CLONING, EXPRESSION AND PURIFICATION OF HFQ PROTEIN FROM *Herbaspirillum seropedicae*

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Hfq, an RNA chaperone, has recently been recognized as an important protein involved in the post-transcriptional regulation in several bacteria. In this work, we analysed the *hfq* gene of *Herbaspirillum seropedicae*, a diazotrophic bacterium found associated to important agricultural crops. The *H. seropedicae hfq* gene was isolated from a genomic library and cloned into the expression vectors pT7-7 and pET28(a), yielding plasmids pKADO1 and pKADO2. These allow the over-expression of the Hfq protein in its native and His-tag forms, respectively. *E.* coli strain BL21(λDE3) was used as host and induction was performed with IPTG. After cell lysis, the soluble fraction was incubated at 80ºC for 15 minutes to allow precipitation of most of host proteins. The Hfq protein was purified by affinity chromatography (His-tag Hfq) using a HiTrap-Chelating-Ni^{2+} column or by hydrophobic interaction chromatography (native Hfq) using a Butil-Sepharose column. In the last one was used a linear gradient of 1.0-0.1mol/L NaCl and 1.5-0mol/L $(NH_4)_2SO_4$ in 50 mmol/L Tris-HCl pH 8.0. The His-tag protein was purified to 90% purity and the native form reached 99% pure protein. Results on the binding of these proteins to RNA will be presented.

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