E333 Cloning and Sequencing of the *sodN* and its flanking regions in *Streptomyces seoulensis*

Yang-In Yim, Hwan Youn, In-Kwon Kim and Sa-Ouk Kang*
Department of Microbiology, College of Natural Sciences, and Research Center for Molecular Microbiology, Seoul National University

Superoxide dismutase (SOD) discovered in *Streptomyces seoulensis* contains nickel as cofactor. The phage clone containing the *sodN* gene was isolated from genomic DNA library constructed in EMBL3 using the *sodN* gene from *S. coelicolor* as probes. About 4.0 kb *Kpn*I fragments containing the entire *sodN* gene and its flanking regions from the phage DNA purified were cloned into pGEM-7Zf(+)(pKnSODN1). DNA sequence reveals that the *sodN* gene is solely homologous with the *sodN* gene encoding NiSOD of *S. coelicolor*. An initiation codon of the *sodN* gene was found 14 amino acid upstream of the N-terminal His codon determined by Edman degradation of NiSOD. According to this results, it was assumed that NiSOD was processed through posttranslational modification. An ORF11 found upstream of the *sodN* gene showed a homology with ORFX, signal peptidase like protein of *S. coelicolor*. Therefore, we thought that the ORF11 protein was required in the process into the mature form of NiSOD.

E334 Disruption of the Genes Associated With NADH-Erythroascorbyl Free Radical(EAFR) Reductase in *Saccharomyces cerevisiae*

Jung-Shin Lee, Won-Ki Huh and Sa-Ouk Kang*
Department of Microbiology, College of Natural Sciences, and Research Center for Molecular Microbiology, Seoul National University

The participation of NADH-cytochrome b_5 reductase in NADH-ascorbyl free radical reductase activity has been reported in rat liver. In this study, *MCR1* and *CBR*, the genes encoding NADH-cytochrome b_5 reductase, were disrupted in *S. cerevisiae* by insertion of *URA3* gene into the genes of *MCR1* and *CBR*, respectively. Disruption of the genes was confirmed by PCR and Southern blot analysis. In *mcr1* disruptant cells and *cbr* disruptant cells, the activity of NADH-erythroascorbyl free radical(EAFR) reductase was reduced to 1% and 70% respectively. So it is thought that the correlation between NADH-EAFR reductase activity and NADH-cytochrome b_5 reductase exists in *S. cerevisiae*. Role of NADH-EAFR reductase in the response of *S. cerevisiae* to oxidative stress, H_2O_2 and menadione, was investigated. The *mcr1* disruptant cells were hypersensitive to H_2O_2 and menadione but *cbr* disruptant cells were not. These results suggested that NADH-EAFR reductase plays an important role in the response in *S. cerevisiae* to oxidative damage.