PROTEOMIC ANALYSIS OF Herbaspirillum seropedicae SECRETED PROTEINS

Chaves, D.F.S.; Monteiro, R.A.; Souza, E.M.; Yates, M.G. and Pedrosa, F.O.

Departamento de Bioquímica e Biologia Molecular – UFPR

Herbaspirillum seropedicae is an endophytic, non-pathogenic diazotroph associated with economically important crops such as rice, sugarcane and wheat. The interactions between plants and bacteria are not well understood but secreted proteins play a key role in this process. The GENOME project (Genopar) revealed that *H.* seropedicae has the structural components of the type III secretion system, which is used by pathogenic bacteria to deliver toxic proteins to eukaryotic cells. This work aimed at identifying proteins secreted by the wild type and a mutant strain (*hrcn⁻*) which does not secrete proteins by the type III system. To obtain secreted proteins, cultures of the wild type and mutant bacteria were centrifuged and the supernatants filtered. The proteins were precipitated using TCA and separated using two-dimensional gel electrophoresis or SDS-PAGE. Proteins were excised from gels and identified using a Maldi-ToF/MS mass spectrometer. Eighty five proteins were identified. Among these were 19 flagellar proteins, 19 ABC type transporters, 6 membrane and 3 hypothetical proteins. Four proteins were absent from the mutant strain. Two of these were identified and their N terminal sequence has a pattern characteristic of type III effector proteins. These results suggest that the type III secretion system may be operational in H. seropedicae. We are currently testing the functionality of this system in vivo using tomato plants.

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