Characterization of a *glnD*$^-$ mutant from *Herbaspirillum seropedicae*

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GlnD is a primary nitrogen sensor found in several proteobacteria. This bifunctional enzyme modifies Pll proteins covalently and reversibly in response to the nitrogen status. Under low levels of glutamine, GlnD has uridylyltransferase activity and transfers UMP groups to PII proteins (uridylylation). Conversely, high glutamine levels induce the uridylyl-removing activity of GlnD, which catalyzes deuridylylation of PII proteins. Depending on their uridylylation status, PII proteins can interact with different targets or transduce specific metabolic signals. Two Pll proteins have been identified in *Herbaspirillum seropedicae* (GlnB and GlnK) which are reversibly uridylylated by GlnD. The *H. seropedicae glnD*$^-$ mutant strain DC2 was obtained by transposon insertion. This strain was unable either to fix nitrogen or use nitrate as a nitrogen source. In this work, plasmid pLAFRglnD, which contains the *H. seropedicae glnD* gene in pLAFR3.18, was introduced into the DC2 strain. The transconjugant strain DC2(pLAFRglnD) was capable of nitrate-dependent growth and able to fix nitrogen. Crude extracts of *H. seropedicae* strains (wild-type, DC2 and DC2(pLAFRglnD)) grown with high or low nitrogen, were analyzed by native polyacrylamide gels followed by immuno-blot assays using antibodies against GlnB or GlnK proteins. The results showed that GlnB and GlnK were permanently deuridylylated in DC2, while in the DC2(pLAFRglnD) strain, the PII proteins were fully uridylylated under low nitrogen levels. The results indicate that GlnD is the sole enzyme required for reversible modification of PII proteins and the uridylylation of PII is essential for nitrogen fixation and nitrate-dependent growth in *H. seropedicae*.

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