

CONSTRUCTION OF CHIMERIC PROTEINS TO INVESTIGATE THE ROLE OF *HERBASPIRILLUM SEROPEDICAE* NIF A DOMAINS IN THE RESPONSE TO FIXED NITROGEN

Monteiro, R.A.¹, Dixon, R.², Little, R.², Oliveira, M.A.S.¹, Bonatto, A.C.¹, Wassem, R.³, Yates, M.G.¹, Chubatsu, L.S.¹, Steffens, M.B.R.¹, Rigo, L.U.¹, Pedrosa, F.O.¹, Souza, E.M.¹

¹Department of Biochemistry and Molecular Biology, Universidade Federal do Paraná, Curitiba, PR, Brazil. ²Department of Molecular Microbiology, John Innes Centre, Norwich, UK. ³Department of Genetic, UFPR

The activity of *Herbaspirillum seropedicae* NifA protein is regulated by the levels of oxygen and fixed nitrogen. The N-terminal GAF domain of this protein is involved in regulation in response to the external ammonium concentration. *Azotobacter vinelandii* NifA protein has its activity controlled by NifL protein that respond to the level of oxygen and fixed nitrogen. The *A. vinelandii* NifA itself is active independent of oxygen and fixed nitrogen levels. To study the regulation of NifA activity by these effectors independently, we decided to construct chimeric proteins containing the N-terminal GAF domain of *H. seropedicae* NifA fused to the AAA+ and C-terminal domains from *A. vinelandii* NifA. *H. seropedicae* NifA is inactive in *Escherichia coli*. In contrast, the chimeric proteins are active in *E. coli* under nitrogen-limiting conditions, but are regulated in response to excess fixed nitrogen. These results indicate that the GAF domain of *H. seropedicae* NifA can regulate the activity of the AAA+ and C-terminal domains of *A. vinelandii* NifA in response to the level of ammonium. We also tested these chimeric proteins in *glnK* and *glnB* mutant strains of *E. coli*. In the *glnK* mutant strain, we observed a reduction in NifA activity, implying that the GlnK protein regulates the GAF domain of *H. seropedicae* NifA.

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