amine group via hydroxylamines. Hydroxylamino-dinitrotoluenes (2- and 4-OHAmDNT) and amino-dinitrotoluenes (2- and 4-AmDNT) were identified as the intermediates. A membrane-associated aromatic nitroreductase activity was detected in a cell-free extract of I. lacteus. This enzyme catalyzed the nitro group reduction of TNT and required NADPH as a cofactor. This enzyme activity was not observed in the presence of molecular oxygen.

B302

Transformation of 2,4,6-Trinitrotoulene (TNT) by the Bacterium Isolated from Activated Sludge

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A 2,4,6-trinitrotoluene (TNT) degrading bacterium was isolated from activated sludge of sewage treatment plant, and its biodegradation capability was examined. TNT was completely disappeared within 6 hours of further incubation when 100 mg/L of TNT was added into the bacterial culture which had been preincubated for one day. However, this bacterium was unable to use TNT as a sole source of carbon. TNT degradation might be accomplished by a cometabolic process using glucose as a growth substrate. 2-Hydroxylamino-4,6-dinitrotoluene and 4-hydroxylamino-2,6-dinitrotoluene were identified as the first detectable degradation products of TNT and their transient accumulation and conversion to other metabolites were observed. 2-Amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene were detected as the following detectable metabolites of hydroxylaminodinitrotoluene (OHAmDNT) isomers and subsequently metabolized to diaminonitrotoluene (DANT) isomers. Dinitrotoluene (DNT) and nitrite were also formed from a denitration pathway, and this suggested that this bacterium also used two different pathways simultaneously for TNT biodegradation as in a study with Irpex lacteus. Some other effects of environmental factors on TNT transformation by the isolated bacterium were also examined.

B303

Cloning and Nucleotide Sequence Analysis of *xylG* Encoding 5C-2HMS Dehydrogenase from *Pseudomonas* sp. S-47.

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Pseudomonas sp. S-47 is capable of degrading 4-chlorobenzoate to produce 5-chloro-2-hydroxymuconic semialdehyde (5C-2HMS) by the enzymes encoding by *xylXYZLTE* cluster. The resulting 5C-2HMS can be transformed to 5-chloro-2-hydroxymuconic acid (5C-2HMA) by 5C-2HMS dehydrogenase. In this study, *xylG* gene encoding 5C-2HMS dehydrogenase was cloned from the chromosomal DNA of strain S-47. The nucleotide sequence of *xylG* showed to be composed of 1,600 bp with ATG initiation and TGA termination codons. A deduced amino acid sequence of the 5C-2HMS dehydrogenase (XylG) exhibited 98%, 93%, and 89% identity with those of the dehydrogenases from *Pseudomonas putida* mt-2, *Pseudomonas putida* G7, and *Pseudomonas* sp. CF600, respectively.

B304

Cloning of 4CBA Dechlorinase Genes (*fcbABC*) from *Pseudomonas* sp. DJ-12 to Expand the Biodegradable

Range of Pollutants

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Dechorination is one of the most critical steps for biodegradation of 4-chlorobenzoate (4CBA) which is a metabolite from various chlorinated herbicides and PCBs. *Pseudomonas* sp. DJ-12 is a natural isolate capable of degrading 4CBA via hydrolytic dechlorination. The hydrolytic dechlorination of 4CBA is carried out in a series of reaction by the enzymes of 4CBA-CoA ligase, 4CBA-CoA dechlorinase, and 4HBA-CoA thioesterase which are encoded by the *fcbA, B,* and *C,* respectively. In this study, the *fcbABC* genes were cloned from chromosomal DNA of *Pseudomonas* sp. DJ-12 using pBluescript SK(+) as a vector to get the pKC117. The transformant cells including pKC117 exhibited dechlorination activity to 4CBA by producing 4-hydroxybenzoate (4HBA) and chloride ions. The pKC117 was also transformed into other bacterial hosts with benzene-ring cleavage activity for aromatic compounds, in order to develope the strains with expanded range of pollutant degradation.

B305

Cloning and Sequencing of Esterase from *Acinetobacter lwoffii* I6C-1

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The esterase-encoding gene, *est*A, was cloned from *Acinetobacter lwoffii* I6C-1 genomic DNA into *Escherichia coli* DH5a with plasmid vector pET-22b (pEM 1). pEM 1 has a 4.4 kb EcoR I insert that contained the complete *est*A gene. A 2.4 kb Ava I-Sph I DNA fragment was subcloned (pEM 3) and sequenced. *est*A gene encodes a protein of 366 amino acids (40,687 Da) with pI of 9.17. The EstA signal peptide was 31 amino acids long, and the mature esterase sequence is 335 amino acids long (37.5 kDa). The conserved catalytic serine residue of EstA is in position 210. The EstA sequence was showed similarity to that of the carboxylesterase from *Acinetobacter calcoaceticus* (75% identity, 85% similarity), *Archaeoglobus fulgidus* (37% identity, 59% similarity), and *Mcobacterium tuberculosis* (35% identity, 51% similarity). These enzymes contained the conserved motif G-X_1-S-X_2-G carrying the active-site serine of hydrolytic enzyme. The EstA formation starts at the end of growth phase, and its activity level remains constant throughout stationary phase. Esterase activity in *E. coil* BL21 (DE3) with pEM 1 was similar to *A. lwoffii* I6C-1.

B306

Observation of Phyllosphere Bacteria on Leaf Surfaces by Epifluorescence Microscopy

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Epifluorescence microscopy was used to observe epiphytic bacteria directly on plant leaf surfaces as well as indirectly in the leaf liberating solution by staining with fluorochromes of 4',6-diamidino-2-phenylindole (DAPI) and acridine orange (AO). Epiphytic bacteria could not be well observed on the leaf surface by staining with AO due to an intrusive orange or red background fluorescence. However, DAPI gave us clear epifluorescent images of the bacteria on the leaf. On the contrary,