

**Expression, purification, and DNA-binding activity of the NtrX protein of
*Herbaspirillum seropedicae***

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Herbaspirillum seropedicae is a diazotrophic, endophytic, gram-negative member of the β -proteobacterial subdivision. This bacterium is found associated with grasses of economic interest such maize, rice and sugarcane. The NtrYX proteins constitute a two-component regulatory system consisting of a sensory kinase (NtrY), containing a conserved kinase domain, and a response regulator protein (NtrX), containing a conserved regulatory domain, involved in aspects of nitrate metabolism in *H. seropedicae*. In this study the NtrX protein of *H. seropedicae* was over-expressed with a polyhistidine-tag fusion using the plasmid pJHO2, containing the *ntrX* gene in the expression vector pET28a, induced with either IPTG (0.5 mmol/L) or lactose (0.5 %) for 3h at 30°C and 37°C. The overexpressed NtrX-His protein was purified first by affinity chromatography on a HiTrap Chelating Ni²⁺ column eluted with 240-280 mmol/L of imidazole, followed by further purification on a HiTrap Heparin column eluted with 400-500 mmol/L of NaCl. DNA band-shift assays showed that the purified NtrX-His protein bound to the *narK* promoter region of *H. seropedicae*. These results suggest that the NtrX protein is important in the respiratory metabolism of nitrate in *H. seropedicae*.

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