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Nickel Binding Protein (NBP1) and Its Gene from *Streptomyces seoulensis*

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Nickel-binding protein (NBP1) was purified from the crude extract of *Streptomyces seoulensis* using Ni^{2+}-charged metal chelate affinity chromatography. The molecular mass of NBP1 determined on SDS-PAGE was 38kDa. An approximately 3 kb DNA fragment containing the structural gene for NBP1 was cloned from lEMBL3 genomic library of *S. seoulensis* using a DNA fragment PCR-amplified with the primers designed from N-terminal and internal amino acid sequences of NBP1. The 3 kb DNA fragment contained 4 open reading frames (ORF I, ORF II, ORF III, ORF IV). The ORF II was identified to encode NBP1. ORF II (*nbp1*) encodes a polypeptide consisted of 306 amino acid residues with a calculated molecular mass of 33.241 Da. The deduced amino acid sequences of *nbp1* shared high similarity to those of CbiX that is known as a cobalt incorporation protein functioning in cobalamin biosynthesis in *Bacillus megaterium*. NBP1 had histidine-rich metal binding motif in its C-terminal region and potential CXXC motif. Because this histidine-rich region and CXXC motif may be related to nickel chelation in NiSOD maturation process, we overexpressed NBP1 using multicopy vector pIJ702 in *S. lividans*. Because NBP1 didn't have its own promoter, we used *sodN* (encoding NiSOD) promoter. NBP1 overexpressed strain had more NiSOD proteins and higher NiSOD activity than strain having just *sodN* promoter.