Shotgun proteomics to prospect the interaction between wheat and *Herbaspirillum seropedicae*

Volpiano, C.G.[1]; Neiverth, A.[1]; Flores, L.V.[1]; Vendruscolo, E.C.G.[1]; Batista, M.[2]; Marchini, F.K.[2]; Santos, M.F.[1]

[1] Dep of Bioscience, Federal University of Parana - UFPR, PR, Brazil; [2] Carlos Chagas Institute / Fiocruz Parana, PR, Brazil

Herbaspirillum seropedicae is a plant growth-promoting bacteria (PGPB) capable of colonizing wheat. PGPB are bacteria beneficially found associated with many plant species. However, the molecular basis of association establishment is still poorly understood. Proteomic analysis may be used as a powerful tool to identify key proteins mediating interaction, especially considering new high-throughput protein identification methods. The aim of this work was to elucidate the molecular responses of wheat roots subjected to *in vitro* co-cultivation with *H. seropedicae* through shotgun proteomics. Proteins were extracted from 28 days *in vitro* cultured wheat roots (*Triticum aestivum* L. cv. CD 120) inoculated (1.5×10^{7} *H. seropedicae* SmR1 UFC.mL^{-1} in 3rd day of seeds germination) or non-inoculated in three biological replicates. The proteins were then reduced, alkylated, digested, desalted and analyzed by RP-LC-ESI-MS/MS with an EASY nLC 1000 coupled to a LTQ-Orbitrap mass spectrometer. MaxQuant (1.5.2.8) was used for peak list picking, protein identification and validation. A t-test was performed using p-values <0.001 for consideration. A total of 1,504 proteins were identified in wheat roots. In inoculated plants and non-inoculated were identified 1,275 and 1,185 proteins, respectively. In addition, 210 proteins varied statically their abundances and about 70% were unknown function proteins. Only an uncharacterized protein (W5DNV0) and a triosephosphate isomerase (TPI) increased their abundances in inoculated plants. Although TPI is a glycolytic enzyme, others enzymes also involved in energy producing decreased their abundances. Some others proteins followed the same decline behavior like peroxidases, profilin-2, annexins and, chorismate synthase. Considering number of protein identification, this work reached the most in-depth proteomic exploitation for *T. aestivum* up to date. This analysis also detected changes in plant protein abundance to handle with PGPB interaction. However, wheat genome databases still need further annotation.

Keywords: PGPB, wheat, shotgun proteomics