S7-1

# Studies on Bacterial Ecology in a PAH-Contaminated Surface Soil and the Biodegradation Potential of *Pseudomonas rhodesiae* KK1 for Aromatic Hydrocarbons

Hyung-Yeel Kahng

*Department of Environmental Education, Sunchon National University*

A few recent studies on genetic diversity have been mainly conducted with microbial populations or many bacteria. This study focuses on analyzing diversity of dioxygenase genes of a bacterium, *Pseudomonas rhodesiae* KK1 capable of utilizing a broad range of monoaromatic compounds including polycyclic and heterocyclic aromatic hydrocarbons such as carbazole and naphthalene previously mentioned. For the analysis of dioxygenase genes, the Rieske-type iron sulfur center sequences of dioxygenases were selected, because every large subunit of a dioxygenase enzyme contains a Rieske-type iron sulfur center, as well as the iron-sulfur center has two characteristic amino acid sequence motifs surrounding a region of amino acids whose sequence varies from enzyme to enzyme.

Analysis of the MGP soil used in this study revealed that it was heavily contaminated with various PAHs such as anthracene, benzo(a)pyrene, carbazole, chrysene, naphthalene, phenanthrene, and pyrene. 1685 μg of naphthalene, 91 μg of benzo[a]pyrene, 700 μg of carbazole, 162 μg of chrysene, 223 μg of fluorene, 539 μg of phenanthrene, and 171 μg of pyrene were extracted per gram soil of the top layer (0-5 feet below surface). The MGP site has been maintained for over 100 years since it was contaminated with polycyclic aromatic hydrocarbons. Accordingly, it was assumed that microorganisms found in the PAH-contaminated environment had developed diverse enzyme systems for survival. A consortium of capable of a variety of PAH was isolated from a MGP site. Molecular analysis of 16S rRNA clonal library revealed that the microbial clones from this site were distributed among the genera *Acidovorax, Arthrobacter, Aquaspirillum, Burkholderia, Clostridium, Escarpia, Geobacter, Pseudomonas, Rhodoferax, Stenotrophomonas*, and unidentified. The most abundant clones were *Stenotrophomonas* (26 %), *Acidovorax* (21 %), *Rhodoferax* (16 %), *Escarpia* (10 %), and *Pseudomonas* (6 %). The consortium obtained from PAH enrichment was composed of *Alcaligenes* sp., *Arthrobacter* sp., *Burkholderia* sp., *Ochromobacter* sp., and *Pseudomonas* sp. KK1. Strain KK1 identified as *Pseudomonas rhodesiae* was selected due to its ability to grow rapidly on a mixture of

PAHs to evaluate its catabolic potential to catabolise the aromatic hydrocarbons including the PAH mentioned above.

KK1 cells grown on TSA were tested for their ability to utilize aromatic hydrocarbons including PAHs under aerobic conditions. KK1 was able to grow on the solid minimal medium containing anthracene, naphthalene, phenanthrene, carbazole or benzene however it could not degrade benzo[a]pyrene, chrysene, and pyrene during an equivalent incubation (Table 2). Also, KK1 cells were found to grow on the solid minimal medium containing 1 mM benzoate, biphenyl, *p*-cumate, or vanillate, but no growth were observed in aniline, fluorene, 2,4-D, dioxin, phthalate, or toluate, or toluene medium at the same incubation time point.

Strain KK1 was tested for its ability to degrade PAHs in mineral salts medium under aerobic conditions using radiorespirometry. KK1 were able to mineralize anthracene, naphthalene, and phenanthrene within 10 days of incubation, however it could not degrade benzo[a]pyrene, chrysene, and pyrene during an equivalent incubation. At the 10-day incubation time point, approx 1.8% of anthracene was mineralized, while 13% of naphthalene, 20% of carbazole, and 22% of phenanthrene were mineralized. A single colony of KK1 cells grown on TSA was also tested in the liquid minimal medium containing aniline, benzoate, biphenyl, *p*-cumate, 2,4-D, toluate or vanillate to evaluate the catabolic potential for 48 hr incubation. Reverse phase HPLC analysis revealed that approx 90% of benzoate, 68% of biphenyl, 78% of *p*-cumate, and 78% of vanillate were transformed at the same incubation time point. However, bioconversion of aniline, 2,4-D, or toluate by strain KK1 was not observed.

Total DNA extracted from strain KK1 was analyzed for the presence of genes for dioxygenases capable of hydroxylating inactivated aromatic nuclei using a specific PCR primer set. PCR products were cloned and 50 randomly selected clones were sequenced. Comparative analysis of predicted amino acid sequences indicated that the dioxygenase clones from strain KK1 could be divided into 5 groups, suggesting that KK1 strain has diverse genes for catabolism of aromatic hydrocarbons. Deduced amino acid sequence of Group 1 (G1: 5'-CRHRGKTLVSVEAGNAKGFVCSYHGW) showed 100% similarity with *NdoC2* of naphthalene dioxygenase from *Pseudomonas putida* ATCC17484. 24% of fifty clones analyzed were included in G1. Group 2 (G2; 5'-CRHRGNKVCFAEAGNARGFICSYHGW) shared 76% similarity in deduced amino acid sequences with *BphA1b* of biphenyl dioxygenase from *Sphingomonas aromaticivorans* F199 and 69% similarity with *PhnAc* of phenanthrene dioxygenase from *Burkholderia* sp. RP007. Group 3 (G3; 5'-CRHRGATVCEHKKGKTNSFVCCYKGW) was found to share 50-64% similarity with carbazole, *p*-cumate, or benzoate dioxygenase based on deduced amino acid sequence, suggesting the genes for carbazole or benzoate catabolism in strain KK1 might be diverged from other known dioxygenase genes. Group 4 (G4; 5'-CRHRGAAPSLGAVENGERVCCYHGW) showed extensive homology with *VanA* of vanillate *o*-demethylase from *Acinetobacter* sp. ADP1, *Ralstonia solanacearum* GM11000, or

*Pseudomonas fluorescens* BF13. 18% of total clones were included in G4. Group 5 (G5; 5'-CRHR(S)GKTLVSVEPAMPKVLFVLITAG; 5'-CRHSGKTLVSVEPAMPKVLFVAITAG) was relatively close to naphthalene dioxygenase, but demonstrated high divergency in deduced amino acid sequence. Besides, unknown dioxygenase has been found.

Northern hybridization was carried out to analyze the expression pattern of KK1 dioxygenases in response to PAHs such as naphthalene and phenanthrene using one representative from G1 or G2 as a probe. It indicated that each one of representative probes gave strong positive signals in response to naphthalene or phenanthrene while all of them gave negative signals in response to cells grown with glucose, providing further evidence for the existence of dioxygenases for catabolism of polycyclic aromatic hydrocarbons in strain KK1.

**Acknowledgement**

This work was supported by Korea Research Foundation Grant (KRF-2003-003-C00128).