EXPRESSION AND PURIFICATION OF THE NAD+ SYNTHETASE (NADE1) FROM *Herbaspirillum seropedicae*

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Herbaspirillum seropedicae is a nitrogen-fixing bacteria found in association with several crops, thus with potential as a biofertilizer. NadE1 catalyses the last step of the NAD^+ synthetic pathway. The *nadE1* gene of *Herbaspirillum seropedicae* is located next to *glnB*, which codes a nitrogen signal transduction protein from the P_{II} family. The *nadE1glnB* gene organization is conserved in many bacteria. The genomic linkage between P_{II} coding genes and *nadE1* suggests that the GlnB protein might regulate NadE1 activity through protein-protein interaction. In the present work we describe the over expression and purification of NadE1. Two primers flanking the *nadE1* gene were designed based on *Herbaspirillum seropedicae* genomic sequence (GENOPAR). The *nadE1* gene was PCR-amplified and cloned into pET28-a vector, generating the pET/NadE1 plasmid that express the NadE1 with an N-terminal His-tag (His-NadE1). *Escherichia coli* BL21(DE3) was transformed with the pET/NadE1 plasmid and His-NadE1 over expression was induced by IPTG. The recombinant protein was purified using affinity chromatography on a HisTrap-chelating-Ni^{2+} column. The His-Tagged protein was eluted with 300 mM imidazole. The protein was 91% pure as revealed by densitometric analysis of SDS-PAGE.

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