PURIFICATION AND CHARACTERIZATION OF THE GLND PROTEIN OF THE NITROGEN-FIXING BACTERIUM *Azospirillum brasilense* STRAIN FP2

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Azospirillum brasilense is a diazotrophic bacterium which associates with important agricultural crops such as maize, wheat and rice and thus has potential as a nitrogen biofertilizer. The GlnD protein, product of the *glnD* gene, is one of the proteins involved in the nitrogen metabolism regulation. This protein is responsible for the covalent modification of the PII proteins, GlnB and GlnZ, according to intracellular nitrogen levels. In this work, we describe the purification of the *A. brasilense* GlnD protein and its activity on the uridylylation of purified *A. brasilense* GlnB and GlnZ. GlnD was over-expressed in *Escherichia coli* as a His-tag fusion protein and purified using affinity chromatography on a HiTrap-Chelating-Ni^{2+} column. The protein, eluted with 500mM imidazole, was 99% pure as revealed by densitometric analysis on SDS-PAGE. The effect of ATP, glutamine and α-ketoglutarate on the uridylylation of purified *A. brasilense* GlnB, GlnB-His and GlnZ proteins by GlnD-His was determined. The GlnD protein (60nmol/L) was able to fully uridylylate GlnB after 30 minutes reaction, but GlnZ was never completely uridylylated in any condition tested. ATP (0.1mmol/L) and α-ketoglutarate (1mmol/L) were required for maximum uridylylation, whereas glutamine (5mmol/L) caused approximately 70% inhibition of the reaction.

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