Iron uptake systems in the grass endophyte *Herbaspirillum seropedicae* Z67: structure and transport of an NRPS-siderophore

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*Herbaspirillum seropedicae* Z67 is a β-Proteobacteria able to colonize the interior of rice plants and to express nitrogenase structural genes in planta. Our aim is to determine the importance of *H. seropedicae* iron uptake systems mediated by siderophores in its interaction with rice plants. Siderophores are low molecular weight compounds with high affinity for iron, produced and secreted by bacteria in low iron media.

By *in silico* analysis we identified a region in the *H. seropedicae* genome as probably involved in the biosynthesis, transcriptional regulation and transport of siderophores, very similar to the biosynthetic and transport locus for ornibactin, the siderophore produced by the related *Burkholderia* genus [1]. Among biosynthetic genes there is one with homology to non ribosomal peptide synthases (NRPS). A *H. seropedicae* mutant strain with this gene interrupted by a transposon insertion, did not produce siderophores. This result corroborates its role in siderophore biosynthesis. The chemical structure of *H. seropedicae* siderophore was predicted by bioinformatic analysis (using the NRPS aminoacid sequence) and LC/MS-MS. The predicted structure was highly similar to the proposed structure for ornibactin. Three ORFs present in this region could be involved in siderophore transport. Two of them were homologous to putative outer membrane siderophore receptors (*cirA* and *fhuA*). The third one presented homology to *orbE*, a gene encoding an inner membrane protein involved in ornibactin export. By proteomic analysis of outer membrane fractions we were able to detect and identify only the product of *fhuA*. Other three iron regulated outer membrane receptors from distant genomic regions were identify by MS: the products of *fiuA*, *shmR* and *fecA* genes. These receptors could be involved in the uptake of exogenous monocatechol siderophores, hemin and ferric citrate respectively.

A probable ORF involved in the transcriptional regulation of siderophore biosynthetic genes was found downstream of the *cirA* gene. This ORF is homolog to *orbS* which is expected to codify an iron starvation (IS) subfamily of extra citoplasmic sigma factors (ECF) involved in transcriptional regulation of ornibactin biosynthetic genes. These ECF direct the RNAp holoenzyme to a conserved DNA sequence called the IS box (consensus TAAAT-N16/17-CGT). Sequences identical to IS consensus were found in two putative promoters.

By directed mutagenesis we expect to identify the specific receptor for the endogenous siderophore of *H. seropedicae* and the implication of *orbS* homologous ORF in the transcriptional regulation of siderophore biosynthetic genes.

Reference


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