

OVER-EXPRESSION AND PURIFICATION OF THE HrpG PROTEIN, A  
PUTATIVE REGULATORY PROTEIN OF THE TYPE III SECRETION SYSTEM IN  
*Herbaspirillum seropedicae*

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*Herbaspirillum seropedicae* is a Gram-negative, nitrogen-fixing bacterium that has been attracting considerable attention as a potential biofertilizer because it associates with roots, and improves the growth, of several plants including maize, wheat and rice. The type III secretion system present in *H.seropedicae* is probably involved in its association with plants. HrpG is a putative regulatory protein of a two-component signal-transducing mechanism of the type III secretion system. The object of this work is to characterize the HrpG protein with respect to the above role by purification and DNA-binding studies. The *hrpG* gene was obtained by PCR using genomic DNA of *H.seropedicae*. The 720 bp DNA fragment was cloned into the expression vector pET28a, allowing the HrpG protein to be over-expressed in *Escherichia coli* BL21(?DE3) as a fusion to a His-tagged sequence. Upon induction with IPTG or lactose, it was over-produced as an insoluble HrpG-His-Tagged fusion protein. HrpG-His was purified from the inclusion body following affinity chromatography on a Hi Trap-Chelating-Ni<sup>2+</sup> column. The purified protein will be used for polyclonal antibody production and tested for DNA-binding activity.

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