Global Transcriptional Shift Analysis of Glutamate Shock in N₂-Fixing Cells of *Azospirillum amazonense* strain CBAmc

Stefan Schwab¹, Ana Luíza Rivello Crivelaro¹², Vinicius Almir Weiss³ Michelle Zibetti Tadra Sfeir³, Helisson Faoro³, Leonardo Magalhães Cruz³, Fábio de Oliveira Pedrosa³, Emanuel Maltempi de Souza³, José Ivo Baldani¹.

¹ Embrapa Agrobiologia, Seropédica, Brazil.
² Federal Rural University of Rio de Janeiro, Seropédica, Brazil.
³ Department of Biochemistry and Molecular Biology, Federal University of Paraná, Curitiba, Brazil.

*Azospirillum* is the best studied genus of plant-growth promoting rhizobacteria (PGPR), and includes fifteen species described so far. *Azospirillum amazonense* is a bacterium initially isolated from Amazon and Rio de Janeiro forage grasses; afterwards, it was also isolated from rice, maize, sorghum, sugarcane, palm trees, and pineapple and banana plants. Results of inoculation assays in rice and sugarcane have shown the potential of that microorganism to be used as inoculant, and indicated a significant contribution from nitrogen fixation for plant growth promotion. Also, nitrogenase activity is only partially inhibited (“switched-off”) by the addition of ammonium. On the other hand, addition of glutamate at 15 mM to derepressed culture completely abolishes nitrogenase activity. In the present study, we examined the shift of global gene expression profile during nitrogenase switch-off in *A. amazonense* strain CBAmc after addition of 15 mM glutamate, by using an RNA-Seq approach. cDNA was prepared and sequenced on the SOLiD platform at the UFPR-INCT facilities (Curitiba, Brazil). Near 2,500,000 raw sequencing reads were obtained for both N₂-fixing and glutamate-shocked cell samples, and these were mapped using Shrimp program against the *A. amazonense* CBAmc genome. 614 genes (~10%, considering the 5,927 CDS of CBAmc) showed more than 2-fold expression decrease during transition from nitrogen fixation to presence of 15 mM glutamate. On the other
hand, 458 genes (~8%) had their expression levels increased more than 2-fold. Among the
downregulated genes were several nif and fix (encoding nitrogenase and accessory
proteins), while some of the upregulated genes code for enzymes involved in the
glutamate/glutamine metabolism, such as glutamate racemase, glutaminase, and glutamine
synthetase. The results show that, in the presence of high glutamate, *A. amazonense*
changes its metabolism to optimize the utilization of the new nitrogen source while shuts
down expensive pathways such as nitrogen fixation.

Keywords: biological nitrogen fixation, nitrogenase switch-off, transcriptome

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