C005

Construction of a Reporter Strain *Pseudomonas putida* for the Detection of Oxidative Stress Caused by Environmental Pollutants

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A potentially cheaper and simpler technique for contaminant detection would be the use of whole-cell reporter based on the inducible promoter responding to environmental stresses A green fluorescent protein-based *Pseudomonas putida* reporter was successfully constructed and shown to be capable of detecting oxidative stress. In this whole-cell reporter, the promoter of paraquat-inducible ferredoxin-NADP$^+$ reductase (*fpr*) gene was fused into a promoterless *gfp* gene on a broad-host-range promoter probe vector *Pseudomonas putida* KT2440 strain harboring this reporter plasmid exhibited increased level of *gfp* expression in the presence of redox-cycling agents (paraquat and menadione), hydrogen peroxide, and environmental chemicals such as toluene, paint thinner, gasoline, and diesel Induction of *fpr* gene in the presence of these environmental chemicals was confirmed using Northern blot analysis This reporter strain provides a useful tool for detecting oxidative stress caused by environmental chemicals.

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C006

Screening of Enantioselective Epoxide Hydrolase from a Marine Bacterium

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Enantioselective synthesis or hydrolysis is getting much more attention due to current concerns about mixed chirality of a lot of chemical, pesticides and medicine To screen strains producing an epoxide hydrolase(EH) which hydrolyzes (R) or (S) - epoxide preferentially, 120 strains isolated from a variety of marine environments primarily by the capability of living on styrene oxide were tested for EH activity using spectrophotometric assay Among those, one strain(JCS358) was selected by enantioselective hydrolysis of styrene oxide, confirmed by gas chromatography(GC)

JCS358 was isolated from the marine sediment The EH from JCS358 preferentially hydrolysed the (R)-epoxides of styrene oxide The GC result showed that enantiopure (S)-epoxides would be obtained with a value of 98% ee (enantiomeric excess)

This study presents a first example which discovered an enantioselective epoxide hydrolase from marine environment successfully

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C007

Optimization of Conjugal Transfer and Properties of *attB* Site from *Streptomyces natalensis* ATCC27448

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For molecular genetic study of *Streptomyces natalensis* ATCC27448 that produces natamycin, a commercially important macrolide antifungal antibiotic, we have developed a system for introducing DNA into *S natalensis* via conjugal transfer from *Escherichia coli* An effective transformation procedure for *S natalensis* was established based on transconjugation from *E. coli* ET12567/pUZ8002 using a ΦC31-derived integration vector, pSET152, containing *oriT* and *attP* fragments. The high frequency was obtained on MS medium containing 10 mM $MgCl_2$ using 6.25×10^8 of *E coli* donor cells without heat treatment of spores. In addition, southern blot analysis of exconjugants and the sequence of plasmids containing DNA flanking the insertion sites from the chromosome revealed that *S natalensis* contain a single ΦC31-*attB* site and at least a secondary or pseudo-*attB* site Similar to the case of various *Streptomyces* species, a single *attB* site of *S natalensis* is present within an ORF encoding a pirin-homolog, but a pseudo-*attB* site is present within a distinct site (GenBank accession no. YP_117731) and also its sequence deviates from the consensus sequences of ΦC31-*attB* site

C008

Identification of the Putative cDNA Clones Encoding Enzymes Required for the Biosynthesis of L-Carnitine from Lysine in *Neurospora crassa*

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L-Carnitine is synthesized by most eukaryotic organisms from lysine as a precursor and the identity of the intermediate metabolites of the L-carnitine biosynthetic pathway has been elucidated in the filamentous fungus *Neurospora crassa* More recently, enzymes required for the catalysis of the reactions in L-carnitine biosynthesis have been characterized at the molecular level in different kinds of eukaryotic organisms However, most of the enzymes responsible for the L-carnitine biosynthesis in *N. crassa* have not been characterized at the molecular level. Here we report on the cloning of the putative *N crassa* cDNA clones encoding enzymes involved in the L-carnitine biosynthesis, based on homology with the recently identified mouse histone-lysine N-methyltransferase, yeast serine hydroxymethyltransferase, human aldehyde dehydrogenase 9, and human gamma-butyrobetaine hydroxylase.

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