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Detection of Bacterial Stress Responses Induced by Environmental Pollutants using GFP Fused to Heat Shock Gene Promoter

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Heat shock response makes it a viable system to exploit as a biomarker for environmental pollutants monitoring. Therefore the molecular chaperone dnaK promoter was transcriptionally fused to gfp as a reporter gene to construct the recombinant plasmid pDNK-Green. When *Escherichia coli* cells transformed with pDNK-Green were stressed by various environmental stresses including $CdCl_2$, phenol, pentachlorophenol(PCP), bispenol A, and tributyltin, the cellular stress response was easily measured with the change of fluorescence intensity. The fluorescence intensity was generally increased in proportion to the concentrations of each pollutant used in this study. Therefore it is suggested that pDNK-Green constructed in this study can be an alternative approach monitoring cellular stress response induced by various environmental pollutants.

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Isolation of the Microbes Having Cyanobacteria Lytic Activity from Blooming Reservoirs

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Samples were obtained at Dochang, Kwalim, Mulwang reservoirs in Kyonggi-Do where cyanobacteria blooming occurred and isolated microbes which could lysis the cyanobacteria. Each 100㎕ of samples were smeared on the *Anabaena cylindrica* lawn and incubated in light chamber on 28℃, 3000lux during 13 days. Fungus having cyanobacteria lytic activity were isolated from the samples of Dochang reservoir. *Molaxella sp.* CK-1, known as bacteria which have cyanobacteria lytic activity, was coinoculate and compared the formation of clear zone to confirm cyanobacteria lytic activity of the fungus. Our results were evaluated that the fungus, obtained from Dochang reservoir have the cyanobacteria lytic activity and that named HYF2000-1 before identification.

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Purification and Characterization of Alkaline and Thermostable Protease from *Bacillus thuringiensis* KS1A-9

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Ninety-one alkalophilic bacteria were isolated from serpentinite soil (>pH9.2). Among 40% of the isolates were found to be product of alkaline protease. All strains were characterized using a variety of phenotypic, chemotaxonomic and 16S rDNA sequence-based phylogenetic analysis. *Bacillus thuringiensis* KS1A-9 was produced alkaline and thermostable protease. The production of alkaline protease by *B. thuringiensis* KS1A-9 on skim milk was maximal on 36hr after cultivation. A alkaline protease purified to homogeneity from the culture filtrate by successive procedures of column chromatographies. Purification factor was calculated as 4.3-folds of the specific protease activity with 24.6 unit/mg. The molecular weight was estimated as 34,000 by

SDS-PAGE. The optimal pH and temperature were pH 10 and 60℃, the enzyme was stable between pH 8 and 11 and below 70℃. The Km and Vmax 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 Adaptative 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-palsmid 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 *Sphingomonasyanoikuyae* Strain B1

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Previous studies showed that the same genes are involved in the degradation of various aromatic hydrocarbons by *Sphingomonasyanoikuyae* 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 C_4 to C_{16} alkanes including