

**F309****Expression of Two Catalase Genes from *Streptomyces coelicolor* (Müller)**

조유희\*, 노정혜

서울대학교 자연과학대학 미생물학과, 분자미생물학 연구센터

Two catalase genes (*catA* and *catB*) were cloned from *Streptomyces coelicolor* (Müller) using PCR primers corresponding to conserved sequences among typical monofunctional catalases. The two genes contained nucleotide sequences for amino acids highly conserved in other catalases. Both *catA* and *catB* genes were expressed in *S. lividans* TK24. Total catalase activities in cells bearing *catA* or *catB* in a multicopy plasmid pJ702 increased about two-fold. In both cases, only one band was enhanced in a native PAGE detected by catalase activity staining. The enhanced activity band from *catA*-expressing cells was identical to that of Cat4, the major catalase in *S. coelicolor* whose level is constitutively high and slightly increased by 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$  treatment. The enhanced band from *catB*-expressing cells had the same mobility to Cat1, one of the minor catalases in *S. coelicolor* which has the slowest mobility in native PAGE and appears only in the stationary growth phase. The transcriptional start sites for *catA* and *catB* were determined by S1 nuclease mapping. The +1 site of *catA* gene was mapped nearest or almost equal to the putative translational start point, whereas for *catB* the +1 site was mapped about 70 bp upstream of the putative translational start codon preceded by a canonical Shine-Dalgarno sequence.

**F310****Isolation and characterization of paraquat inducible genes from *Escherichia coli*.**

이준희\*, 고영삼, 최제니, 노정혜

서울대학교 자연과학대학 미생물학과, 분자미생물학 연구센터.

We isolated several promoters inducible by paraquat, a superoxide-generating agent, from *Escherichia coli*, using a promoter-probing plasmid pRS415. From sequence analysis we found out that one promoter is for *fpr* encoding NADPH:ferredoxin reductase, the other is for *ribA* encoding GTP cyclohydrolase II which is the first enzyme in the biosynthetic pathway of riboflavin and the third was mapped at the upstream region of *rfaY*, which is one gene of lipopolysaccharide core biosynthetic gene cluster. We constructed an operon fusion of *lacZ* gene with these promoters to monitor the expression of the genes in the single-copy state. *LacZ* expression by *ribA*, *fpr* and *rfaY* promoters were induced about 10, 20, and 8 folds, respectively, by 800  $\mu\text{M}$  paraquat. Other known superoxide generator, menadione and plumbagin, also induced the expression of  $\beta$ -galactosidase in these fusion strains. On the other hand, no significant induction was observed by treatment with hydrogen peroxide, ethanol and heat shock. Induction of  $\beta$ -galactosidase was significantly reduced by introducing a  $\Delta\text{sox-8::cat}$  or  $\text{soxS3::Tn10}$  mutation into the fusion strains, indicating that these genes are members of the *soxRS* regulon. The transcription start site of *ribA* and *fpr* promoters were determined by primer extension analysis and putative binding sites for SoxS were identified. GTP cyclohydrolaseII activity in soluble extracts of *E. coli* increased about 60% by treatment with paraquat. However, flavin pools did not change significantly.