

### **Functional characterization of the NtrX protein *Herbaspirillum seropedicae***

Osaki, J. H.<sup>1</sup>; Chubatsu, L.S.<sup>1</sup>; Monteiro, R. A.<sup>1</sup>; Kadowaki, M.A.<sup>1</sup>; Oliveira, M.A.S.<sup>1</sup>,  
Souza, E. M.<sup>1</sup>; Pedrosa, F. O.<sup>1</sup>; Yates, M.G.<sup>1</sup>; Rigo, L. U.<sup>1</sup>

<sup>1</sup>Departamento de Bioquímica e Biologia Molecular, UFPR, Curitiba, Paraná.

The NtrYX proteins are members of a two-component regulatory system, NtrY is a sensor protein and NtrX is a response regulator protein. The NtrX protein of *H. seropedicae* contains two domains, the signal receiver domain and the DNA binding domain (HTH). Previous studies reported the construction of NtrY mutant of *H. seropedicae* and showed that this mutation affected the expression of genes involved in the metabolism of nitrate and nitrite, suggesting the involvement of NtrYX in the regulation of the expression of these genes in *H. seropedicae*. In order to contribute to a better understanding of the role of NtrYX system in *H. seropedicae*, the *ntrX* gene was cloned expressed in *E. coli* and the recombinant protein His-NtrX was purified by affinity chromatography. The purified protein was used to show the interaction to the promoter region of the operons *narXL*, *narKnirBDCnasA*, *narK1KGHJI* using electrophoretic mobility shift assays. The band-shift assays showed that the His-NtrX protein bound specifically to these promoter regions and that protein phosphorylation probably increases their affinity for DNA. This result suggests the involvement of NtrX protein in the regulation of the operons *narXL*, *narKnirBDCnasA* and *narK1KGHJI* expression.

**Key words:** *Herbaspirillum seropedicae*, NtrX protein, electrophoretic mobility shift assays.

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