

Cloning, Expression, Purification and Characterization of a Transthyretin-Related Protein from *Herbaspirillum seropedicae*

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Transthyretin-related proteins (TRPs) belong to a highly conserved family of homologous proteins related to the human transporter protein transthyretin (TTR). They are present in a large range of bacterial, fungal, plant, invertebrate and vertebrate species and, despite their structural similarities with TTR, TRPs are not able to bind thyroid hormones. TRPs are known to function as 5-hydroxyisourate hydrolases in the purine degradation pathway. A putative gene coding for a TRP has been identified from the complete genome sequence of *Herbaspirillum seropedicae*, a β -proteobacteria and an obligate endophytic diazotroph found in association with grasses, rice, and sugarcane. In this work, we describe the cloning, expression, purification, structural and functional characterization of this enzyme. Its amino acid sequence shows high identity with TRPs and TTRs from other organisms. *H. seropedicae* TRP contains the conserved TRP signature sequence (YRGS) at its C-terminal end. Mass spectrometry data confirmed the identity of this protein. CD spectroscopic analysis suggests a proper folding and a predominant β -strand content. Functional enzymatic assays confirms the activity of this protein as a hydroxyisourate hydrolase. Site-directed mutagenesis assays (H7A, H101A and Y114F) confirmed that these three conserved residues, previously described to be located at a TRP active site, are critical to the enzyme activity.

Keywords: Transthyretin; Transthyretin-related proteins; 5-hydroxyisourate; *Herbaspirillum seropedicae*; purine catabolism.

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