F310 Cloning and characterization of catA, which encodes the major catalase of *Streptomyces coelicolor*

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Conservation of domains in all eukaryotic and several prokaryotic typical catalases enabled the design of PCR primers to amplify catalase gene fragments. We observed the production of a single DNA fragment amplified when *Streptomyces coelicolor* ATCC 10147 genome was used as the template. The PCR fragment had a significant sequence homology to other typical catalases and was used as a probe to screen *S. coelicolor* genomic phage library. One of the isolated positive clones contained 4.5 kb insert, which had the identical sequence to the PCR product. The DNA sequence of this gene named *catA*, encodes a 488-amino acid polypeptide with strong homology to typical catalases. Total catalase activity of cells bearing *catA* gene in a multicopy plasmid increased about two-fold. The one enhanced activity band in *catA*-expressing cells had the same mobility to Cat4, the major catalase in *S. coelicolor*, and was detectable by the anti-Cat4 antiserum. The transcriptional start site for *catA* gene was mapped by high resolution S1 nuclease mapping and the putative promoter region, the Shine-Dalgarno sequence and the translation initiation codon, TTG. Northern and Western blot analyses as well as the enzyme assay revealed that the expression of *catA* gene was increased by about two fold upon H_2O_2 pre-treatment in logarithmic growth phase mainly due to the increased transcriptional level and gradulally decreased as culture progresses into the stationary growth phase even though the total catalase activity was four fold increased, which might be attributed to other catalase activities than CatA.

F311 Molecular Cloning of *psd1*$^+$ encoding Cu/Zn Superoxide Dismutase in *Schizosaccharomyces pombe* and Analysis of its Regulation

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The genomic clone encoding *psd1*$^+$, Cu/Zn superoxide dismutase (SOD) gene from *Schizosaccharomyces pombe* was isolated. The ORF sequence was prepared by PCR and 2.9 kb genomic DNA encoding *psd1*$^+$ was obtained by screening the λ EMBL3 library with this PCR fragment. The nucleotide sequence was determined with construction of deletion series. Its amino acid sequence was deduced from the coding region and highly conserved region was shown in comparison with *S. cerevisiae*, mouse, bovine, and human Cu/ZnSOD. The RNA start site was determined by S1 nuclease mapping and primer extension. In order to analyze its regulation of expression, the mRNA level of *psd1*$^+$ was measured at the conditions of oxidants, metal, osmotic shock, heat shock, and growth phase by Northern analysis.