Title: FUNCTIONAL GENOMIC OF *HERBASPIRILLUM RUBRISUBALBICANS* HCC103 STRAIN GROWN IN THE PRESENCE OF SUGARCANE APOPLASTIC LIQUID

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Summary:

Brazil is the world´s largest sugarcane producer with this crop playing an important role in the Brazilian agribusiness. However, the sugarcane crop may requires large amounts of nitrogen fertilizer to increase the yield and consequently increasing costs and risks of environmental damage. Endophytic diazotrophic bacteria, able to perform biological N fixation inside the sugarcane tissues, could be an alternative to alleviate the requirement for part of the nitrogen. It is believed that the plant apoplast, defined as the set of extracellular compartments containing carbohydrates, mineral salts, proteins, lignin and water, is an appropriated niche for the establishment of the bacteria. In order to increases the knowledge on the mechanisms associated to the apoplastic colonization, the *Herbaspirillum rubrisubalbicans* strain HCC103, a sugarcane associated bacterium, was grown in the presence or absence of sugarcane apoplastic liquid and analyzed for differentially gene expression with a high-through put sequencing based method (RNA-Seq). Strain HCC103 was inoculated in flasks with 100 ml of JNFb liquid medium supplied with NH_4Cl as N source at a concentration of 10^5 UFC.ml^{-1} and incubated at 150 rpm and 30°C. At the exponential growth phase, the grown culture was divided in three portions and supplemented with 50% of sterile water or fresh JNFb liquid medium or liquid of apoplast (from RB867515 sugarcane variety). Two to three hours after incubation, the cells were harvested for total RNA extraction. The total RNA was isolated with Trizol reagent® and treated with DNAseI. After ribosomal RNA (rRNA) depletion, rRNA depleted RNA was used to construct the sequencing libraries that were analyzed in the Ion Proton platform. The generated data were analyzed using the CLC Genomics Workbench and the results showed a minimal of 8.2% and maximum of 14.1% mapping against the HCC103 genome. Genes with fold change higher than 2 were considered induced and that one with fold change lower than 2 were considered repressed, at *p*-value 0.05. Genes functional annotations were done with Blast2Go program. In general, most of the differentially expressed genes was involved with the secondary metabolism and the synthesis of organic and nitrogen compounds. It is expected that this study allow the identification of key genes of *H. rubrisubalbicans* HCC103 strain involved in the interaction with the sugarcane plant, stimulated or suppressed by the constituents of the apoplast liquid.

Keywords: diazotrophic bacteria, transcriptomics, plant-bacteria interaction.

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