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.

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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.

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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,

epiphytic bacteria in the liberating leaf solution were well observed on filters stained by both types of fluorochrome, although DAPI showed better fluorescent images than AO and not necessarily required a washing step of the filters stained. The optimum conditions of the DAPI stains were 5 mg ml^{-1} for 5 min both for leaves and for filters of the liberating solution. It was confirmed that a critical step in the epifluorescence microscopy of leaf surfaces was to minimize release of water from the leaf. For this, the stained leaf samples were put on a filter paper, kept in a dry oven at 70℃ for 2 min instead of air-drying, and then immediately observed by epifluorescence microscopy. The established technique was applied to enumerate epiphytic bacteria on oak tree leaf surfaces.

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Isolation and Phenotypic Characterization of 2-(2,4-Dichlorophenoxy)-Propionic Acid Degrading Bacteria from Soils

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Eight numerically dominant 2-(2,4-dichlorophenoxy)-propionic acid(2,4-DP)-degrading bacteria and four pairs of bacteria showing syntrophic metabolism of 2,4-DP were isolated from soils, and their phylogenetic and phenotypic characteristics were investigated. The isolates were able to utilize the herbicide 2,4-DP as a sole source of carbon and energy and their 2,4-DP degradative enzymes were induced by the presence of 2,4-DP. Analysis of 16SrDNA sequences indicated that the isolates were related to members of the genera, Sphingomonas, Herbaspirillum, and Afipia. The chromosomal DNA patterns of the isolates obtained by polymerase chain reaction(PCR) amplification of repetitive extragenic palindromic(REP) sequences were distinct from each other. Nine of the isolates were observed to have plasmids, but none of them was transmissible. Many of the isolates degraded 2,4-D, MCPP, and MCPA as well as 2,4-DP. Oxygen uptake experiments indicated that most of the isolates degraded 2,4-DP through 2,4-dichlorophenol.

B308

Catabolic Potential of *Pseudomonas rhodesiae* Strain KK1 Isolated from PAH-Contaminated Soil at a Former Manufactured Gas Plant (MGP) Facility

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Catabolic potential of strain KK1 from polycyclic aromatic hydrocarbons (PAH)-contaminated soil at a former manufactured gas plant (MGP) facility was analyzed using dioxygenase clonal library and radiospirometry. The coal tar-contaminated soils at the MGP site had experienced significant PAH contamination for at least 100 years. Strain KK1 was identified as *Pseudomonas rhodesiae* using BIOLOG analysis system, fatty acid analysis, and 16S rDNA sequencing, and designated *Pseudomonas rhodesiae* KK1. To evaluate catabolic potential of *Pseudomonas rhodesiae* KK1, total DNA was extracted from KK1 cell and dioxygenase genes were amplified using the PCR process with random dioxygenase