM3912² (95.3%) and *Ulvibacter antarcticus* IMCC3101¹ (94.9%). The major fatty acid were iso-C₁₅:₀ (26.2%) and iso-C₁₅:₁. G (10.5%). The G+C content of the DNA was 38.1 mol%. The strain contained MK-6 as the respiratory quinone. Polar lipids were
consisted of phosphatidylethanolamine, unknown aminolipids, unknown aminophospholipid and unknown polar lipids. On the basis of phylogenetic distinction and differential phenotypic characteristics, strain IMCC12008T (KCTC 32322T) should be assigned to the genus *Ulvibacter* as the type strain of a new species, for which the name *Ulvibacter marinus* sp. nov. is proposed.

*Keywords: Ulvibacter, Yellow Sea*

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*Mucilaginibacter soyangensis* sp. nov., Isolated from Lake Soyang

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A gram-staining-negative, aerobic, round, convex and reddish-pigmented, strain HME8677T, was isolated from Lake Soyang in Korea. The DNA G+C content was 46.1 mol%. Phylogenetic tree based on 16S rRNA gene sequence showed that strain HME8677T formed a lineage within the genus *Mucilaginibacter*. The strain HME8677T was closely related to *Mucilaginibacter lutimaris* (94.8% sequence similarity), *Mucilaginibacter litoreus* (94.6%) and *Mucilaginibacter soli* (94.5%). The major fatty acids were summed feature 3 (comprising C16:1ω7c and/or C16:1ω6c; 36.4%), iso-C15:0 (26.7%) and C16:1ω5c (6.6%). On the basis of the evidence presented in this study, strain HME8677T represent a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter soyangensis* sp. nov., is proposed the type strain HME8677T (= KACC 17311T = CECT 17311T).

*Keywords: Mucilaginibacter sp.*

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Comparison of the phosphate-accumulating bacteria in anaerobic, anoxic, and oxic chambers of a pilot A2O process

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A2O process is a sequential wastewater treatment process that uses anaerobic, anoxic, and oxic chambers for nitrogen and phosphorus removal. Each of these chambers performs different function in the process, and bacteria in each reactor directly involved in the removal of nitrogen and phosphorus. In a study for bacterial community of A2O process, the diversity of *Candidatus Accumulibacter*, involved in phosphorus removal, was surveyed. A pilot-scale A2O process (50 m3 day–1) was operated for more than 6 months, and bacterial 16S rRNA gene diversity was analyzed using pyrosequencing. A total of 7,447 bacterial sequence reads were obtained from anaerobic (1,546), anoxic (2,158), and oxic (3,743) chambers. Even though there were differences in the atmospheric condition and functionality, no prominent differences could be found in the bacterial community of the three chambers of the pilot A2O process. All sequence reads, which were taxonomically analyzed using the Eztaxon-e database were assigned into 638 approved or tentative genera. Among them, Phosphate-accumulating bacteria, *Candidatus Accumulibacter phosophatis* and two other phylotypes in the same genus were found to constitute 3.4, 2.9, and 3.5% of the identified genera in anaerobic, anoxic, and oxic chambers, respectively.

*Keywords: A2O process, phosphate-accumulating bacteria*

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Identification and Characterization of Opportunistic Bacteria Isolated from the Photo-bioreactors for the Cultivation of *Chlorella* sp. KR-1

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This world is urgently forced to solve global warming and increasing energy demand. Many researches
prospect that the production and utilization of microalgae-based energy cover the problems for near-term future. Considering its price competition in energy markets, microalgae would be cultured under a rough condition rather than aseptic conditions well controlled. Thirteen bacteria were isolated from the photo-bioreactors operating for Chlorella sp. KR-1 and six bacteria among them were randomly selected to understand the effectiveness on the co-culture with Chlorella sp. KR-1. The initial cell numbers of each bacterium and Chlorella sp. KR-1 were adjusted to \(1 \times 10^7\) cells and \(5 \times 10^7\) cells per one milliliter, respectively. The culture was continuously illuminated by 150 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) cool-white fluorescent light. The maximum biomass was obtained from each photo-bioreactor on the fourth day after the cultivation, one strain which identified as a Pseudomonas sp. promoted the growth of Chlorella sp. KR-1 as much as 1.2 fold while two isolates showed the growth inhibition of Chlorella sp. KR-1. Therefore, more studies on co-existing bacterial activity have been required for a stable bioenergy production.

*Keywords: microalgae, co-culture, bacterial activity

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Diversity of Culturable Bacterial Flora in Oligotrophic Cover Soils Over Ultramafic Rocks
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Serpentine soil is a heavy metal-rich soil typically generated by denaturing of ultramafic rocks. Oldhamiana (Gypsophila oldhamiana) is species with a feature of living in oligotrophic soil due to its high Ni-resistance. In this study, we hypothesized that the heavy metal tolerance of oldhamiana is partly due to its ability to modulate bacterial community in its rhizosphere. To identify rhizobacteria specific to this function, we compared bacterial community in rhizosphere of oldhamiana (SCS3) versus that in unplanted serpentine cover soil (SCS1). Number of viable bacteria in SCS1 was \(2.1 \times 10^5\) CFU g\(^{-1}\) at TSA with neutral pH while no colony was cultured on TSA with alkaline pH. From SCS3, \(4.1 \times 10^4\) and \(3.6 \times 10^3\) CFU g\(^{-1}\) were cultured on TSA media with neutral and alkaline pH, respectively. In alkali medium inoculated with SCS3, Enterobacter cancerogenus predominated by 52%. E. cancerogenus was often used to promote growth of plants. E. cancerogenus was known to survive well in alkaline environments. Therefore, it was concluded that survival of oldhamiana in serpentine soils has dependence on activities of non-oligotrophic growth-promoting rhizobacteria with tolerance to alkaline condition.

*Keywords: Oldhamiana, Oligotrophic soil, Rhizobacteria

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Isolation of Flavobacterium spp. for Potential Biocontrol Application
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Ralstonia solanacearum causes the bacteria wilt infecting wide range of Solanaceae plants. Resistant mechanisms of tomato plant lines against bacteria wilt were uncovered. Interaction of microbial communities in rhizosphere with plants may affect disease resistant mechanisms. We investigated bacterial communities of several rhizosphere soil from resistant tomato cultivar Hawaii 7996, susceptible tomato cultivar Moneymaker and a non-host Korean cabbage. The analysis of bacterial communities revealed that phylum Bacteroidetes increased in rhizosphere of tomato plants than bulk soil. Thus, we isolated random colonies using the TSA or R2A medium to select members of phylum Bacteroidetes. Isolates in genus Flavobacterium of phylum Bacteroidetes were selected based on analysis of the 16S rRNA gene sequence. A total of 28 Flavobacterium isolates was further characterized. Several isolates formed biofilm on culture plate by producing exopolysaccharides. All of them were tolerant to kanamycin and gentamycin. Many of Flavobacterium isolates did not inhibit Ralstonia solanacearum but inhibited mycelial growth of Rhizoctonia solani and Phytophthora infestans. We are investigating biocontrol potential of those bacteria and interaction with plants.

*Keywords: rhizosphere, Flavobacterium, biocontrol

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Diversity and Phylogenetic Characteristics of Culturable Oceanic Bacteria Isolated from Rhizosphere Soil of Suaeda japonica Makino in Suncheon Bay
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Total of 122 incubatable oceanic bacteria isolated from rhizosphere of crowds of *Suaeda japonica* Makino in Suncheon Bay, their biological diversity and phylogenetic characteristics were investigated. One hundred twenty-two bacterial isolates were identified by comparing 16S rDNA sequences amplified with PCR using primer 27F and 1492R. Total isolates were sorted into 5 groups: Actinobacteria (5.7%), Cytophagia (3.3%), Flavobacteria (1.6%), Bacilli (53.3%), α-proteobacteria (7.4%), γ-proteobacteria (28.7%). Similarity of 16S rDNA was distributed between 97.3-100% and 15 isolates which were below 98.5% were grouped as candidates of new species. Some of isolates were known for plant growth promoting activity and inhibition of phytopathogenic microorganism. On the basis of 16S rDNA sequences, bacterial phylogenetic tree was made to confirm the taxonomic relationship and it was able to compare the bacterial diversity of each 5 sampling sites by application of Shannon's diversity index.

*Keywords: Bacterial diversity, Suaeda japonica Makino, Suncheon bay*

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**Brevundimonas soojongensis** sp. nov., isolated from forest soil collected in Suwon area

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A Gram-negative bacterium, NH1-13 was isolated from soil collected in a mountain area of Suwon. Colonies on agar plates were circular, raised, translucent and moderate white in color with entire margin. 16S rDNA sequence analysis showed that strain NH1-13 formed a coherent cluster with members of the genus *Brevundimonas*. Its similarities were 98% with *B. aurantiaca*, 97.85% with *B. vesicularis*, 97.64% with *B. intermedia*, 97.53% with *B. nasdae*, 97.07% with *B. olei*, 97.07% with *B. mediterranea* and 97% with *B. pontoexterær*. Strain NH1-13 grew optimally at R2A medium and 30°C in the presence of 1–4% (w/v) NaCl. It may be proven through other phenotypic and chemotaxonomic analyses as a new species.

*Keywords: Brevundimonas sujeongensis, soil*

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**Rapid Detection of Pathogens in Human Blood by Direct PCR Using AnyDirect Solution**

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The rapid and accurate identification of the pathogens responsible for bloodstream infections is crucial for prompt initiation of appropriate therapy, as this would decrease morbidity and mortality rates. Early diagnosis and rapid bacterial identification are of primary importance for outcome of septic patients. The high mortality rate associated with sepsis necessitates a timely identification of the causative organism in order to optimize antimicrobial therapy. PCR assays are increasingly being used for this purpose. We have established a PCR method using AnyDirect solution, which enhances PCR from whole blood, for direct detection of pathogens from human blood without prior DNA purification. When we compared the efficiency of conventional PCR amplification of target genes from blood supplemented with pathogens in the presence or absence of AnyDirect solution, amplification products were detected only in reactions to which AnyDirect solution had been added. This results shows that our direct PCR assay with AnyDirect solution is simple, rapid and specific.

*Keywords: direct PCR, whole blood, pathogen*
prompt initiation of appropriate therapy, as this would decrease morbidity and mortality rates. Early diagnosis and rapid bacterial identification are of primary importance for outcome of septic patients. The high mortality rate associated with sepsis necessitates a timely identification of the causative organism in order to optimize antimicrobial therapy. PCR assays are increasingly being used for this purpose. We have established a PCR method using AnyDirect solution, which enhances PCR from whole blood, for direct detection of pathogens from human blood without prior DNA purification. When we compared the efficiency of conventional PCR amplification of target genes from blood supplemented with pathogens in the presence or absence of AnyDirect solution, amplification products were detected only in reactions to which AnyDirect solution had been added. This results shows that our direct PCR assay with AnyDirect solution is simple, rapid and specific.

*Keywords: direct PCR, whole blood, pathogens

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Bacterial Community Structure in Rainwater Influenced by Typhoon Events in South Korea
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Bacteria are ubiquitous in the atmosphere. The presence of bacteria in the air could impact cloud formation by acting as cloud condensation nuclei. Studies showed that bacteria metabolize, grow and reproduce within cloud droplets. This study is focusing on the bacteria community structure within rainwater influenced by typhoon events using pyrosequencing. A total of 14 rainwater samples were collected from July to October 2012, including four Typhoon rainwater samples. There is no extreme bacterial community changes observed in Typhoon rainwater samples as compared by taxonomic analysis and Principle Coordinate Analysis (PCoA). At the Class level, Alphaproteobacteria, Betaproteobacteria, Cyanobacteria, and Gammaproteobacteria are constantly observed throughout the samples. Specifically Betaproteobacteria dominant by Massilia and Herbaspirillum was observed in high proportion in rainwater collected in July whereas Cyanobacteria dominant by Calothrix and Chroococcidiopsis was conserved in rainwater from August to October. Some rainwater samples showing similar distribution of bacterial structure, was further analyzed by PCoA which grouped these samples together and suggested that the bacteria in the rainwater might be origin from the same source.

*Keywords: Pyrosequencing, rainwater, bacterial community

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Isolation and Characterization of Cellulose and Xylose degrading Bacteria from Mushroom Substrates
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To isolate thermophilic Cellulose and Xylose degrading Bacteria, spent mushroom substrates with sawdust were collected from mushroom cultivation farm, Jinju, Gyeongnam in Korea. Among of the isolates, one strain, designated SH06 was selected by agar diffusion method. The strain SH06 was identified as members of the Bacillus amyloliqufaciens by biochemical characteristics using Bacillus ID kit and VITEK 2 system. Comparative 16S rDNA gene sequence analysis showed that strain SH06 formed a distinct phylogenetic tree within the genus Bacillus and was most closely related to Bacillus subtilis with 16S rDNA gene sequence similarity of 99%. On the basis of its physiological properties, biochemical characteristics and phylogenetic distinctiveness, strain SH06 was classified within the genus Bacillus, for which the name Bacillus sp. SH06 is proposed. The cellulase and xylanase activity of Bacillus sp. SH06 was slightly increased according to bacterial population from exponential phase to stationary phase in growth curve for Bacillus sp. SH06.

*Keywords: Bacillus sp. SH06, Cellulase, Xylanase

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Development of Antimicrobial Agents Against American Foulbrood Disease, Stonebrood Disease and Chalkbrood Disease by Using Actinobacteria.
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In nature, there are a great number of micro-organisms. Until now, many microbial species have been found,
but only a few thousand species out of them have been isolated by pure cultures. Actinobacteria also are abundant in soil, and only little portion of them has been known. So there are lots of actinobacteria that are uncultured and hidden in their functions. This study introduced a new culture method to cultivate more diverse actinobacteria by using different culture media, such as R2A, humic acid-vitamin, brain heart infusion (BHI), Bennett’s (B), and Benedict’s modification of the Lindenbein. Further, this study will try to apply to the well-known diseases of honey bees, such as american foulbrood (AFB), stonebrood and chalkbrood, by using new actinobacteria isolated from diverse soil samples. We have around 1,000 isolates in the library. Among them, 162 isolates were already proven to inhibit the growth of Paenibacillus larvae, a bacterial agent of AFB.

*Keywords: Actinobacteria, Paenibacillus larvae, american foulbrood

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Diversity of probiotic lactic acid bacteria in the Korean gastrointestinal tract

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Human intestinal tract is one of the most varied and complex ecosystems, and dietary life an important role in the presence and prevalence of probiotics. The composition and dynamics of fecal microbial communities during the study were analyzed by the PCR-DGGE using group and genus-specific primers. DGGE of fecal 16S rRNA amplicons from 6 volunteer individuals showed host-specific populations of Lactobacilli and Bifidobacterium that were stable over a period of 5 weeks. Group specific Lactobacilli such as Lactobacillus sp., Leuconostoc sp., and Weisella sp. and genus specific bifidobacterium such as B. longum, B. coryneforme, B. adolescentis, B. breve, and Bacteroides fragilis were detected in human feces. These results indicate that the community of Lactobacilli and Bifidobacterium in human feces is unique because of the presence of specific species for human feces and a minority of the Lactobacilli and Bifidobacterium group may be caused by dietary life.

*Keywords: Human intestinal tract, Probiotics

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Diversity of Plant Growth-Promoting Rhizobacteria Isolated from Pepper in Saline Soil

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Plant growth-promoting rhizobacteria (PGPR) stimulate the growth of their host plants and reduce the damages caused by stressful environmental conditions. To investigate the diversity of PGPR under salt stress, rhizospheric soil was collected from pepper plants from Mir-Yang, Korea. The value of electrical conductivity in rhizospheric soil showed 7.78 ds/m suggesting a highly saline environment. In this study, about 400 rhizobacteria were isolated from pepper rhizosphere. The 16S rDNA genes of the 139 isolates were amplified and sequenced, and isolates could be categorized into 24 genera where the genera Arthrobacter, Bacillus, Brevibacterium, Paenibacillus and Pseudomonas were the most abundant. Bacillus and Bacillus-derived genera were dominant which showed 68.35% of all isolates. These isolates were characterized as PGPR: production of Indole-3-acetic acid (IAA), siderophore production, phosphate solubility and production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Among these, 44.6% were produce IAA, 74.8% of isolates the produced siderophore, 31.2% were able to solubilize phosphate. Three isolates were produce ACC deaminase. Isolates positive for these characteristics could be effective in promoting plant growth under stressful conditions.

*Keywords: Plant growth-promoting rhizobacteria (PGPR), Microbial Diversity, pepper

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Succession of Bacterial Community in Mesocosms of Carcass Landfills Treated with Quicklime at Various Concentrations

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Quicklime is calcium oxide (CaO) and reacts with water to generate heat and very strong alkaline compounds. Quicklime is usually treated both inside and outside of carcasses burials for complete deactivation of potential pathogens. However, quicklime may kill normal soil-flora besides pathogens. Inhibition of
activities of soil bacteria can lead to disturbance or destruction of soil ecosystem processes. In this study, we assumed that detrimental effects of quicklime may depend on concentration of quicklime, and we examined differences in responses of bacterial community to different concentrations of quicklime. Results of cluster analysis indicated strongest dependence of community composition and its succession to incubation time, followed by dependence to quicklime concentration. pH of samples treated with 5% and 10% sustained pH values ≥ 12, indicating insufficient neutralizing activities under high concentration of quicklime. When treating soil of more than 3% quicklime, soil bacteria appear to fail to survive due to too high pH. Therefore, it was concluded that bacterial decomposition of carcasses in landfills can be maintained by careful selection of quicklime concentration because metabolisms in landfill has very strong dependence on quicklime concentration.

*Keywords: Quicklime, Landfill, Bacterial community

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Metagenomic Analysis of Fungal Communities Inhabiting the Fairy Ring Zone of *Tricholoma matsutake*

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*Keywords: Tricholoma matsutake, Fungal communities, Metagenomic analysis

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Diversity Analysis of Endophytic Fungi Isolated from the Native Plants of Seven Species in Dokdo Islands

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Endophytic fungi were isolated from the roots of native plants collected in Dokdo islands. The native plants of seven species, such as *Aster sphathulifolius* Maxim, *Agropyron tsukushiense* var. transiens Ohwi, *Tetragonion tetragonoides* Kunze, *Sedum oryzifolium* Makino, *Lilium lancifolium* Thunb, *Commelina communis* L. and *Rumex crispus* L. were collected from Dongdo and Seodo. Endophytic fungal strains were analyzed by internal transcribed spacer (ITS: containing ITS1, 5.8s and ITS2 regions) regions with universal primers ITS-1 and ITS-4. As the results of identification, ninety-three endophytic fungi comprised two phyla, and phylum Ascomycota (91 fungal strains) was more dominant than Zygomycota (2 fungal strains). All endophytic fungi were confirmed to be the six orders, and Eurotiales (60%) were more abundant than the other orders. At genus level, all endophytic fungi were composed of *Absidia*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Drechslera*, *Elaphocordyceps*, *Fusarium*, *Levia*, *Penicillium*, *Phomopsis*, *Purpureocillium*, *Trichoderma* and *Umbelopsis*. *Penicillium* sp. was the most dominant species in this study. The analysis of diversity index confirmed that *A. tsukushiense* var. transiens Ohwi and *S. oryzifolium* Makino were more diverse compared to the endophytic fungi in the other plants.

*Keywords: Dokdo islands, Endophytic fungi, Fungal diversity

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Bacterial Community Analysis in Chicken Gastrointestinal Tracts using Pyrosequencing

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Microbial communities from different regions of the gastrointestinal (GI) tract of broiler chickens were analyzed using pyrosequencing approach. Seven different regions along the GI tract from three chickens, such as crop, gizzard, duodenum, jejunum, ileum, cecum and large intestine, were collected for bacterial community analysis. Major bacterial phyla in the chicken GI tract were found to include Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria and Acidobacteria. The genera Lactobacillus and Enterococcus were the most dominant from crop to ileum, whereas members belonging to the families Lachnospiraceae, Ruminococcaceae and Clostridiaceae were dominant in the cecum and large intestine. The genus Gallibacterium of the Proteobacteria was found to be more dominant in upper gut (crop, gizzard and duodenum) than others. Fast UniFrac analysis showed that microbial communities of different individuals tend to be grouped independent of different GI tracts, except for ileum, cecum and large intestine, which exhibited more GI tract-specific community compositions. Our results suggest that gut microbiota of broiler chickens were influenced by individual variations and also distinctive according to the different segments of GI tract.

*Keywords: Chicken gut microbiome, Pyrosequencing, Gastrointestinal Tracts

A strain, GJW-30\(^T\), was isolated from soil of the lava forest, Jeju, Korea. It was strictly aerobic, Gram-negative, non-motile rods. The isolate produced white colonies on R2A agar. It contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unidentified aminolipid and an unidentified lipid in the polar lipid, Q-10 as the predominant ubiquinone, C\(_{18:1}\)\(\omega 7c\) and C\(_{17:0}\) as major fatty acids, meso-diaminopimelic acid as the diagnostic diaminoc acid in the cell-wall peptidoglycan, and glucose, ribose and rhamnose as whole-cell sugars. The Phylogenetic analysis based on 16S rRNA gene sequencing showed that strain GJW-30\(^T\) formed a deep branch within the order Rhizobiales, sharing the highest level of sequence similarity with Rhodoplanes elegans AS130\(^T\) (93.5%). Combined genotypic and phenotypic data suggested that the strain should be placed a novel genus and species, for which the name Gotjawalibacter aewolensis gen. nov., sp. nov. is proposed. The type strain is GJW-30\(^T\) (= KCTC 32391\(^T\)).

*Keywords: Gotjawalibacter, lava forest

MicroRNA 122 (miR-122) is a liver-specific micro RNA and appears as the most highly expressed miRNA in the adult liver where it makes up 70% of all miRNAs. MiR-122 has previously been found to increase the accumulation and translation of hepatitis C virus (HCV) RNA through direct interaction with the 5’ untranslated region of HCV RNA genome. HCV core protein is the viral nucleocapsid protein that binds and packages the viral RNA genome. Besides its function as a viral structural protein, the core protein is implicated in HCV chronic infection-associated liver diseases by induction of reactive oxygen species and modulation of apoptosis. Here, we show that HCV core protein regulates the abundance of miR-122 level through interaction with GLD2, a non-canonical poly(A) polymerase in the cytoplasm. Expression of the core protein transiently or in a stable cell line derived from the human hepatoma cell line Huh7 led to reduction of the miR-122 abundance, as assessed by Northern blot analysis and real-time quantitative RT-PCR. The decrease of miR-122 abundance was associated with suppression of HCV RNA replication.

*Keywords:

The Effect of Metformin Treatment on Gut Microbiome in High-fat Diet-induced Obese Mouse Model

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Gut microbiome might be involved in the development of metabolic syndromes such as obesity and type 2 diabetes (T2D). However, mechanistic interactions between gut microbiome and metabolic disorders are still largely unknown. In this study, the effect of metformin treatment on gut microbiome has been investigated using high-fat diet-induced obese mouse model. The metformin treatment in high-fat diet induced obese mouse causing gut microbiota to have low diversity and unique microbiota in comparison. Especially, the genome of Akkermansia muciniphila which was suggested as an intestinal mucin-degrading bacterium was highly increased during metformin treatment. In the analysis of functional genes, lipopolysaccharide biosynthesis, sphingolipid metabolism and fatty acid metabolism were significantly upregulated by metformin treatment. These results demonstrated that metabolic disorders and recovery were highly associated with gut microbiome, and the change of gut microbiome composition by metformin treatment was strongly linked to improvement of metabolic syndrome.

*Keywords: Gut microbiome, Metformin, Metabolic syndrome

Marinobacterium granuli sp. nov., isolated from the Yellow Sea
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A Gram-reaction negative, non-motile, non-pigmented rod, designated IMCC1424T, was isolated from a surface seawater sample of the Yellow Sea of Korea. Optimal growth of strain IMCC1424 was observed at 30°C, at pH 7.5-8.0 and with 1.5-2% (w/v) NaCl. Based on 16S rRNA gene sequence comparisons, the strain was most closely related to "Marinobacterium marisflavi" IMCC4074T (96.7%) and shared low 16S rRNA gene sequence similarities with other members of the genus Marinobacterium (91.0-93.8%). The G+C content of the DNA was 53.6 mol% and the predominant cellular fatty acids were C16:1ω7c and/or C16:1ω6c (42.6%), C16:0 (29.1%), C18:1ω7c (20.3 %). Strain IMCC1424T contained Q-8 as the respiratory quinone and phosphatidylethanolamine, phosphatidylglycerol as the major polar lipids. On the basis of phylogenetic distinction and differential phenotypic characteristics, strain IMCC1424T (KCTC 32321T) should be assigned to the genus Marinobacterium as the type strain of a new species, for which the name Marinobacterium granuli sp. nov. is proposed.

*Keywords: Marinobacterium, Yellow Sea

Bowmanella UDC354 sp. nov., Isolated from Seawater of Dokdo, Korea
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The UDC354 isolated from a seawater sample collected in the shallow coastal region of Dokdo of Korea. A phylogenetic analysis based on 16s rRNA gene sequence indicated that strain UDC354 belonged to the genus Bowmanella. The most closely related type strains were B. pacifica (94.64%) and B. denitrificans (94.49%). Members are Gram-negative belonging to the class Gammaproteobacteria. Cells grow well on marine agar plate. Colonies are circular and smooth grey-white color after 48 h incubation at 30°C. Cells grown in broth cultures were curved rods that were motile. Growth occurred between 4 and 40°C, with an optimum at 30-37°C. NaCl stimulates growth but is not an absolute requirement. This strain is oxidase- and catalase-positive, and does not hydrolyze cellulose, xanthine and hypoxanthin. It is tween 20, 40, 60, 80 and Dnase-positive. The major fatty acids were C16:0 (23.48%), summed feature 3 (C16:1ω7c and/or C15:0 2-OH, 17.46%) and C18:1ω7c (14.38%). The G+C content of the DNA of the type strain is 54.06 mol%. On the basis of combined phenotypic properties and phylogenetic and genetic data, strain UDC354 is considered as a novel species of the genus Bowmanella.

*Keywords: novel species, Bowmanella, Dokdo

Microbulbifer gwanganensis sp. nov., a novel species belonging to the genus Microbulbifer isolated from seawater collected in Busan, South Korea
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A Gram-negative, non-motile and strictly aerobic rod (strain M9 T) was isolated from seawater off the coast of Gwangan Beach in Busan, South Korea. The optimum temperature for growth of strain M9 is 30 °C and is able to grow at 10 °C to 50 °C. Growth is optimal in the presence of 2% (w/v) NaCl and it requires salt for growth. Strain M9 grows optimally at pH ranging from pH 6.0 to pH 8.0 and has a DNA G+C content of 60.5 mol %. Phylogenetic analysis based on 16S rRNA sequences showed that it has a closest similarity to Microbulbifer salipaludis (strain SM-1 T) and the level of 16S rRNA identity between strain M9 and Microbulbifer salipaludis (strain SM-1 T) is 97.7%. Thus, based on phenotypic and properties and phylogeny, strain M9 T should be represented as a novel species of the genus Microbulbifer, for which the name Microbulbifer gwanganensis sp. nov. is hereby proposed.

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**Keywords:**

Isolation and Taxonomic Characterization of Two Marine Bacteria Sulfitobacter sp. Strains NB-68 and NB-77

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The genus Sulfitobacter is a member of the Roseobacter clade, which contains highly abundant marine heterotrophs in the coastal region and has diverse roles such as sulfur oxidation, dimethylsulfoniopropionate demethylation, carbon monoxide oxidation and aerobic anoxicogenic photosynthesis. Recently, we isolated two Sulfitobacter bacteria, designated NB-68 and NB-77, from a region of sea-sparkle bloom in the Geoje island. The identity values of the nearly full-length 16S rDNA sequences of stains NB-68 and NB-77 with that of Sulfitobacter mediterraneus DSM 12244 are 97.72% and 97.83%, respectively. Between NB-68 and NB-77, the identity was 98.7%. Cells are Gram-negative, aerobic, non-motile, and rod-shaped, and they form convex and slightly yellowish colonies within 3 days on marine agar at 25°C. Physiological, biochemical and chemotaxonomic characteristics as well as results of a phylogenetic analysis indicate that each represent novel species in the genus Sulfitobacter. [Financial support from the National Research Foundation and the Global Frontier Intelligent Synthetic Biology Center of the Ministry of Science, ICT &Future Planning, Republic of Korea]

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**Keywords:** Sulfitobacter,Roseobacter clade,Sulfur metabolism

Novosphingobium suncheonensis sp. nov., isolated from soil collected a forest in Suncheon, South Korea

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A Gram-negative and short rod-shaped bacterium, designated strain r2a-b, was isolated soil collected in a forest region in Sucheon, Jeollabuk-do. On the basis of 16S rRNA gene sequence similarity, this strain is related to Novosphingobium capsulatum (98.75%), N. aromaticivorans (97.87%), N. subterraneum (97.43%), and N. taihuense (97.15%). Colonies are 0.5-1.5 mm in diameter, circular, raised, entire, and yellow. Cells can grow at 20-40°C (optimum, 20-40°C), at pH 5-9 (optimum, pH 5-9), and at NaCl concentrations of 0-3% (optimum, 0-1%). Growth occurs on LB, NA, TSA, and R2A (best). The strain is catalase-positive. Esculin ferric citrate, D-glucose, L-arabinose, D-maltose are assimilated (API ID 20NE). Alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, α-glucosidase, β-glucosidase are produced (API ZYM). The predominant respiratory quinone is Q-10. The major fatty acid is 68.04% sum in feature 8 (C18:1 ω7c).

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**Keywords:** Novosphingobium suncheonensis,soil

Comamonas osanensis sp. nov., isolated from soil collected a forest in Osan, South Korea

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A Gram-negative and aerobic bacterium, designated strain BG, was isolated soil collected in a forest region in Osan, Gyeonggi-do. On the basis of 16S rRNA gene sequence similarity, this strain is related to *Comamonas guangdongensis* (98%), *C. odontotermitis* (97.15%), *C. composti* (96.84%), *C. terrae* (96.77%), and *C. koreensis* (96.52%). Cells can grow optimally at 30°C, pH 5.5-9 (range, pH 5-9.5), and NaCl concentrations of 0-1% (range, 0-4%). Growth occurs on LB, NA, TSB, and R2A optimally and on MacConkey normally. Its enzyme activity was negative on lipase (C14) and cystine arylamidase and weekly positive on trypsin and N-acetyl-β-glucosaminidase. Assimilation occurred on capric acid, adipic acid, itaconic acid, 3-hydroxybenzoic acid, L-histidine, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and -proline. The major fatty acids were sum in feature 3 (C16:1 ω7c/C16:1 ω6c/), C16:0, and sum in feature 8 (C18:1 ω7c).

*Keywords: Comamonas osanensis, 16S rRNA gene sequence similarity, major fatty acids*

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**Genotypic Changes and Sepearation in Escherichia coli** Phylogenetic Groups in the Yeongsan River Basin, Republic of Korea

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In this study we examined 3,480 *E. coli* strains from the Yeongsan River basin to determine if there was a correlation between phylogenetic groups and HFERP genotypes and to determine if there was genomic plasticity among environmental *E. coli* isolates. Interestingly, multidimensional scaling (MDS) analysis based on HFERP DNA fingerprint data indicated that *E. coli* in phylogenetic groups A and B1 were uniquely clustered. Results of self-organized maps (SOMs) analysis also indicated that *E. coli* phylogenetic groups were seasonally affected by water temperature, with greater occurrences of phylogenetic groups A and B1 in low and high temperature seasons, respectively. The presence of *E. coli* in phylogenetic groups A and B1 were inversely related. Results of this study indicated that while *E. coli* strains could be clustered based on their genotypes and environment conditions, their phylogenetic groups did not changed in relation to these same conditions. The distributional differences of phylogenetic groups among *E. coli* populations in different environments may be caused by different genomic adaptability and plasticity of *E. coli* strains belonging to each phylogenetic group.

*Keywords: Escherichia coli, fecal indicator bacteria, microbial ecology*

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**Leuconostoc kyungheense** sp. nov., lactic acid bacteria isolated from Chonggakkimchi

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A Gram-stain-positive, non-motile, non-spore forming, catalase-negative, facultative anaerobic, spherical or oval-shaped lactic acid bacterium, designated strain THK-X10T, was isolated from Korean food Chonggakkimchi in Suwon, Korea, and Strain THK-X10T taxonomic position was investigated by using a polyphasic approach. The temperature range for growth was 4-37°C (optimum, 30°C) and the pH range was 5.0-10.0. On the basis of 16S rRNA gene sequence similarity data, strain THK-X10T was shown to belong to the genus *Leuconostoc* (L). THK-X10T was related to *L. miyukkimchii* M2 (99.86%), *L. gelidum* NCFB 2775T (97.70%), *L. inhae* IH003T (97.70%), *L. holzapfelii* BFE 7001T (97.59%) and *L. gasicomitatum* LMG 18811T (97.56%). The G+C content of the genomic DNA was 50.3 mol%. The DNA-DNA hybridization values between strain THK-X10T and *L. miyukkimchii* M2 were 46.6±4.2% and 2.0±1.0%, respectively. On the basis of phenotypic and molecular properties, strain THK-X10T represents a novel species within the genus *Leuconostoc*, for which the name *Leuconostoc kyungheense* sp. nov. is proposed. The type strain is THK-X10T (=KACC 16238 =LMG 26591 =JCM 18025).

*Keywords: Leuconostoc kyungheense sp. nov., Chonggakkimchi, 16s rRNA gene sequences*

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**Leuconostoc yonginensis** sp. nov., a β-glucosidase-producing bacterium, isolated from Kimchi

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A Gram-stain-positive, non-motile, non-spore forming, catalase-negative, facultative anaerobic, spherical or oval-shaped lactic acid bacterium, designated strain THK-X10T, was isolated from Korean food Chonggakkimchi in Suwon, Korea, and Strain THK-X10T taxonomic position was investigated by using a polyphasic approach. The temperature range for growth was 4-37°C (optimum, 30°C) and the pH range was 5.0-10.0. On the basis of 16S rRNA gene sequence similarity data, strain THK-X10T was shown to belong to the genus *Leuconostoc* (L). THK-X10T was related to *L. miyukkimchii* M2 (99.86%), *L. gelidum* NCFB 2775T (97.70%), *L. inhae* IH003T (97.70%), *L. holzapfelii* BFE 7001T (97.59%) and *L. gasicomitatum* LMG 18811T (97.56%). The G+C content of the genomic DNA was 50.3 mol%. The DNA-DNA hybridization values between strain THK-X10T and *L. miyukkimchii* M2 were 46.6±4.2% and 2.0±1.0%, respectively. On the basis of phenotypic and molecular properties, strain THK-X10T represents a novel species within the genus *Leuconostoc*, for which the name *Leuconostoc kyungheense* sp. nov. is proposed. The type strain is THK-X10T (=KACC 16238 =LMG 26591 =JCM 18025).

*Keywords: Leuconostoc kyungheense sp. nov., Chonggakkimchi, 16s rRNA gene sequences*
A Gram-positive, non-motile, non-spore forming, catalase-negative, facultative anaerobic, oval-shaped, β-glucosidase producing lactic acid bacterium, designated strain THK-W39T, was isolated from fermented food, Kimchi in Yongin, Korea. And its taxonomic position was investigated by using a polyphasic approach. Strain THK-W39T was able to grow at 4-37°C (optimum, 30°C) and pH 4.0-9.0. Growth was occurred in MRS broth supplemented with NaCl of 0-9.0% (w/v). On the basis of 16S rRNA gene sequence similarity data, strain THK-W39T was shown to belong to the genus *Leuconostoc* (L.) and was related to *L. mesenteroides* subsp. *mesenteroides* ATCC 8293T (98.8% sequence similarity), *L. miyukkimchii* M2T (98.0%), *L. gasicomitatum* LMG 18811T (96.9%), *L. kimchii* IH25T (96.7%). The G+C content of the genomic DNA was 38.3 mol%. The DNA-DNA hybridization values between strain THK-W39T, and *L. mesenteroides* subsp. *mesenteroides* ATCC 8293T and *L. miyukkimchii* M2T were 41.7±6.0% and 17.6±3.1%, respectively. On the basis of phenotypic and molecular properties, strain THK-W39T represents a novel species within the genus *Leuconostoc*, for which the name *Leuconostoc yonginensis* sp. nov. is proposed. The type strain is THK-W39T (=KACC 16237T=LMG 26590T=JCM 18024T).

**Keywords:** *Leuconostoc yonginensis* sp. nov., Kimchi, 16s rRNA gene sequences

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*Bacillus pukyongensis* sp. nov., a novel species of the genus *Bacillus* from seawater in the coast of Busan, Korea

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A low-G+C content Gram-positive bacterium, designated as M7-8T, phylogenetically related to species of the genus *Bacillus*, was isolated from seawater in the coast of Busan, Korea. It is rod shaped, aerobic, and has an optimal growth temperature of 37°C and an optimal pH of about 7.0~8.0. Growth was observed at NaCl concentrations of 0~5.0%. The major respiratory quinone was MK-7. The predominant fatty acids were palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), and oleic acid (C18:1n9c). The G+C content of the DNA was 47 mol%. Phylogenetic analyses of the 16S rRNA gene sequence revealed that the novel isolate is closely related to the type strain of *Bacillus foraminis*, forming a cluster with it. The pairwise similarity of the 16S rRNA gene sequences of the strains is 97.9 %. On the basis of the phylogenetic analyses and the distinct phenotypic characteristics, strain M7-8T represents a novel species within the genus *Bacillus*, for which we propose the name *Bacillus pukyongensis* sp. nov. The type strain is M7-8T.

*Keywords: