

ABSTRACT

LEANDRO FERNANDES ANDRADE; D.Sc; Universidade Estadual do Norte Fluminense Darcy Ribeiro, March, 2017. Proteomic analysis of the response to sucrose stress in *Gluconacetobacter diazotrophicus* PAI 5 and identification of a new gene essential to the abiotic stress tolerance and to the plant interaction. Advisor: D.Sc. Gonçalo Apolinário de Souza Filho; Counselors: D.Sc. Rosana Rodrigues e D.Sc. Vanildo Silveira.

Environmental stresses are characterized as limiting factors for global agricultural production. This scenario has required the use of technologies that aim to mitigate the productivity loss, among them, it highlights the plant breeding. Among the sources of genes for tolerance to environmental stresses, the bacteria are distinguished by the diversity of adaptive mechanisms to establish themselves in different environments and by your applicability using genetic engineer. *Gluconacetobacter diazotrophicus* is a bacteria isolated from sugarcane and it is considered plant growth promoting bacteria (PGPB). This bacteria is highlights to your ability to grow in the presence of 30% sucrose, besides to present characteristics of tolerance to different stressors. These characteristics suggest the existence of molecular mechanisms of tolerance to environmental stresses, which are great biotechnological potential. In this sense, the present work aimed to identify mechanisms of sucrose tolerance in *G. diazotrophicus* PAI 5, and to characterize new genes involved in the response to abiotic stresses and in the plant growth promotion of the *Arabidopsis thaliana*. For this, the bacteria was cultured in medium under control condition and in medium supplemented with sucrose 600 mM. The stress caused for high sucrose reduced the cell growth by 30% but It did not induce morphological changes in the cells. The comparative proteomic analysis in the study conditions identified 869 proteins, in which 77 Differential Accumulated Proteins (DAP) increase were identified, among them carbon metabolism proteins and osmoprotective transporters; and 197 DAPs reduced, among them proteins porins and sugar transporters. The proteomic analyzes performed identified 53 proteins described as hypothetical in *G. diazotrophicus* PAI 5, which considered now as new proteins

involved in the response to sucrose stress. To proceed to the molecular characterization of these proteins, it was search for insertional mutants on Mutant Libray of *G. diazotrophicus* PAI 5. Although it was not recognized mutants of the hypothetical proteins identified in this proteomics study, it was possible the selection of the mutant GDP09A11, that coding a hypothetical predicted protein in *G. diazotrophicus* PAI 5. Assays was performed with the mutant with subsequent confirmation of its high sensitivity to sucrose stress. This characteristic allowed renaming the gene to *GdSucre01*. Assays performed with the $\Delta GdSucre01$ mutant showed the *GdSucre01* gene was identified as important to the stress tolerance by cadmium, Na₂SO₄, PEG, sucrose, as well as to the fixation biological process and the interaction and promotion growth of *A. thaliana* growth. The results obtained in this work revealed new genes involved in the tolerance of *G. diazotrophicus* to sucrose stress. In addition, it was possible to identify a new GdSucre01 protein with an important role for a tolerance to abiotic stresses and to plant growth promotion. Our study opens new perspectives for the understanding of mechanisms of stress resistance in microorganisms and in the process of interaction and plant growth promotion.

Keywords: endophytic bacteria, bacteria-plant interaction, osmotic stress, PGPB, mutagenesis, comparative proteomics.