F029 Induction of the *sufA* Operon Encoding Fe-S Assembly Proteins by Superoxide Generators and Hydrogen Peroxide in *Escherichia coli*: Involvement of OxyR, IHF and an Unidentified Oxidant-Responsive Factor.

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A promoter (*sufAp*), inducible by various oxidants, directs transcription of the *sufABCDSE* operon encoding an alternative Fe-S cluster assembly system in *Escherichia coli*. We found three *cis*-acting oxidant-responsive elements (OREs) and gel mobility shift assays with a 50bp DNA probe containing ORE-III revealed the presence of an ORE-III specific factor that binds only when cells are treated with oxidants. S1 mapping analysis revealed that phenazine methosulfate (PMS) and H_2O_2 induced *sufA* expression by more that 40-fold. In *oxyR* mutant, *sufA* was still induced more than 10-fold. Fur, a ferric uptake regulator that negatively regulates this operon in response to iron availability, did not mediate the oxidant induction. Deletion of the *suf* operon caused cells to be more sensitive to superoxide-generating agents without affecting sensitivity to H_2O_2. We propose that the oxidant induction of the *sufA* operon is mediated through OxyR, IHF, plus an unidentified oxidant-responsive factor, and that the *suf* gene products are needed to defend cells against oxidative stress caused by superoxide generators.

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F030 Substrate Specificities of Adenylation Domains in Nonribosomal Peptide Synthetase Modules of Biosynthetic Gene Cluster for Cephabacins in *Lysobacter lactamgenus*

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Cephabacin F and H are cephem antibiotics having oligopeptide at C-3 position, produced by *Lysobacter Lactamgenus*. In a previous study, 11 putative ORFs involved in cephabacin biosynthesis were identified, and the gene products of two ORFs 9 and 11 are presumed to encode nonribosomal peptide synthetases (NRPSs). The ORF 9 contains three NRPS modules, and the ORF 11 contains one. The amino acid specificity of each NRPS modules was predicted by generating specific binding pockets, and the genes encoding adenylation domain of each NRPS module were cloned into pET expression vectors and expressed in *E.coli* BL21 (DE3) host strain. The overexpressed recombinant proteins in soluble form was purified with two sequential passages through nickel affinity column and subjected to ATP-PPi exchange assay for the examination of substrate specificity. The NRPS 1 adenylation domain in ORF9 showed relatively high activity for L-arginine whereas NRPS 2, 3 (ORF9), and 4 (ORF11)-adenylation domains preferably catalyzed the activation of L-alanine, but all adenylation domains showed somewhat relaxed specificities. These results disclose new perspectives about the mode of substrate selection by NRPS.

F031 Molecular Cloning and Expression of a Novel Chitinase Gene from *Vibrio* sp. 98CJ11027

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The *chiK* gene encoding chitinase ChiK was isolated from a marine bacterium, *Vibrio* sp. 98CJ11027. We amplified a partial sequence of the *chiK* using chitinase-specific primer sets. Using inverse PCR cloning method with two specific sets of PCR primers rather than single set, we identified the 4 kb DNA fragment containing *chiK* gene. From the fragment, we amplified the full *chiK* gene and cloned into pUC18 vector designated as pChiK. The recombinant *E. coli* clones harboring pChiK produced a clear zone around the colony in the colloidal chitin plates. Open reading frame of the *chiK* is 2553 bp long encoding a ChiK precursor protein of 851 amino acids, which consist of N-terminal signal peptide of 21 amino acids and a mature ChiK protein with 830 amino acids. The deduced amino acid sequences of *chiK* show 89% identity with that of *Vibrio carchariae* chitinase. The conserved module of *Vibrio* sp. 98CJ11027 ChiK indicates that the ChiK belongs to the family 18 of glycoside hydrolases.

F032 Functional Analysis of the Genome of *Leuconostoc kimchii* under Stress Condition

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Leuconostoc kimchii is a novel lactic acid bacterium isolated from the traditional Korean vegetable product kimchi. The genome project was initiated and its genome consists of 2.1 Mb containing about 2,000 genes. We analysed the genome sequences over 99% and constructed a DNA microarray for genome-wide gene expression analysis. The first version of the microarray contains PCR-amplified DNA fragments which cover 1121 genes and a number of control sequences. The expression profiles under various stress conditions such as osmotic shock, pH change and oxidative stress were examined. The genes in responsive to oxidative stress contained several thioredoxin genes. There are some genes highly expressed under all stress conditions, suggesting a general regulator to control the responses against various stresses.

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