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

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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 pIJ702 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 μM H_2O_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.

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

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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, repectively, by 800 μM paraquat. Other known superoxide generator, menadione and plumbagin, also induced the expression of β-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 β-galactosidase was significantly reduced by introducing a Δ*sox-8*::cat or *soxS3*::Tn*10* 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.