DIRECT INTERACTION OF THE N-TERMINAL DOMAIN OF *Azospirillum brasilense* NIFA WITH THE GLNB PROTEIN

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Azospirillum brasilense is a nitrogen-fixing bacterium that associates with important agricultural crops such as maize, wheat and rice. The *A. brasilense* transcription regulator NifA is required to activate transcription of nitrogen-fixing genes *(nif)* in response to both nitrogen and oxygen. The NifA N-terminal domain is involved in the control by fixed nitrogen. This control also involves the nitrogen-status-signalling protein GlnB probably by direct interaction between the NifA N-terminal domain and GlnB. A PCR-amplified DNA fragment coding for the NifA N-terminal region was cloned into the expression vector pET28a allowing the N-terminal domain to be over-expressed in *Escherichia coli* as a fusion to a His-tagged sequence. The N-terminal-His fusion protein was purified by affinity chromatography to 99% purity as revealed by densitometric analysis of SDS-PAGE. GlnB protein was also over-expressed in *E. coli* and purified as a native protein. The direct interaction of the NfA-N-terminal domain and the native GlnB protein was measured by pull-down assays using Ni^{++}-NTA magnetic beads. These results showed that *A. brasilense* GlnB interacts directly with the NifA N-terminal domain as predicted. The GlnB co-factors, ATP and α-ketoglutarate, alone or together, did not influence this interaction under the conditions tested.

Supported by CAPES, Instituto do Milênio/CNPq/MCT, CNPq, and Fundação Araucária.