Antibacterial activity of Bacillus Subtilis WL-7 Isolated from Korean Fermented Soybean Paste.

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Bacillus subtilis WL-7 was isolated from Korean fermented soybean paste. We could conclude from our experiments that this strain has valuable characteristics, which can be very useful for many kinds of enzymatic industries. At first some important microbial substances produced by B. subtilis WL-7 were studied. One of these substances can be used as a natural food preservative and a protease depending on the different temperature. This strain also produces an antibacterial substance, which is secreted into the medium. Antibacterial activities against growth of E. coli were found from their clear zone about 16–23 mm. Also protease activity was found from the clear zone about 30 mm.

Keywords: Bacillus subtilis, antibacterial substance, soybean paste

Screening of an Antagonistic Bacterium for control of Red-Pepper (Capsicum annum L.) Anthracnose and Phytophthora Capsici

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Pepper (Capsicum annum L.) is one of the most important market vegetables grown worldwide, but the yield and quality of marketable peppers are frequently limited by Phytophthora blight caused by the pathogen Anthracnose and Phytophthora capsici. This study was conducted to select antagonistic rhizobacteria against Phytophthora capsici (KACC 40157, KACC 40476) and Anthracnose (Colletotrichum gloeosporioides KACC 4003, Colletotrichum coccodes KACC 40011, Colletotrichum acutatum KACC 40689) using a sequential screening procedure and to evaluate control efficacy of disk paper method and in vitro test with the selected strains against Phytophthora blight of pepper in the field. 85 bacterial strains, 19 potentially antagonistic strains were screened through disk paper method and in plant trials, and five candidate strains, CS-31, CS-43, CS-52, CS-61, and CS-72, were selected for pot tests. Several of these isolates may have potential for development as biofertilizers or biopesticides for red pepper (Capsicum annum L) crops. [This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. 20100101-054-068-001-01-00)" Rural Development Administration, Republic of Korea]

Keywords: Phytophthora capsici, Anthracnose, biopesticides

Screening and Isolation of Sulfur-oxidizing Microorganism from in Jeju

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A study on the screening and isolation of microorganism was performed for the removal of malodor, such as sulfur, produced from the swine waste. Using a novel sulfur-oxidizing bacterium (SOB) media, 50 bacterial strains species isolated from the swine waste. The PCR yielded a soxB fragment of approximately 700-1000 bp from most of the bacteria. Isolates strains were compared and used to infer relationships of soxB between sulfur-oxidizing bacteria belonging to various 16S rDNA-based phylogenetic groups. Major phylogenetic lines derived from 16S rDNA were confirmed by soxB phylogeny. The microorganism that grow in the liquid mineral medium containing of sodium thiosulfate as sole nitrogen source at 25 mM was isolated and selected. The isolated The Optical 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. This study provides the information on bacterial species living in livestock farm and suggests that these bacteria have metabolic abilities to utilize sulfur-oxidizing derived from removal of main malodor.

Keywords: sulfur-oxidizing microorganism, soxB gene, 16s rDNA
Acute Toxicity Testing of the Reference Toxicants and Difference Medium using Daphnia Magna Strauss

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As emphasized in all guidelines and norms on standard acute toxicity test, "Sensitivity" and "Precision" are the two major key factors for the credibility of the test results at the intra-laboratory as well as the inter-laboratory level. A simple way to get an estimate of the health and the sensitivity of test organisms is the perform test on reference toxicant. All standard ecotoxicological methods to date therefore advise, and some even impose, that "control" assays be performed in reference toxicants in the same manner and under the same conditions as the assays on the samples under investigation. In this study, reference toxicants such as substances including potassium dichromate(K2Cr2O7), sodium chloride (NaCl), potassium chloride(KCl), cadmium chloride(CdCl2), copper sulfate(CuSO4), and sodium dodecyl sulfate(SDS) were evaluated for acute toxicity using Daphnia magna for EPA Medium and OECD medium.(M4).

All acute immobilization tests have to performed with young daphnias less than 24h old at the start of the test. The average 50% effect concentrations of K2Cr2O7, NaCl, KCl, CdCl2, CuSO4, and SDS for EPA Medium and OECD M4 were 0.96(M4: 1.3), 6,400(4,665), 300(560), 0.05(0.06), 0.05(0.025), 5.8(4.9) mg/L, respectively. Keywords : Daphnia magna Strauss, reference toxicant, EC50, EPA medium, M4.

Quantitative and Qualitative Detection of Agricultural Microorganisms Expressing Inulin and Mop Cyclase in Soils

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The environmental release of genetically engineered microorganisms (GEMs), contributed to improve agriculture or remediate environmental hazards, has raised concern over the fate of the organisms and their engineered genes. To detect the microorganisms released into the environment at the molecular level, P. fluorescens carrying the mannityl catabolism region, and B. subtilis producing iturin were employed as model microorganisms. Using specific fusion primers and the TaqMan probe c, qualitative and quantitative detections of model microorganisms by PCR and real-time PCR were conducted employing a small-scale soil core device, designed to track drifting microorganisms due to rain and surface water. The genomic DNAs from P. fluorescens and B. subtilis isolated from an enriched culture in Congo Red Medium was analysed by 16s rDNA sequencing. It was identified as Microbacterium hominis and B. subtilis KCTC1914, Staphylococcus aureus KACC10196, E. coli K12, S. typhimurium KCTC2515, Calbicicans KCTC7270 and antifungal activity of coast of south and east sea in korea. The seaweed species(Rhodophyceae 11, brown 17, green algae 7) have 35 seaweeds species(Rhodophyceae 11, brown 17, green algae 7) have been used in traditional and folk medicine by coastal peoples in many areas of the world. Historically, Asian countries have used seaweeds for various medicinal purposes; early records of herbal medicinal seaweeds (utilized by boiling in water and using in decoctions drugs) appeared in Chinese literature 'Pen Tsae Kan Mu' about 2000 years ago. The aim of this study was to examine the activity spectrum as well as isolation and characterization of the bioactive secondary metabolites from the ethanol extracts of the marine seaweeds collected from coast of south and east sea in korea. 35 seaweeds species(Rhodophyceae 11, brown 47, green algae 7, and east sea in korea. The seaweeds with ethanol extracts were tested in laboratory assay (disk paper method) and in vitro (in plant trials) assay.

Keywords : seaweed, antifungal activities

Screening for Antibacterial and Antifungal Activities in Seaweeds Extract from Coast of South and East sea in Korea

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Marine seaweeds, have been used in traditional and folk medicine by coastal peoples in many areas of the world. Historically, Asian countries have used seaweeds for various medicinal purposes; early records of herbal medicinal seaweeds (utilized by boiling in water and using in decoctions drugs) appeared in Chinese literature 'Pen Tsae Kan Mu' about 2000 years ago. The aim of this study was to examine the activity spectrum as well as isolation and characterization of the bioactive secondary metabolites from the ethanol extracts of the marine seaweeds collected from coast of south and east sea in korea. The seaweeds species utilized by boiling in water and using in decoctions drugs) appeared in Chinese literature 'Pen Tsae Kan Mu' about 2000 years ago. The aim of this study was to investigate the activity spectrum as well as isolation and characterization of the bioactive secondary metabolites from the ethanol extracts of the marine seaweeds collected from coast of south and east sea in korea. The seaweeds species utilized by boiling in water and using in decoctions drugs) appeared in Chinese literature 'Pen Tsae Kan Mu' about 2000 years ago. The aim of this study was to investigate the activity spectrum as well as isolation and characterization of the bioactive secondary metabolites from the ethanol extracts of the marine seaweeds collected from coast of south and east sea in korea. The seaweeds with ethanol extracts were tested in laboratory assay (disk paper method) and in vitro (in plant trials) assay.

Keywords : seaweed, antifungal activities

Characterization of Nitrogen Fixing Bacteria isolated from a Reed

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Nitrogen is one of key elements needed for plant growth. Plants take up nitrogen in the form of nitrate and ammonium. Most of plants absorb nitrogen source by fertilizers. But from 50 to 70% of fertilizers applied was washed away. It contributes to the pollution of the environment such as groundwater and rivers. This study was conducted to isolate free-living nitrogen fixing bacteria from reed and to examine its beneficial traits for developing sustainable biofertilizers. Enriched consortium obtained from a reed in Ansan was developed for the fixing of nitrogen. The fifteen strains were selected, and they were evaluated for efficient nitrogen fixing ability using acetylene reduction activity(ARA) assay by gas chromatograph. One strain showing nitrogenase activity above 200 nmol-ethylene h-1 mg protein-1 was selected. Nitrogen fixing bacteria isolated from an enriched culture in Congo Red Medium was analysed by 16s rDNA sequencing. It was identified as Microbacterium hominis [AKC-10 (similarity : 99%)]. This strain was found to IAA(indole acetic acid) positive activity with an efficiency of approximately 19 mg/L.

Keywords : nitrogen fixing bacteria, Acetylene reduction activity assay, IAA
Identification and Characterization of Bacteria from Spent Mushroom (Flammulina velutipes) Substrates
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Spent mushroom substrate (SMS) is a by-product remaining after a crop of mushrooms. The fresh SMS was sample within 24 hours of removal from the production facility in DOJUN farm. About 5 bacterial species were isolated from fresh SMS on TSA medium. Among them, one isolate showed the antifungal activity against Aspergillus flavus, Aspergillus ochraceus and Penicillium viridicatum producing mycotoxin on PDA medium, potentially. The isolate with growth-temperature maxima near 65°C was produced cellulase, xylanase and mannanase as hydrolyase. Chemotaxonomic data (G+C content: 46%, major fatty acids: anteiso-C15:0, C15:0, and iso-C16:0) supported the affiliation of the isolate to the genus Bacillus. Analysis of comparative 16S-rDNA sequence showe that the isolate formed a distinct phylogenetic tree within the genus Bacillus and was most closely related to Bacillus amyloliquefaciens with 97% of 16S rRNA sequence information. Additional PCR-based genome screening was performed to identify the stains whose genes might associate with production of valuable secondary metabolite. The presence of potentially-valuable genetic information such as type I PKS, type II PKS and aminoglycoside phosphotransferase was screened from these NSRA strains followed by the establishment of genetic manipulation techniques.

Keywords: spent mushroom substrates, Bacillus amyloliquefaciens, hydrolyase

Formulation of Multi-Functional PGPR Bacillus subtilis and Pseudomonas Strains Having Phytophthora Blight Suppressing and Plant Growth Promoting Functions
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Multi-functional PGPR Bacillus subtilis AH18, B. licheniformis K11 and Pseudomonas fluorescens 2112 could promote the growth of root and stem on red pepper and suppress phytophthora blight against Phytophthora capsici. For the commercial production of these strains, Bacillus strains were formulated by heat-shocked endospores which could enhance their heat resistance. An optimal medium composition of Bacillus spp. is 2% glucose, 2% CLS, 1% ammonium sulfate, 0.6% K2HPO4, 0.4% K2HPO4, 0.3% MgSO4·7H2O and 0.05% MnSO4·5H2O and the optimal culture conditions are 37°C, 200 rpm, 2.5 air and 72 hrs. P. fluorescens 2112 cultured at 30°C, 200 rpm, 2.5 air and 24 hrs in same medium composition. For the maltreatment storage test, the heat-resistant endospores of Bacillus formulators were maintained for 6 weeks at 54°C and 7 days at 4°C. And that of P. fluorescens 2112 which was formulated with the inorganic carrier of zeolite was maintained for 12 weeks at 30°C and 7 days at 4°C. When P. capsici were treated by the concentration of 1x105 bacterial spores to the infested red-pepper plant on in vivo pot test, the only water-treated pepper in control pot were died above 40% of infested plant, whereas 3 PGPR consortium formulator-treated pepper plants could be survived up to 80%.

Keywords: heat-resistant endospores, PGPR, formulation

Isolation of Potentially Valuable Actinomycetes Strains via PCR-based Genome Screening
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Actinomycetes, gram-positive soil bacteria, are valuable microorganisms which produce useful secondary metabolites including antibiotics, antiparasitic substances, anti-cancer drugs, and immunosuppressants. Although a major family of actinomycetes called streptomycetes has been intensively investigated, a potentially-valuable non-streptomycetes rare-actinomycetes (NSRA) family is largely uncharacterized. In order to isolate NSRA strains among actinomycetes strain collection, a PCR-based genome screening was performed from 180 independently-isolated antifungal actinomycetes strains (kindly provided by KRIBB). Using a streptomycetes-specific PCR screening strategy [1, 2], we were able to screen out most of streptomyces species from the strain collection. The remaining several NSRA strains were then confirmed and classified based on 16S rRNA sequence information. Additional PCR-based genome screening was performed to identify the stains whose genes might associate with production of valuable secondary metabolite. The presence of potentially-valuable genetic information such as PKS, type I PKS and aminoglycoside phosphotransferase was screened from these NSRA strains followed by the establishment of genetic manipulation techniques.

Keywords: Actinomycetes, rare-actinomycetes, PCR-based genome screening

Effect of Biotic Cathode under Limited DO Concentration on the Current Generation
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DO conc. in cathode of microbial fuel cell (MFC) is very important factor to generate the current. DO conc. should be kept up to the 6.6 mg/L using the bare electrode and sustained up to 2.0 mg/L using the Pt electrode. This value is higher than 0.5 mg/L the critical DO conc. of aerobic bacteria. To know the effect of microorganism as catalyst in cathode, this study used the abiotic cathode of two chambers MFC with bare electrode and Pt electrode and the biotic cathode of single chamber MFC. microbial community of biotic cathode at open and closed circuits was composed of α-, β-, γ-, δ-proteobacteria and environmental samples. However the composition ratio of β-proteobacteria in closed circuit was 22.72% higher than that of open circuit. In the limited DO condition, the current of bare electrode and Pt electrode was greatly dropped from 2.5±0.1 and 4.1±0.1 mA to 1.6±0.1, 1.5±0.1, respectively. However the current generated from single chamber MFC dropped slightly and maintained 2.1±0.1mA. The current from MFC with biotic cathode was higher compared with that of abiotic cathode. From this result, microorganisms in cathode electrode influenced in current generation under limited DO condition. It might be including the bacteria which can use the electron as electron donor.

Keywords: Microbial fuel cell, Abiotic cathode, Biotic cathode, Electron donor
Characterization of an Esterase Possessing Chloramphenicol Acetate Esterase Activity
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Nowadays, Korea’s coastal sand dune is threatened by coastal erosion, desertification, overdevelopment and sea level rise caused by climate change. So, using sand dune plant rhizobacteria is by far the most effectively used method for restoration of decreasing sand dune plant ecosystem. Pseudomonas aurantiaca IBS-10 and IBS-14 could be verified production capacity of plant growth regulating hormone (abscisic acid, jasmonic acid, and natural cytokinin) as well as auxin (indole-3-propionic acid) by HPLC and GC-MS analysis. Also, Two strains had mechanism for microbial levan production. Microbial levan can increase tolerance against environmental stresses such as drought, salt and cold. The powder pilot products of P. aurantiaca IBS-10 and IBS-14 were stable on the 18 weeks at 30°C and 4°C. This result could set up guaranteed for a year by improvement and standard of thermal stability test (Rural development administration, Korea). When pilot products were treated on Imperata cylindrica var. kornigii under the high drought and salt condition, the stressed plant could be survive up to 80% for 10 days and the seed germination of Cylustegia soldanella was over 35% higher than non-treatment on the field test in Hwa-jin beach
Keywords: PGPR, microbial agent, sand dune plant

Application of Biofilm-Forming Bacteria for Improvement of Compressive Strength in Cement-Sand Mortar
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This study shows an application of biofilm-forming bacteria for improving compressive strength in cement-sand mortar. Thirteen strains were isolated from tetrapod blocks on the West Sea of Korea. Among these strains, Exiguobacterium marinum KNUC405 which showed the highest biofilm formation was selected for further study. The cement-sand mortar consolidated with the KNUC405 showed a 25% increase of compressive strength compared with negative control treated only with water. The strength improvement might be due to the biofilm formation within the pores of the cement-sand matrix as shown by the scanning electron microscopy. To verify the effect of biofilm formation, we used Dnase I for suppression of biofilm formation by KNUC405 in cement-sand mortar. Treated sample with Dnase I showed a bit lower compressive strength than that of non Dnase I treated one. This result suggests that biofilm formed by bacteria addition could roll as a binder to increase compressive strength in concrete mortar.
Keywords: Bioconcrete, compressive strength, Exiguobacterium marinum

Pseudomonas Alkylphenolia KL28 forms Specialized Multicellular Floating Bodies at the Air-Liquid Interface
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Pseudomonas often forms multicellular communities such as biofilms which allow them to survive under stressful conditions such as low nutrients, antibiotics, and/or desiccation. Pseudomonas alkylphenolia KL28 has been shown to form aerial structures in the presence of vaporized p-cresol. Interestingly when this strain was inoculated in Petri dish containing LB liquid medium, it formed unique circular flatten floating bodies. These structures are completely different from normal pellicles which usually appear like thin mats of cells at the air-liquid interface. Establishment of this architecture follows temporal distinct stages and entire process was completed within 48 h. These structures are usually stedy, hydrophobic and exhibit characteristic boundaries. Electron microscopy (SEM and TEM) study showed that fully matured structures are encased in a matrix containing extracellular polymeric substance. Mutations that alter the adhesion, motility and matrix formation showed direct influence on floating body development. The unique floating communities described in this investigation will be helpful in future studies aiming at understanding of bacterial multicellularity.
Keywords: Pseudomonas, multicellularity, biofilm

Compressive Strength in Cement-Sand Mortar
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Keywords: Bioconcrete, compressive strength, Exiguobacterium marinum

Sand Dune Plant Growth Promotion and Inducing Environment Stress Resistance by Multi-Functional PGPR from the Rhizosphere of Coastal Plants
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Keywords: Bioconcrete, compressive strength, Exiguobacterium marinum

Pseudomonas Alkylphenolia KL28 forms Specialized Multicellular Floating Bodies at the Air-Liquid Interface
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Pseudomonas often forms multicellular communities such as biofilms which allow them to survive under stressful conditions such as low nutrients, antibiotics, and/or desiccation. Pseudomonas alkylphenolia KL28 has been shown to form aerial structures in the presence of vaporized p-cresol. Interestingly when this strain was inoculated in Petri dish containing LB liquid medium, it formed unique circular flatten floating bodies. These structures are completely different from normal pellicles which usually appear like thin mats of cells at the air-liquid interface. Establishment of this architecture follows temporal distinct stages and entire process was completed within 48 h. These structures are usually stedy, hydrophobic and exhibit characteristic boundaries. Electron microscopy (SEM and TEM) study showed that fully matured structures are encased in a matrix containing extracellular polymeric substance. Mutations that alter the adhesion, motility and matrix formation showed direct influence on floating body development. The unique floating communities described in this investigation will be helpful in future studies aiming at understanding of bacterial multicellularity.
Keywords: Pseudomonas, multicellularity, biofilm
Novel Derivatives of Thiazolidinedione as Selective Algicides Against Red-Tide Harmful Algae: Heterosigma Akashiwo, Chattonella Marina and Cochlodinium Polykrikoides
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We screened the algicidal activity of 61 thiazolidinedione derivatives against microalgae causing harmful algal blooming. Also we designed the derivatives of TDs which showed selective and effective activity against harmful algae based on the analysis of structure-activity relations. Among the 28(1-series) compounds tested, most showed effective algicidal activity against Heterosigma akashiwo, Chattonella marina and Cochlodinium polykrikoides, while non-harmful algae were relatively tolerant to these thiazolidinedione derivatives. Compounds 1-6, 1-13 and 1-22 were the most potent against C. polykrikoides with IC50 values < 0.5 mM. The introduction of a chloride at the C2 and methyl group at the C3 of TD showed a great increase in inhibitory potency against red tied algae. The introduction of a methyl group at the amine group (2-29) rendered the derivative (2-14) increased the inhibitory potency of compound 2-1, whereas addition of more methylene group decreased the inhibitory potency. These results show that some thiazolidinedione derivatives can act as potent algicides to manage the growth of harmful algal species.

Keywords: Harmful algal blooms, Toxicity, Thiazolidinediones

Isolation and Characteristics of Hydrogen Sulfide Reduction Bacteria, Brevundimonas Diminuta
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Mixing oil cakes are known as sulfides, ammonia, and amins are emitted. The microorganism removes odor gas was purified from colonies eniched in vial where oil cakes were contained. In order to measure sulfides reduction ratio of the isolates, hydrogen sulfide reduction activity test was performed. Control groups are settled as follows: oil cakes(0.25g) in vial(100ml) was contained without inoculation. And experimental groups are prepared with inoculation as the same condition of control. Hydrogen sulfide removal efficiency of greater than 90% of isolate was identified as Brevundimonas diminuta by the morphological, and 16S rDNA sequence analysis. Brevundimonas diminuta was reported in the study for the oxidation of ammonia in other report.

Keywords: Brevundimonas diminuta, Hydrogen sulfide
**J-20**

**Growth of Dehalococcoides sp. During Degradation of Chlorinated Ethenes**

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An anaerobic enrichment culture derived from creek sediment reductively dechlorinated trichloroethylene (TCE) to ethene. We investigated the distribution and activity of chloroethenes-degrading microorganisms during reductive dechlorination of TCE to ethene in the enrichment culture, FMC-12T, which consistently and completely converted 2 mM TCE to ethene after 31 days and could dechlorinate TCE to ethene at an average rate of 64.5 μmole bottle-1 day-1. We investigated the bacterial dynamics in FMC-12T by the quantification of total bacteria, Dehalococcoides sp., Dehalobacter sp., and Geobacter lovleyi using real time PCR method. The 16S rRNA gene copy number were 1.51×10⁶ ± 2.47×10⁴, 1.14×10⁶ ± 1.48×10⁶, 7.91×10¹ ± 1.18×10¹, and 3.21×10¹ ± 2.19×10¹, respectively after dechlorination of TCE (after 31 days’ incubation). It has been known that the member of the genera Dehalococcoides sp., Dehalobacter sp., and Geobacter lovleyi could reduce tetrachloroethene (PCE) and TCE in terminal electron accepting processes. Dehalococcoides population increased much more than the other bacterial species during TCE dechlorination in FMC-12T. Therefore, the domination of Dehalococcoides sp. would seem to explain why FMC-12T was able to completely reduce TCE to ethene.

**Keywords**: Anaerobic culture, Ethene, Reductive Dechlorination

**J-21**

**Comparison of Disinfectants to Control Biofilm in Domestic Pipe System: Case with Ozone-GAC Treated Water**

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Feasibility of chlorine for the secondary disinfectant was investigated as the city plans to supply the citizens with drinking water treated by ozone and GAC. The advanced treatment water with ozone and GAC process was applied to the annular reactors with the sample pipe coupons of two pipe materials (copper, stainless steel=STS) were operated for 3 months. After confirming the biofilm was formed, chlorine and chloramines were added considering the field condition with the concentrations of 0.3 and 2.2 mg/L, respectively for another 3 months. The LRV’s to control biofilm were estimated to be 2.5 log for chlorine and 3.8 log for chloramines. When evaluating the effect of disinfection by pipe materials, copper was more effective in controlling suspended bacteria although the residual concentration of disinfectant was lower than in STS. Dissolution of pipe material was not detectable for STS while more copper was dissolved with chlorine than with chloramine (paired t-test, =0.05). THMs were reduced by 30~56% within the range of test. Although chlorine can be more effective with the same level of concentration, chloramine may be better in applying under the field condition as chloramine has better penetrating capability and little problem in DBPs, chlorine odor, and corrosion.

**Keywords**: Biofilm, Chlorine, Chloramine

**J-22**

**Peptide Guided Shape- and Size- Tunable Synthesis of Gold Nanostructures**

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Recently, there has been increasing interests in synthesizing well-designed, functional nanomaterials using biometric methods which mimic naturally occurring inorganic material synthesis systems in the organisms under environmentally benign conditions. Moreover, engineered bio-origin peptides that specifically recognize inorganic surfaces have been shown to be useful for the assembly and synthesis of inorganic nanostructures. In this report, we show the controlled, size- and shape-specific production of gold nanostructures under physiological conditions using a deodecapeptide, Midas-2 which is originally selected from a phage-displayed combinatorial peptide library. With analyses of TEM, SEM, AFM, UV-vis and AAS, the results show that the size (from a few nanometer to close to one hundred micrometer) and shape (nanoparticles, nanoribbons, nanowires and nanoplatelets) of the gold nanostructures produced are greatly influenced by single amino acid changes in Midas-2 and adjusting solution pH and gold ion concentration. The tunability to control the size and shape of the gold nanostructures by changing the peptide structure and reaction conditions will lead to many potential applications because of their unique size- and shape-dependent optical and electrical properties.

**Keywords**: peptide, gold, biological synthesis

**J-23**

**Indole as an Intercellular Signal in Microbial Community**

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Bacteria can utilize signal molecules to coordinate their behavior so as to survive in dynamic multispecies communities. Indole is widespread in the natural environment since a variety of both Gram-positive and Gram-negative bacteria (to date, 85 species) produce a large quantity of indole. Although it has been known for over a hundred years that many bacteria produce indole, the real biological roles of this molecule are only now beginning to be unveiled. As an intercellular signal molecule, indole controls diverse aspects of bacterial physiology, such as spore formation, plasmid stability, drug resistance, biofilm formation, and virulence in indole-producing bacteria. In contrast, many non-indole-producing bacteria, plants, and animals produce diverse oxygenases which may interfere indole signaling. It appears indole plays an important role in bacterial physiology, ecological balance, and possibly human health. Here we discuss our current knowledge and perspectives on indole signaling.

**Keywords**: indole, signal molecule, biofilm
Bioremediation of Contaminated Harbor Sediments Using Effective Microorganisms

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Effective microorganisms (EM) have been applied to the treatment of sediments contaminated with organics. Treatment with higher concentrations of EM loess balls (4%) and with a lower concentration of EM (0.1%) containing molasses (0.05%) contributed to a more rapid removal of malodor. GC analysis of the various treated samples as well as the EM fermented culture showed the variable profiles of organic acids produced. Acetic acid, propionic acid, valeric acid, capronic acid, and lactic acid were rapidly removed when molasses (0.05%, w/w) were used as a supplemental nutrient source, indicating the EM activity enhanced the removal of malodor. GC analysis of the various treated samples as well as EM (0.1%) containing molasses (0.05%) contributed to a more rapid remediation of sediments contaminated with organics. Treatment with higher concentrations of EM loess balls (4%) and with a lower concentration of EM (0.1%) containing molasses (0.05%) contributed to a more rapid removal of malodor. GC analysis of the various treated samples as well as the EM fermented culture showed the variable profiles of organic acids produced. Acetic acid, propionic acid, valeric acid, capronic acid, and lactic acid were rapidly removed when molasses (0.05%, w/w) were used as a supplemental nutrient source, indicating the EM activity enhanced the removal of malodor. GC analysis of the various treated samples as well as EM (0.1%) containing molasses (0.05%) contributed to a more rapid remediation of sediments contaminated with organics.

Keywords: effective microorganisms; sediments; organic acids, bioremediation
Application of Inclusion Bodies as Interaction Trap and Biocatalyst in living Escherichia coli cells
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Inclusion bodies are in vivo particles of misfolded polypeptides. The high-level expression of polypeptides with a C-terminal cellulose binding domain (CBD) provokes the synthesis of insoluble protein aggregates in Escherichia coli that are highly functional and catalytically active. In this study, green or red fluorescence proteins (GFP or RFP) with a C-terminal CBD produced fluorescent protein particles and the β-glycoside-hydrolyzing enzymes formed inclusion bodies with high catalytic activities, resulting in a 7–2.5 fold higher activity in the inclusion bodies than in the soluble fraction. Furthermore, the particles showed an increased catalytic activity when disaggregated by severe sonification, implying that the enzymes inside the particles were also highly functional. When a bait-CBD protein was expressed in E. coli together with a free prey protein, the latter was captured in the inclusion body, probably based on a specific interaction with the bait protein. Therefore, inclusion bodies induced by a C-terminal CBD can be a useful indicator of molecular interaction in living microbial cells. Plus, inclusion bodies with β-glycosidase activity can be repeatedly used as an immobilized biocatalyst for the hydrolysis of β-glycosides.

Keywords: cellulose binding domain (CBD), active protein particle, protein interaction

Shewanella-mediated Synthesis of Selenium Nanowires and Nanoribbons
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Amorphous selenium nanospheres, originally produced by Shewanella sp. strain HN-41 under the anaerobic conditions, can be rapidly transformed into extensive, long and thin, polycrystalline Se nanowires and nanoribbons (~100 μm x 57 nm) in 80% DMSO with bacterial pellets at physiological temperature. Scanning and transmission electron microscopic analyses indicated that the Se nanowires and nanospheres were crystalline structures indexed into the hexagonal plane of Se. The structures possessed an unusually high crystalline peak (100), suggesting a preferential [001] growth direction. Electron microscopic analyses and incubation studies suggested that the cell membrane of Shewanella sp. strain HN-41 likely plays an important role in the formation of amorphous Se nanospheres from soluble Se (IV) and the formation of long and thin h-Se nanowires and nanoribbons. The formation of zero- and one-dimensional h-Se nanostructures by this bacterium may provide a facile strategy to recover soluble Se (IV) from the environment and generate new materials that will be useful for advanced nanotechnologies.

Keywords: Shewanella, Selenium, Nanosphere and Nanoribbon

Ethanol and Acetate Production and Bacterial Community Structure During Syngas Fermentation by a Mixed Microbial Culture
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A strictly anaerobic bacterial consortium was enriched on carbon monoxide from an anaerobic digester sludge using repeated subculturing. The consortium utilized CO as the sole carbon and energy source and metabolized CO to ethanol, acetate, and CO2 as end products. At the early half stage of enrichment, there was much more production of acetate (1 - 2 g/L) than ethanol (40 - 200 mg/L); after that, ethanol production increased rapidly to 2 - 3 g/L. The change in the metabolites from CO gave evidence of ethanol fermenter enrichment and was further confirmed in the data of cloning and sequencing. While acetate was the major metabolite, the major clones were matched with the well known acetogens, Acetobacterium malicum and A. woodii similarities over 95%. However, the representative ethanol fermenters, Clostridium autoethanogenum and C. ljungdahlii were the closest relatives of the major clones with similarities over 93% at the end of the enrichment period. The characteristics of CO utilization and the composition of the obtained consortium are under study.

Keywords: CO, syngas fermentation, ethanol

Physiological and Metabolic Responses for Alkane Degradation in Acinetobacter sp. strain DRI
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The hexadecane degradation of Acinetobacter sp. strain DRI was evaluated with changes in temperature and ionic salt contents. Hexadecane degradation of strain DRI1 was reduced markedly by the presence of sodium chloride (but not potassium chloride) and high temperature (37°C ) was also shown to inhibit the motility, biofilm formation, and hexadecane biodegradation. The biofilm formation of strain DRI on the oil-water interface might prove to be a critical physiological feature for the degradation of hexadecane. The positive relationship between biofilm formation and hexadecane could be expected with changes in salts at 30°C, but not at low temperatures (25°C). Alterations in cell hydrophobicity and EPS production by temperature and salts were not correlated with hexadecane biodegradation. The results of proteomic analyses have demonstrated that proteins involved in fatty acid oxidation, the glyoxylate pathway, and gluconeogenesis are highly upregulated and many oxidative stress proteins have been identified as important for the efficient biodegradation of hexadecane.
Production of Bacteriocins by Coagulase-Negative Staphylococci Isolated from Vegetables
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Staphylococcus spp. including Staphylococcus aureus and coagulase-negative staphylococci (CNS) such as S. epidermidis are gram-positive cocci that are widespread in nature as the most important pathogen. Recently, the increasing prevalence of staphylococcal MRSA (methicillin-resistant Staphylococcus aureus) and MRSA accumulate additional resistance determinant, making them difficult to manage therapeutically. Because of these facts, new strategies for controlling MRSA and multiresistant staphylococci are required. Bacteriocins are a class of ribosomally synthesized antimicrobial peptides produced by bacteria. In this work the multiresistant S. aureus and Staphylococcus spp. were collected from various sources. They were tested antibiotics susceptibility, screened bacteriocin genes and bacteriocin activity to find new alternative agent for control. Our results showed that S. pasteurii and S. xylosus strains isolated vegetables in Korea produce broad spectrum to multiresistant S. aureus and gram positive bacteria. And S. pasteurii strains produce novel bacteriocin that is good candidate to control multiresistant staphylococcal strains and gram positive strains.

Keywords : MRSA, bacteriocin, Staphylococcus aureus

Microfluidic Analysis of Bacterial Chemotaxis
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This study present a microfluidic approach for the rapid analysis of bacterial chemotaxis using the generation of precise and stable gradients of signaling molecules. The laminar flow and diffusional mixing in the microfluidic device create a stable chemical gradient of concentration. The visual analysis of chemotactic responses results in the rapid analysis of chemotactic responses to chemoaffectors because bacteria can express green fluorescent protein (GFP). For the proof of concept, we have applied the microfluidic device to investigate the effect of attractant (peptone) and repellent (trichloroethylene; TCE) on the behavior of chemotactic responses with wild type Pseudomonas aeruginosa PAO1 and chemotactic mutant (PC4). The profile of population of bacteria indicates that Pseudomonas aeruginosa PAO1 are attracted to peptone and repelled from TCE but chemotactic mutant always shows nonchemotactic behavior.

Keywords : Chemotaxis, Microfluidics, Rapid analysis

Change of Quality of Apples by Using Isolation Bacteria min-sub JO 1, Keun-Hyung LEE1, Jung-Bok LEE2, Chun-Pyo JEON1, Do-Nyeon KIM2 and Gi-Seok Kwon*1
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Biological control can be used as an alternative to fungicides. It employs saprophytic microorganisms to protect fruits and vegetables from infection by pathogens. However, the control of postharvest diseases with antagonistic microorganisms is not often as consistent as control with synthetic fungicides. The period between harvesting and placing fruit in storage, from less than a day to a few days, requires rapid antagonist action. In this way, antagonistic microorganisms have been used in combination with fruit curing methods. In this study, the research was conducted to measure the effect of isolated bacteria of the quality(totalpolyphenol, hardness, acidity, soluble solid contents, decay rate) maintenance on apple during storage for 160 days at 0 0C. As a result, totalpolyphenol, hardness and soluble solid contents in isolated bacteria was higher value than control and pesticide. Decay rate in control, pesticide and isolated bacteria were 15%, 10%, 10% in 160 days. Therefore, it could be suggested that the isolated bacteria is more effective than other storage method in storage of apple. [This work (Grants No. 00037884) was supported by Business for Cooperative R&D between Industry, Academy, and Research Institute funded Korea Small and Medium Business Administration in 2010]

Keywords : Postharvest diseases, Apple
Evaluation of Humic Acid, Heavy Metals, and Salt on the Recovery of Murine Norovirus by Electropositive Filters

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The virus adsorption-elution technique (VIRADEL) using electropositive charged filters are commonly applied method for recovering enteric viruses from water. Molecular assay such as RT-PCR was to detect virus eluted from electropositive filters. For example, human norovirus (HuNoV), one of the most important waterborne pathogens, cannot be cultivated and is typically detected by RT-PCR assay. However, it is plausible that various inhibitors could be simultaneously concentrated during VIRADEL process and affect RT-PCR assays. We evaluated the effect of typical inhibitors on the recovery from two different electropositive filters using murine norovirus (MNV) as a surrogate for HuNoV. Viruses were analyzed by both real-time TaqMan reverse transcription (RT)-PCR assay and plaque assay. A known amount MNV was inoculated onto 1 L of surface water. Each of inoculated water samples include various concentrations of humic acid, heavy metals [Cd, Pb] and NaCl. Our results indicated that the presence of heavy metals, salt, or NaCl significantly reduced the recoveries of virus from two electropositive filters. These results suggest that it should be very cautious to analyze waterborne HuNoV using electropositive filters in environments with high concentrations of inhibitors or salts.

Keywords: Electropositive filter, Murine norovirus, Humic acid and Heavy metals

Isolation of Bacteria Capable of Both Heterotrophic and Autotrophic Denitrification from a Wastewater Treatment Process Fed with Spent Caustic

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Spent caustic (SC) from petrochemical plant could be applied to the sewage treatment process as an electron donor for autotrophic denitrification. We isolated the SC-utilizing denitrifier from the sewage treatment process fed with SC, and evaluated their denitrification characteristics. Three colonies were isolated from the anoxic tank of pilot treatment process fed with SC, and evaluated their denitrification. We isolated the SC-utilizing denitrifier from the sewage charged filters are commonly applied method for recovering enteric viruses from water. Molecular assay such as RT-PCR was to detect virus eluted from electropositive filters. For example, human norovirus (HuNoV), one of the most important waterborne pathogens, cannot be cultivated and is typically detected by RT-PCR assay. However, it is plausible that various inhibitors could be simultaneously concentrated during VIRADEL process and affect RT-PCR assays. We evaluated the effect of typical inhibitors on the recovery from two different electropositive filters using murine norovirus (MNV) as a surrogate for HuNoV. Viruses were analyzed by both real-time TaqMan reverse transcription (RT)-PCR assay and plaque assay. A known amount MNV was inoculated onto 1 L of surface water. Each of inoculated water samples include various concentrations of humic acid, heavy metals [Cd, Pb] and NaCl. Our results indicated that the presence of heavy metals, salt, or NaCl significantly reduced the recoveries of virus from two electropositive filters. These results suggest that it should be very cautious to analyze waterborne HuNoV using electropositive filters in environments with high concentrations of inhibitors or salts.

Keywords: Electropositive filter, Murine norovirus, Humic acid and Heavy metals

Modeling Transport of E. coli O157:H7 in Soils under Groundwater Irrigation

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It is unclear to what degree pathogen cells leach into the surrounding environment during irrigation events. However, it is expected that numbers will depend on a range of parameters, including the bacterial species and initial population, survival rate in the irrigated soil media, and soil type. Breakthrough column experiments were conducted with disturbed, 50-cm long sandy loam and river sand soil columns under conditions that would maximize the potential for bacterial transport. Flow velocity were 1.9-2.4, 2.3-2.6 cm hr-1 for sandy loam and sand soils, respectively. Escherichia coli O157:H7 concentrations spiked to the columns and in effluent were measured. Because of the apparent low recovery of E. coli O157:H7 in the sandy loam, only the observed data in the sand were used for modeling. Two-site model solution programmed in the HYDRUS-1D computer codes provided the optimized pathogen transport parameters as well as the R-square correlation coefficient between observed and simulated transport of E. coli O157:H7. It can be inferred that attachment and blocking are major mechanisms to retard bacterial mobility in the soils since the 2-site model has been developed to include terms of attachment and blocking in the governing equation of the water and solute transport.

Keywords: modeling, transport, E. coli O157:H7

Comparison of Dilution to Extinction Method and Plating Method for Isolation of Exoelectrogens from a Microbial Fuel Cell

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In microbial fuel cell (MFC), exoelectrogens which transfer electrons to electrode have an important role for electricity production. Identification and characterization of exoelectrogens are the most significant factors of increasing electricity yield. Most of the exoelectrogens are known as Fe (III)-reducing bacteria capable of reducing insoluble iron oxides. In order to isolate exoelectrogens, two different methods were used: dilution to extinction using U-tube MFCs and plating. Disparate microorganisms were founded depending on isolation methods in spite of the same source. DGGE analysis showed that certain microorganism became dominant through the dilution cycles using U-tube MFCs. Most dominant bacteria was identified as Ochrobactrum anthropi str. W-7. Plating method was conducted using enrichment medium for Fe (III)-reducing bacteria on agar plates. 6 isolates were identified and all of the strains were identified as Gram-positive Firmicutes. Through the electricity production test apply to U-tube MFCs, all strains produced current and maximum current density (23.8 mA/m2) was obtained. It is suggested that exoelectrogens also function as Fe (III)-reducing bacteria possible to be isolated from plating method and isolated bacteria could be vary with isolation methods.

Keywords: isolation, exoelectrode, dilution to extinction
Search for Heavy Metal Resistance Genes from Soil Metagenomic Library
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The presence of heavy metals in the environment can lead to the development of resistance in microorganisms. This study was undertaken to search for novel heavy metal resistance genes present in the environment. Soil samples were collected from an industrially contaminated site situated in Busan, South Korea. For the past 40 years, this site has been contaminated with industrial waste and organisms from phyla Proteobacteria, Firmicutes, Bacteroidetes and uncultured bacteria were found at this location as revealed by terminal-restriction fragment length polymorphism analysis. A metagenomic library, made in E. coli EPI300 using pCCIFOS fosmid vector, comprising of approximately 66,200 metagenomic clones was screened in order to search for clones that contained cadmium resistance genes. Fosmid DNA was isolated from selected clones, digested with BamHI and clones with similar restriction profile were removed. By performing transposon mutagenesis, we were able to find heavy metal resistance genes whose products showed high similarity with heavy metal translocating P-type ATPases. Further work related to defining the heavy metal resistance gene in the selected metagenomic clones is under progress. These clones are also being tested to check their resistance against other heavy metals.  
Keywords: Soil metagenome, Cadmium resistance genes, Industrially contaminated soil

Genomic Analysis of Novel BTEX Degrader, Pseudoxanthomonas Spadix BD-a59
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Pseudoxanthomonas spadix BD-a59, found to be responsible for the degradation of all BTEX compounds, was isolated on Minimal Salts Basal (MSB) agar containing 0.01% yeast extract with BTEX as the sole carbon source (Kim et al., 2008 AEM). We sequenced the whole genome of strain BD-a59 for the identification of the enzymes and regulators corresponding to the entire BTEX degradation pathways, using a 454 pyrosequencing technique. However, approximately 1,360 contigs were obtained, which might be caused by high G+C contents and limitations of pyrosequencing. By using the BLAST tool, we discovered TBDMD(toluene/benzene monooxygenase) and TMOA(toluene monooxygenase α subunit) genes responsible for BTEX degradation (contig 1154) and Xylene monooxygenase (XMO, contig 1338) as well as benzaldehyde dehydrogenase (BZDH, contig 548) that may be related to xylene degradation upper pathway. Fosmid libraries were constructed to assemble each gene and to discover the additional flanking regions between contigs and the others. Hybridization was carried out each target gene. A inter space between BZDH and XMO was found by using PCR amplification. Genomic constitution of tolulene/xylene degradation pathway and activities of each gene will be revealed soon.  
Keywords: BTEX, biodegradation, Pseudoxanthomonas sp.

Isolation and Characterization of Ethanogenic Acetogens from Animal Feces using Syngas Fermentation Bioreactor
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Due to rapid development of petroleum-based industry, there are many negative effects: decrease of reserve of petroleum and environmental pollution. Thus, possibility of bioethanol has been recognized as a kind of alternative energy. Syngas can be produced by the gasification of biomass at high pressure and temperature, and also it is utilized by microorganisms that can transform syngas into biochemical feedstocks such as fatty acids and alcohols. The main purpose of this research is to isolate and characterize ethanogenic acetogens from animal feces. In this research, bubble column reactor in which sintered glass filter was installed was used for improving mass transfer rate, carbonated buffered medium was used as medium (pH 7.0); mixed gas: CO, H2, CO2 (4:4:2) was supplied at the rate of 77.2 mL/min; animal feces (0.5% (w/v)) were used as inoculum sources. The reactor was operated at temperature of 30℃. As a result, 18 isolates were isolated and identified by 16s rRNA gene sequence.  
Keywords: Bioethanol, Synthesis gas, Acetogens, Bubble column reactor, 16s rRNA gene analysis

Characterization of Photosynthetically Activating Compounds (PACs) produced from Purple Nonsulfur Bacteria and Its Application to Enhance Physiological Responses of Fermenting Microorganisms
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In purple nonsulfur photosynthetic bacteria, carbon dioxide fixation has been intensively investigated; and through the Calvin reductive pentose phosphate pathway by ribulose 1,5-bisphosphate carboxylase/oxygenase, this organism can produce organic compounds such amino acids, the nucleic acid material, a vitamin, and bio-active substances. Therefore, this study is focused on analyzing the properties of organic compounds and the impact on physiological activation of other microorganisms by carbon dioxide fixed organic compounds (PACs). In this study, the changing in saccharide, amino acid content and viscosity of the PACs was investigated through culture conditions of the purple nonsulfur bacteria. In addition, our data indicated that the addition of the PACs into culture media accelerated growth rates of Escherichia coli, Saccharomyces cerevisiae and Clostridium acetobutylicum as well as enhanced the utilization of xylose and production of ethanol in recombinant S. cerevisiae. Therefore, the data provides strong evidence that PACs have the multifunctional potential to substantially increase the physiological activity of other organisms. It is determined as the new material which can force to activate the fermentation promotion of the industrial fermentation organisms.  
Keywords: Purple nonsulfur photosynthetic bacteria, Physiological activation, Fermenting Microorganisms
Variation of Bacterial Community in Soil Culture without or with Containing Xenobiotics and Isolation of Xenobiotic-degrading Bacteria

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Forest soil was incubated at 20°C for 10 weeks, to which 7 xenobiotics that are tetrathylenglycol (TEG), 1-aminopropan-2-ol (APOL), diethylene glycolmonomethylether (DGMME), diethylene glycolmonoethyl ether (DGMEE), diethylene glycol (DGE), monomethylammonium hydroxide (TMAH) were added at 1%. DNA was directly extracted from the soil culture before and after addition of xenobiotics at the intervals of 3 weeks during incubation. Temperature gradient gel electrophoresis (TGGE) patterns for variable region (V3) of 16S-rDNA amplified with DNA extracted from both soil cultures without and with containing xenobiotics were a little changed according to incubation time. Xenobiotics variation in the soil culture was analyzed at the intervals of 1 week using GCMS and LCMS. TEG, DGMME, DGMEE and TMAH were more effectively mineralized in comparison with APOL, MP, and THT in the soil culture. TEG-, APOL-, DGMME-, DGMEE-, THT-, MP-, TMAH-degrading bacterium was Burkholderia sp SK100101, Burkholderia sp SK100102, Burkholderia sp SK100103, Burkholderia sp SK100104, Phyllobacterium sp SK100105, Burkholderia sp SK100106, Acidovorax sp SK100107, respectively, isolated from the soil culture containing xenobiotics.

Keywords: xenobiotic compounds, TGGE, bacterial community, soil microcosm, forest soil

Prevalence of Pathogens in a Survey of Organic Fertilizer in Korea

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In recent years, there has been an increasing public concern for fecal contamination of water, air and produce by pathogens residing in organic fertilizer such as manure, compost and agricultural by-products. Efforts are now being made to control or eliminate the pathogen populations at on-farm level. Development of efficient on-farm strategies to mitigate the potential risk posed by the pathogens requires data about how the pathogens prevail in the organic fertilizer. Microbiological analyses of commercial organic fertilizer from 28 companies were conducted to determine the total aerobic bacteria and coliform counts and the prevalence of E. coli, S. aureus, B. cereus, Salmonella spp., E. coli O157:H7, L. monocytogenes, and E. sakazakii. The range of aerobic plate counts was 3 to 7 log10CFU/g. Coliforms were detected only in samples from 4 companies and the range was 1 to 3 log10CFU/g. E. coli and E. sakazakii were detected in 4 and 11% of samples, respectively, while the other pathogens were not detected. Although we cannot conclude that pathogens of the organic fertilizer are a major source of environmental and produce contamination, there is no doubt that they could be involved in cross-contamination if appropriate measures are not taken.

Keywords: fertilizer, prevalence, pathogen

Characterization of a Novel Lipase Responsible for 3-Hydroxypropylmalic Acid Methyl Ester Hydrolysis from Industrial Waste Contaminated Soil Metagenome

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Soil metagenomic libraries were constructed in Escherichia coli using fosmid and metagenomic DNA isolated from industrial waste contaminated soils samples. A total of 142 lipolytic fosmid clones were found by screening 123,800 clones based on tributyrin-hydrolyzing activity. A clone designated LP96, which showed degradation activity towards 3-hydroxypropylmalic acid methyl ester (3-OH PAME), which is a quorum-sensing signal molecule that controls virulence in Ralstonia solanacearum, was completely sequenced. A subclone, designated LP96B, which contained only the lipolytic gene, showed 3-OH PAME degradation activity. DNA sequence analysis revealed that LP96B contains an ORF encoding a 278 amino acid protein with a predicted molecular weight of 30.98 KDa. Phylogenetic analysis of the deduced amino acid sequence revealed that the lipolytic protein belongs to the lipase/esterase family V. Lipolytic gene was over-expressed using pET-30B (+) in E. coli BL21(DE3). The over-expressed, purified lipolytic enzyme showed degradation activity towards 3-OH PAME. Our results indicate that a novel lipase (beta-hydroxypropyl malic acid methyl ester hydrolase) was discovered from the soil metagenome that will be used to control plant infection by Ralstonia solanacearum.

Keywords: Metagenome, Novel lipase, 3-OH PAME
In Vivo Antifungal Activities of Microbial Agents against Brown Patch of Creeping Bentgrass

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Brown patch by Rhizoctonia solani AG-1(1B) is a fungal soil disease that causes a serious threat to turfgrass in Korea. Pseudomonas spp. are gram-negative soil bacteria that have been widely used as biocontrol and growth-promoting agents. Microbial agents (P. trivialis YDU-O-5G-1-3, P. jessenii YDU-O-5G-3-3, P. mandelii YDU-F-6F-1, and P. fluorescens YDU-F-3R-3) originated from soil were evaluated for in vivo control effect of brown patch disease in creeping bentgrass. The four biocontrol agents showed strong in vitro antagonistic activities against the brown patch pathogen. After freeze vacuum drying of liquid cultures in several nutrients and with cryoprotectants, the formulated powder of four isolate were also elucidated to be effective for high antifungal in vivo activities against the pathogen. In particular, two bacterial agents (P. jessenii YDU-O-5G-3-3 and P. mandelii YDU-F-6F-1) with 105 cells/ml had inhibition values over 75% against the pathogenic fungus. Based on these results, the isolates would seem to be good candidates as biocontrol agents. They are currently undergoing a field study.

Keywords: microbial agent, Pseudomonas spp., antifungal

Drought Stress Resistance Induction of Pepper by Multi-Functional PGPR Bacillus licheniformis K11

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Plant tissues increase ethylene level under the environmental excessive stress condition such as salt or dryness. An increased concentration of ethylene in plants can cause the inhibition of plant growth. The ACC deaminase produced by PGPR can reduce the plant’s ethylene concentration by cleaving the ethylene precursor ACC. B. licheniformis K11 has ACC deaminase activity for the reduction of environmental stress. Even under high salt stress, pepper plants could subsist 15 days of drought, whereas the PGPRs treated pepper plants to tolerate such as salt and drought. Untreated pepper and tomato were dead after 15 days of drought, whereas the PGPR treated pepper plants were able to tolerate the drought stress. Even under high salt stress, pepper plants could subsist such as salt and drought.

Keywords: ACC deaminase, PGPR, Drought stress

Mutation of a Gene Encoding Glycosyltransferase Affects Lipopolysaccharide Biosynthesis and Cell Surface-Related Properties in Pseudomonas Alkylphenolia KL28

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Cell surface components such as lipopolysaccharide (LPS) and exopolysaccharide (EPS) play an important role in the bacterial adaptation in various environments. In the presence of vaporized p-cresol, Pseudomonas alkylphenolia KL28 forms specialized aerial structures (SAS). A transposon mutant of strain KL28 (C23) incapable of forming mature SAS was isolated to characterize the gene involved. Genetic analysis of the C23 mutant revealed the transposon insertion in a gene encoding an uncharacterized glycosyltransferase (named Ssg protein). The ssg deletion mutant of KL28 produced truncated LPS in which O antigen was completely absent. The ssg mutation also greatly influenced the composition of EPS. Wild type strain KL28 produced fucose, glucose, and mannose-rich EPS, while the mutant EPS completely lacked fucose and mannose, with being a glucose as a major component. The mutant strain showed reduced activities of spreading on soft agar, attachment to surface and biofilm formation. Our results show that the ssg gene of KL28 is involved in both EPS and EPS biosynthesis and plays an important role in multicellular behavior of strain KL28.

Keywords: Pseudomonas, glycosyl transferase, lipopolysaccharide

Phenotypic and Physiological Changes in Acinetobacter sp. strain DRI with Exogenous Plasmid

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The genus Acinetobacter is well-known to uptake exogenous DNA from the environment. In this study, we performed natural transformation with a novel diesel-degrading Acinetobacter sp. strain DRI, using the broad host range plasmid, pRK415. Many factors, including temperature, quantities of DNA, and aeration have proven important for efficient natural transformation. Interestingly, Acinetobacter sp. strain DRI(pRK415) differed phenotypically and physiologically from the wild-type strain in several aspects, including motility, biofilm formation ability, and responses to oxidative stress: the transformed cells became more sensitive to hydrogen peroxide and cumene hydroperoxide, and their motilities and biofilm formation activity decreased. Our data indicated that caution should be exercised when performing genetic manipulation with plasmids, owing to the possibility of phenotypic and physiological changes of the host occurring along with plasmid uptake.

Keywords: Acinetobacter, Natural transformation, Exogenous plasmid
**Selection of Heptamer Peptides Specifically Binding to Lead Ion**
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Lead is widely recognized as a toxic heavy metal causing serious nerve diseases. Most of the lead poisoning patients suffered from the lead substances of paint and drinking water which are supplied through pipeline. For these reasons, sensing lead contained in paint and drinking water is necessary for public health. In spite of these needs, its research still is not developed as well. Peptide display phage library is a strong screening tool that is more flexible and easier to handle than other peptide screening systems. It can be used to screen against not only organic compound but also inorganic substances, such as metal ions. To select peptides specifically binding to lead ion, we conducted biopanning using lead ion immobilized on a chelating sepharose with peptide display phage library. We selected five peptide sequences which appeared most frequently. These repeated sequences showed an average of 30% higher binding affinity than negative control in enzyme immunoassay (EIA). One peptide sequence, STLPLPP, caused thermodynamic change when bound to lead ion, whereas it did not cause any thermodynamic change with Cadmium as determined by isothermal calorimetry.

**Keywords**: lead, phage display library, biopanning

**Analysis of Microbial Communities in Soil Cultivated with Genetically Modified Chinese Cabbage using Real-Time PCR-DGGE**
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The potential impacts of transgenic cabbage SKCP (Brassica rapa L. ssp. pekinensis, overexpression of myrosinase gene) on soil microbial communities were analyzed using both culture-dependent and culture-independent methods. The microbial population dynamics (bacteria, actinomycetes, and fungi) were not different between SKCP and control cabbage SK throughout the experiments. PCA of the fatty acid methyl ester (FAME) profiles and Real-time PCR-DGGE analysis of 16S rDNA were quite influenced by environmental factors such as seasonal fluctuations and field site.

**Electrochemical Suppression of Methane Generation from the Lake Sediment.**
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In this study, suppression of methane gas generation from the lake sediment was investigated using an electrochemical method. The gas suppression system was constructed with a conventional three-electrode system and a potentiostat. The working electrode was put into the lake sediment. Counter electrode and reference electrode were installed around working electrode, and potential (vs reference electrode) was applied. In the present applied potential (0.3V), the generation of CH4 was decreased by 25% compared with that of control (without potential). After 40 days of operation, the maximum suppression rate of CH4 gas generation was observed. In the presence of the applied potential, about 50% (2,980 mg/day) of decrease in CH4 gas generation achieved (control:5,940 mg/day). These experimental results suggested that the attachment of electrode may induce the change of environment of sediment, and change the conventional metabolism of the CH4 producing microorganisms.

**Production and Optimization of a Protease from the Micrococcus sp.**
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Microbial proteases are the most important group of industrial enzymes such as food, leather, detergent, pharmaceutical and waste management industries. We isolated prolylendopeptidase bacteria, which was identified as Micrococcus sp. based on 16S rDNA sequence and biochemical characterization. In the present investigation, the protease production and activity optimum were determined for a newly isolated, under various chemical and physical conditions. A modification of kunitz method was used for protease assay. As a result, the maximum protease was produced in the presence of 2% skim milk at 30°C. Also maximum enzyme activity was exhibited at pH and temperature of 9.0 and 37°C, respectively. Moreover, the enzyme activity was strongly inhibited by PMSF, EDTA and EGTA. The result demonstrates that it probably belongs to the subclass of serine-metallo proteases.

**Keywords**: Alkaline protease, Micrococcus sp., Characterization protease