

TITLE: COMPARATIVE PROTEOMIC ANALYSIS IN *Gluconacetobacter diazotrophicus* PAL5 STRAIN IN RESPONSE TO HYDROGEN PEROXIDE

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ABSTRACT:

Among the alternatives of N input to ecosystems, biological nitrogen fixation (BNF) is considered as the most viable ones, since it reduces the costs and damage to the environment, reduces the dependence of nitrogen fertilizers and contributes to soil fertility. This process is performed by nitrogenase which requires large amounts of ATP provided by aerobic respiration. During this process, reactive oxygen species (ROS) may be generated, damaging the cells, inhibiting the activity of nitrogenase and affecting the processes of photosynthesis and colonization. To accomplish BNF and colonize plants, endophytic bacteria must resist efficiently or repair the damage caused by reactive oxygen species. *Gluconacetobacter diazotrophicus* is an endophytic nitrogen fixing bacteria which, when subjected to oxidative stress, express proteins that directly eliminate the oxidant. This study analyzed the effect of oxidative stress induced by H₂O₂ in protein expression profile of *G. diazotrophicus* strain PAL5. The wild type *G. diazotrophicus* was grown in biological triplicate in modified LGI-P, followed by exposure of cells either to the presence or absence of 100 µM H₂O₂. Cell sampling, was performed 15, 30 and 60 minutes after induction with H₂O₂ or not. Total protein from each treatment were obtained and submitted to liquid chromatography analysis coupled to mass spectrometry (ESI-LC-MS/MS). The mass spectrometry analysis identified proteins involved in various cellular processes such as replication, transcription, translation, cell division, proteolysis, NAD biosynthetic process, RNA, amino acids, cofactors, transport, among others. Proteins involved in cellular defense against oxidative stress were identified 30 and 60 minutes after exposing to H₂O₂, with an increase in the abundance of a peroxidase and superoxide dismutase at 30 minutes and a peroxiredoxin at 60 minutes. In addition, several other proteins related to oxidation-reduction processes were also observed with increased differential abundance. The results suggest that *G. diazotrophicus* strain PAL5 expresses H₂O₂ detoxifying proteins not belonging to the catalase family, confirming results already published in the literature. The peroxidases are the first enzymes to act when low doses (micromolar range) of hydrogen peroxide are applied.

Keywords: Functional genomics, Proteomics, Oxidative stress, Inoculation, Diazotrophic bacteria

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