

E317 Purification of Nickel-binding Protein (NBP1) and Cloning of Its Gene (*nbp1*) from *Streptomyces seoulensis*

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

Several accessory proteins may be associated with the incorporation of nickel to apo-protein of nickel-containing superoxide dismutase (NiSOD) newly found from *Streptomyces seoulensis*. To find one of these accessory proteins, nickel-binding protein (NBP1) was purified in one step with nickel chelating affinity chromatography. The molecular weight of protein monomer in SDS/polyacrylamide electrophoresis was 38,000. N-terminal and internal sequence of the fragments digested by endoproteinase Lys-C were obtained. Using 294-bp PCR product obtained from the primer prepared on the basis of these amino acid sequences, phage containing the gene encoding NBP1 was isolated from λ -EMBL3 library of *S. seoulensis* by plaque hybridization. Full sequencing of 3-kb *SalI* fragment containing the gene for NBP1 was carried and structural gene encoding NBP1, named for *nbp1*, was obtained. The deduced product of *nbp1* showed homology with CbiX, a cobalt incorporation protein functioning in cobalamine biosynthesis in *Bacillus megaterium*.

E318 Molecular Cloning and Characterization of the Putative Sigma and Anti-Sigma Factors in *Bacillus subtilis*

Han-Bong Ryu* , Sung-Woon Yu, Mi-Nae Yun and Sa-Ouk Kang
Laboratory of biophysics, Department of Microbiology, College of Natural Sciences, and Research Center for Molecular Microbiology, Seoul National University

Two open reading frames, designated as *yla-c* and *yla-d* in the *Bacillus subtilis* genome sequencing project, were cloned using pRB374 vector which is shuttle vector in *E. coli* and *B. subtilis*. They showed the sequence homology with *ybbL* and *ybbM* that are known to be ECF family sigma and anti-sigma factor. Thus their function was suggested to be sigma and anti-sigma factor, respectively. The *yla-c*-encoded product was overexpressed using pET-32a(+) vector in *E. coli* AD494 and purified using nickel affinity column followed by enterokinase treatment. The *yla-c* and *yla-d* gene cloned in pRB374 vector were overexpressed in *B. subtilis* PS832. The *yla-c*-encoded product was not detected by Western blotting. Strains transformed by each of *yla-c* and *yla-d* genes showed the retardation of the initiation of exponential growth stage with the reduced sporulation rate and cell density at the stationary stage. From this result, we propose that *yla-c* and *yla-d*-encoded products may exert physiological effects on the growth, especially on the sporulation of *B. subtilis*.