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

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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 *Sal*I 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***

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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 *ybb*L and *ybb*M 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*.