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In vitro characterization of AspA, NadK and ThrA proteins of *Herbaspirillum seropedicae*

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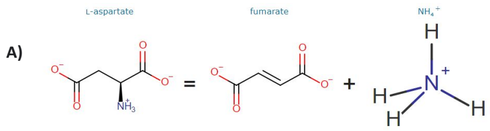
Abstract

The PII family of proteins are ubiquitously spread between Bacteria, Archaea and plants. These proteins have a pivotal role in the so called Ntr system, responsible for the regulation of the nitrogen metabolism in bacteria. In this system, the PII proteins transduce molecular clues through protein-protein interactions, modulating the activity of many enzymatic targets. In order to describe the role of the *Herbaspirillum seropedicae* GlnB protein, member of the PII family, we previously characterized its interactome, indicating several candidate interaction target-proteins, which are potentially regulated by GlnB. Amongst the identified proteins at the interactome we picked AspA, NadK and ThrA, the focus of the present work. AspA has an aspartate ammonia lyase activity, forming fumarate and ammonium from aspartate. NadK phosphorylates NAD^+, consuming ATP, to form $NADP^+$. ThrA, also known as HSD, is a NADP-dependent dehydrogenase that catalyses the oxidation of L-homoserine to form L-aspartate 4-semialdehyde. *H. seropedicae* is a nitrogen fixing bacteria that lives in association with Poaceae plants, having the potential to transfer nitrogen to them, and can then be used as biofertilizer to promote plant growth.The present work aims to *in vitro* characterize the activity of the AspA, NadK and ThrA proteins from *H. seropedicae*, and the ability of GlnB to modulate their activities.

Introduction

The nitrogen metabolism of Proteobacteria is controlled by the general molecular system called Ntr, which is composed by determined proteins capable to sense the nitrogen status command a metabolic response upon shifts in environmental nitrogen availability[1]. The PII family of proteins play a pivotal role at the Ntr system as transducing elements, which control the activity of target proteins to modulate the metabolism, allowing the bacterial cell to respond to environmental nitrogen shifts [2]. Recently, roles of PII proteins in the regulation of protein targets unrelated to nitrogen metabolism are been reported. These novel data indicate that the PII proteins have a much broader function in the cell than solely regulation of nitrogen metabolism. In attempt to explore the potential target proteins of the GlnB, a PII-like protein, of *Herbaspirillum seropedicae*, a former study identified possible interacting proteins by using a chromatography method. Amongst the proteins identified in that study are subjects of the present work: AspA, NadK and ThrA (Fig.1). The present work aims to clone the genes that code for such proteins, express and purify the recombinant proteins and test the capacity of the GlnB to interact and regulate these proteins in vitro.



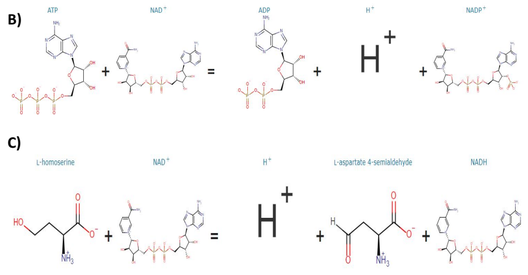


Fig.1. Reactions catalyzed by enzymes. A) AspA enzyme; B) NadK enzyme; C) ThrA enzyme.

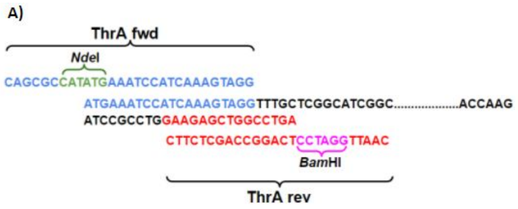
Materials and methods

Extraction of genomic DNA from *Herbaspirillum seropedicae* was performed by boiling a culture aliquot at 100°C for 5 min.

For the amplification of each gene, direct primers containing a restriction site for the NdeI enzyme and reverse primers for the BamHI enzyme were designed. The PCR reactions contained the 1X enzyme buffer, dNTP, the primers, DNA polymerase, and the genomic DNA of the bacterium. The genes are currently being cloned into pETM11 expression vector by using T4 DNA ligase. Cloning confirmation will be done using the enzymes *Xba*I and *Bam*HI and checked on a 1% agarose gel.

Results

The designed primers (Fig.2) direct primers containing a restriction site for the NdeI enzyme and reverse primers for the BamHI enzyme were efficient in amplifying both NadK and ThrA genes of *H. seropedicae.* For the NadK primer set a 918 bp fragment was obtained, for the ThrA set a 1311 bp fragment was obtained, and for the AspA set no amplified fragment was obtained yet. Fig.3 shows the genes amplified at temperatures at 58°C, 60°C, and 62°C, with primer pairs containing restriction sites for *Nde*I-*Bam*HI.



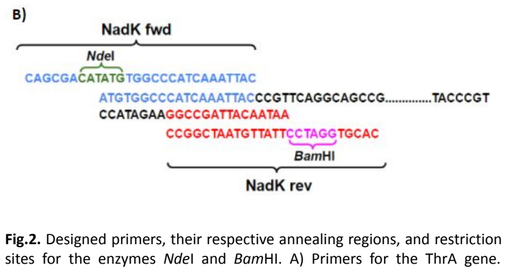


Fig.2. Designed primers, their respective annealing regions, and restriction sites for the enzymes *Nde*I and *Bam*HI. A) Primers for the ThrA gene. B)Primers for the NadK gene.

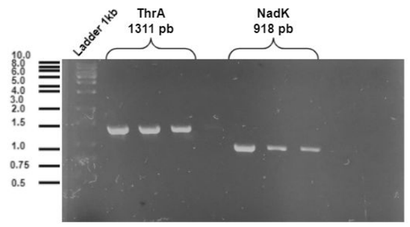


Fig.3. PCR reaction for amplification of ThrA and NadK gene from *H. seropedicae*. 1% agarose gel showing the amplified PCR products of 1331 bp for ThrA and 918 bp for NadK, at 58ºC, 60ºC and 62ºC, with primer pair containing restriction site for *Nde*I-*Bam*HI.

For expression the genes will be inserted into the pETM11 vector (Fig.4) which has a T7 promoter under LacI control. The gene for vector selection is Km, which confers resistance to the antibiotic kanamycin. In Fig.3 the vector with some of the important restriction sites is shown.

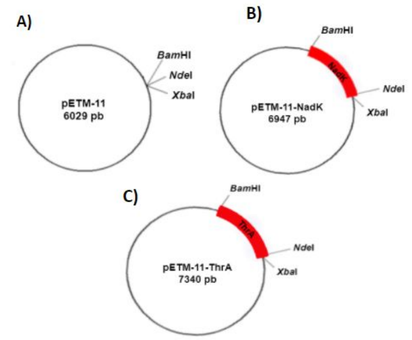


Fig. 4. The plasmid pETM11 and the NadK and ThrA genes. A) The empty pETM11 plasmid and some restriction sites. B) Plasmid pETM11-NadK with the important restriction sites represented. C) Plasmid pETM11-ThrA with the important restriction sites represented.

Conclusion

In the present study the ThrA and NadK genes from *Herbaspirillum seropedicae* were amplified. To continue the work, the fragments and the vector will be digested to link them together. Once the cloning is confirmed, the proteins will be overexpressed and their activity and interaction with PII will be characterized.

Recommendations

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