

## F307

### Characterization of hydrogen peroxide resistant mutants of *Streptomyces coelicolor*

한지숙\*, 노정혜

서울대학교 자연과학대학 미생물학과, 분자미생물학 연구센터

To study the defense mechanism against hydrogen peroxide stress in *Streptomyces coelicolor*, mutants resistant to hydrogen peroxide were isolated and characterized. Five mutants resistant to 2–40 mM hydrogen peroxide were selected at a frequency of  $1 \times 10^{-5}$  per survivor following UV mutagenesis of spores of *S. coelicolor* J1501. These mutants overexpressed the major type of catalase (Cat4) of *S. coelicolor* and one of them expressed as much as 50 fold more than the wild type. They also produced two catalase bands which were not observed in the wild type. The activities of glucose 6-phosphate dehydrogenase and superoxide dismutase exhibited little differences between the mutants and the wild type. In addition, these mutants were more resistant than the wild type to killing by heat shock. The mutation site in one mutant HR40 was mapped on the chromosome by genetic crossings. The catalase overproducing phenotype was due to a single mutation and mapped close to *mthB2* locus which do not overlap with the locus of *catA* gene encoding the major catalase (Cat4).

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### Analysis of Superoxide Dismutases (SOD) and Cloning of *psd1* Gene Encoding CuZnSOD in *Schizosaccharomyces pombe*

이 준\*, 정재훈, 김동욱, 노정혜

서울대학교 자연과학대학 미생물학과, 분자미생물학 연구센터

The fission yeast *S. pombe*, like other eukaryotes, was shown to contain two kinds of SODs, CuZnSOD and MnSOD. Their activities were differentiated either by incubating with  $\text{CN}^-$  which selectively inhibits CuZnSOD or by fractionating the cytosolic and organellar components. Menadione (MD), a redox-cycling agent, could induce both SOD activities up to 2-fold in early exponential phase. The total SOD activity remained unchanged even when cells grew into stationary phase. However, the ratio of two SODs changed drastically, i.e., CuZnSOD constituted about 80% of the total in exponential phase, but it lost most of its activity in stationary phase. This indicates that MnSOD can make up for the lost CuZnSOD activity in stationary phase and suggests that MnSOD gene expression may be under the C-source control. The open reading frame region of CuZnSOD was prepared by PCR amplification. Using this fragment as a probe, 2.9 kb genomic DNA encoding CuZnSOD, *psd1* was cloned. Northern analysis showed that CuZnSOD was induced by MD,  $\text{Cu}^{2+}$  and not by  $\text{H}_2\text{O}_2$ . Also its mRNA could not be detected in prolonged culture, indicating that the expression of *psd1* gene is regulated mainly at the transcriptional level.