

## F303

### Cloning of Two Catalase Genes from *Streptomyces coelicolor*

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The existence of domains conserved in all eucaryotic and several procaryotic catalases (type I catalases) enable the design of PCR primers to amplify catalase gene fragments. We observed the production of a single DNA fragment amplified when *S. coelicolor* (Müller) genome was used as the template for PCR. This fragment (YH301) had a significant sequence homology to other type I catalases. *S. coelicolor* genomic phage library was screened using YH301 as a probe, and several positive clones were isolated. One clone (YH311, *catY*) of 4.5 kb insert contained the identical sequences to YH301, and was expected to cover the entire ORF of type I catalase. YH301 fragment hybridized to another band in genomic Southern analysis when performed in a lower stringency. 5.8 kb *SalI* fragment was cloned from a sub-library and sequenced partially. It was different from *catY* sequences but still showed a remarkable homology to type I catalases and *catY*. This gene was named *catJ*. The finding of two different catalase genes is consistent with our previous observations that there exist more than two catalases in *S. coelicolor* whose gene expression is regulated differently during growth.

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### Screening and Characterization of Paraquat Inducible (*pqi*) Genes in *Escherichia coli*

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We screened promoters inducible by paraquat, a superoxide generating agent from *Escherichia coli*. For this purpose, we constructed a random promoter library from *E. coli* MG1655 using a promoter-probing plasmid, pRS415.  $\beta$ -galactosidase assay was carried out on this library in microwell plates. Of about one thousand clones with weak to medium promoter strength, five promoters (*pqi* G59, G79, E16, E23, and H73) were detected to be induced by paraquat. The promoter strength increased about 3.6-fold in G59, 2.5-fold in G79, 1.9-fold in E16, 1.8-fold in E23, and 2-fold in H73 upon induction. We constructed an operon fusion of the *E. coli lacZ* gene to the *pqi* promoters to monitor the transcriptional level in the single copy state. Transcription from *pqi* G59, G79, E16, E23, and H73 promoters was induced about 20, 10, 2, 2, and 3 folds, respectively. Other superoxide generators, 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. Mapping analysis using the *E. coli* phage library made by Kohara was carried out.