EXPRESSION, PURIFICATION AND FUNCTIONAL CHARACTERIZATION OF PhaR: A POLYHYDROXYBUTYRATE (PHB) BIOSYNTHESIS REGULATORY PROTEIN FROM *Herbaspirillum seropedicae*

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Herbaspirillum seropedicae is a nitrogen-fixing and endophytic bacterium which associates with several agricultural crops, improving the plant growth. This bacterium can accumulate polyhydroxybutyrate (PHB) polymer as a carbon and energy source. The PHB synthesis is negatively controlled by PhaR, a DNA-binding protein. In this work the *pha*R gene was amplified from *H. seropedicae* genome and cloned into the expression vector pET28(a). Expression of the PhaR protein was induced in *E. coli* BL21(DE3) strain using 0.3 mM IPTG and incubation at 20ºC for 12 hours. The expressed protein was purified in the presence of 0.05% Triton X-100, in order to avoid protein aggregation. By using a Ni^{2+}-binding affinity chromatography and a linear gradient of 10 to 500 mM imidazole in 100 mM NaCl and 50 mM Tris-HCl pH 7.5, the *H. seropedicae* PhaR protein reached 98% purity. The purified PhaR protein was able to bind the intergenic region between the *phb*B and *pha*R genes as observed by Electrophoretic Mobility Shift Assay (EMSA). Also it was able to bind PHB granules as determined by pull-down assays. Our results indicate that 0.05% Triton X-100 does not affected the folding of purified *H. seropedicae* PhaR protein to the active conformation. The purified PhaR protein is being submitted to crystallization for further structural studies.

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Keywords: *Herbaspirillum seropedicae*, protein expression and polyhydroxybutyrate.