

SDS-PAGE. The optimal pH and temperature were pH 10 and 60 °C, the enzyme was stable between pH 8 and 11 and below 70 °C. The K_m and V_{max} were estimated as 4.8 mg/ml and 58 unit/ml, respectively. The enzyme was markedly inhibited by Cu^{++} and Ca^{++} at 1mM concentration.

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Evidence for the Role of Glutathione S-transferase (BphK) in Adaptive Responses during the Degradation of Aromatic Compounds by *Sphingomonas yanoikuyae* Strain B1

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Sphingomonas yanoikuyae strain B1 is able to metabolize a wide variety of aromatic compounds. This versatile catabolic ability is apparently due to the relaxed specificity of the initial degradative enzymes of separate upper metabolic pathways, which channel many structurally different aromatics into central metabolites such as benzoate and *m*-toluate. The latter compounds are further degraded by products of a TOL-palmsmid type *meta*-operon. Interestingly, a putative gene for glutathione S-transferase (*bphK*) was found in the *meta*-operon of B1. Even though the function of this gene product is unknown, GST has also been found in operons for other aromatic degradation pathways. In an effort to determine the biological function(s) of the GST in B1 an insertional knockout mutant strain MB3 (*bphK::Km*) was constructed. Unfortunately, the mutant strain showed no phenotypic changes when the growth of MB3 was analyzed on aromatic compounds, except

that it shows much longer lag period on *m*-toluate than B1. Subsequently, the induction patterns of GST were examined in the cells of B1 grown on *m*-toluate, benzoate, *m*-xylene and biphenyl, respectively. GST was found to be induced in B1 grown on *m*-toluate or *m*-xylene while no GST activity was detected in the cells grown on benzoate or biphenyl. This means that GST is induced specifically by *m*-toluate or its metabolites. Subsequent complementation experiments with MB3 demonstrated that a subclone containing only the *bphK* gene is able to reduce the lag period of MB3 on *m*-toluate same as that of B1. Based on current experimental data, GST in B1 is thought to play a role in an adaptation response(s) to chemical stress, which might be caused by certain aromatic compounds or their metabolic intermediates.

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A Novel Acetaldehyde Dehydrogenase (XylQ) Implicated in the Degradation of Both Aromatic and Aliphatic Hydrocarbons by *Sphingomonas yanoikuyae* Strain B1

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Previous studies showed that the same genes are involved in the degradation of various aromatic hydrocarbons by *Sphingomonas yanoikuyae* strain B1. In particular the ferredoxin (BphA3) and reductase (BphA4) components are involved not only as components in the initial ring-oxidizing dioxygenase but also as components in toluate dioxygenase. Recently, it was found that B1 is also able to mineralize C₄ to C₁₆ alkanes including

butane, *n*-hexane, *n*-octane, *n*-dodecane, and hexadecane. It is interesting to observe that an insertional mutant strains EK385 (*bphC::Km*) and MB1 (*bphA4::Km*), which lost BphA3 and BphA4 activities, are also unable to grow on all of the alkanes tested. This observation indicates that the same ferredoxin and reductase components are associated even with the aliphatic-oxidizing oxygenase. Furthermore another mutant strain EK121 (*xylE::Km*), which lost the downstream gene activities (*xylGJQKIHCBphB*) due to polar effects, was found to be unable to grow on the alkanes tested. However, it should be noted that EK121 still grows on dodecanoic acid although it lost the ability to grow on the upstream metabolic intermediates of alkane degradation including dodecyl alcohol and dodecyl aldehyde. Complementation experiments with different subclones showed that 2.2-kb *Cla*I restriction fragment, containing only the *xylQ* gene for a putative acetaldehyde dehydrogenase, allowed EK121 to recover the ability to grow on alkanes as well as the intermediate compounds. These data suggest that XylQ converts aliphatic aldehydes to corresponding acids.

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Distribution of PAH concentrations and Degradation Potential for PAHs by Natural Microorganisms in Marine Sediments of Kwangyang Bay and Ulsan Bay

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The distribution of PAH concentrations in sediments of Kwangyang Bay and Ulsan Bay and degradation potential for PAHs by

indigenous microorganisms were investigated. The concentrations of PAHs in Kwangyang Bay and Ulsan Bay were 0.11~55.1 ppm and 0.54~76.05 ppm, respectively. The evolution of carbon dioxide was increased to 9.6~184.9% by the additional PAH mixture (ca. 1000 ppm) after 25 days incubation. For the evaluation of more direct evidence of degradation potential by indigenous microorganisms, we tried to measure the decrease of specific PAHs by GC. The amount of artificially added each 100 ppm of phenanthrene, pyrene and benzo[a]pyrene were checked for 2 to 4 weeks of incubation. In the sediments of Ulsan Bay, 73.8~89.8% of phenanthrene and 24.0~76.7% of pyrene were degraded by natural microorganisms at aerobic condition and ~18.1% of phenanthrene and ~6.5% of pyrene were degraded at anaerobic condition after 2 weeks of incubation. In the sediments of Kwangyang Bay, 100% of phenanthrene and 89.3~95.9% of pyrene were decreased after 4 weeks. But the benzo[a]pyrene was shown to be recalcitrant. From these results we concluded that, 1) the availability of oxygen seemed to be one of the important limiting factor in degradation of PAHs smaller than 4 ring in natural environment and 2) another remediation techniques such as bioaugmentation is required to remove large PAH molecules like as benzo[a]pyrene.

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Phylogenetic Characterization of Bacterial Isolated from Acid Mine Drainage-Contaminated of Stream and Ground water

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Twenty-six pigment forming bacteria isolated from acid mine drainage-contaminated of stream and ground water were characterized by