

Identification of Putative HrpL-dependent Promoters in *Herbaspirillum seropedicae*

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Herbaspirillum seropedicae is a nitrogen-fixing bacterium found in endophytic association with economically important *Gramineae*. Many bacteria rely on the type III secretion system (T3SS), a specialized machinery for protein secretion, to interact with their hosts. This system is encoded by a cluster of approximately 20 hypersensitive response and pathogenicity (*hrp*) genes, which falls into two major groups according to the *hrp* operon structure and the regulatory system controlling gene expression. In the organisms of group I the *hrp* genes are regulated by HrpL, an alternative sigma factor. The *hrpL* gene expression is controlled by RpoN and HrpRS proteins. HrpL proteins are highly conserved and recognize a consensus sequence, named ‘*hrp box*’. In group II, the *hrp* operons are activated by an AraC-like activator. The gene for the activator protein is activated by HrpG, which belongs to the OmpR family of the two-components response regulators. In this work we found that *H. seropedicae* has a unique mechanism for *hrp* expression regulation. This system is a hybrid between group I and II regulatory cascades since in this microorganism an external signal seems to activate *hrpG* expression which in turn activates *hrpL*, responsible for the expression of the *hrp* genes. Four putative *hrp-boxes* were identified upstream *hrp* genes of *H. seropedicae*. To study the expression of *hrp* genes, the putative HrpL-dependent promoters were amplified and subcloned into the vector pPW452, which contains a promoter-less *lacZ* gene, and their expression analysed in the wild type and *hrpL* mutant strains of *H. seropedicae*.

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