

Effect of ATP and α -Ketoglutarate on the Direct Interaction of the N-Terminal Domain of *Azospirillum brasilense* NifA with the GlnB Protein

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Azospirillum brasilense is a diazotrophic bacterium which associates with important agricultural crops such as maize, wheat and rice and it has been indicated as an inoculant for graminea. In this organism the nitrogen fixation process is highly regulated by ammonia and oxygen. At transcription level, this control requires the NifA protein, a transcriptional activator. In *A. brasilense*, the ammonium control depends on the NifA N-terminal domain and requires GlnB, an N-signaling PII-like protein. A direct interaction between the NifA N-terminal domain and the GlnB protein has been proposed as a mechanism for this N control. In this work, both proteins were expressed and purified from *E. coli*: the NifA N-terminal domain as a His-tag fusion protein (99% purity), and GlnB as a native protein (95% purity). The direct interaction assay for the NifA-N-terminal domain and the native GlnB protein was performed using Ni²⁺-NTA magnetic beads in “pull-down” assays. Our results showed a direct interaction between *A. brasilense* GlnB and the NifA N-terminal domain dependent on the co-factors, ATP and α -ketoglutarate. The minimal α -ketoglutarate concentration required to observe proteins interaction was 2mmol/L, under our conditions. In the presence of 3.5mmol/L ATP and 2mmol/L α -ketoglutarate a higher level of proteins interaction was observed. These results contribute in clarifying the mechanism of nitrogen control of NifA in *A. brasilense*.

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