Abiotic stress tolerance in *Rhizobium tropici* CIAT 899: response to atrazine toxicity

TULLIO, L.D.¹, PAULITSCH, F.¹, REICHERT, P.R.S.¹, GRAVINA, F.²; PILEGGI, M.¹, BATISTA, J.S.S.¹

¹ Universidade Estadual de Ponta Grossa (Av. General Carlos Cavalcanti, 4748 – Uvaranas-Ponta Grossa – PR)

² Universidade Federal do Paraná (Av. Cel. Francisco H. dos Santos, 210, Jardim das Américas, Curitiba – PR)

Rhizobium tropici CIAT 899 is recognized by its outstanding efficiency in fixing nitrogen, competitiveness and tolerance to abiotic stresses, including toxicity stress of xenobiotics. The capacity to cope efficiently with oxidative stress is central in response to a variety of stress conditions. Then, the aim of the present study was to analyze the ability of tolerance and degradation of the herbicide atrazine by R. tropici CIAT 899, and the association with the response to oxidative stress. Bacteria were grown in both Yeast Mannitol medium (YM) and YM supplemented with field dose (23,57µM) of atrazine (YM+A). For the growth kinetics by measuring the optical density and degradation of atrazine assays, aliquots were obtained every 2h, during 26h. Whole-cell proteins was extracted of 10, 15 and 25h aliguots, and were separated by non-denaturating polyacrylamide gel electrophoresis for determination of superoxide dismutase (SOD) e catalase (CAT) activities, and identification of SOD isoforms. Growth curves show that the growth in YM+A was maintained below the control until 20h, when a redution in growth rate occurs, superior than in YM, in which the exponential growth is maintained until 24h. CIAT 899 initiates the degration of about 40% of atrazine after 20h. However, for not being able to mineralize the molecule, the generation of more toxic metabolites may have negatively affected the CIAT 899 growth. SOD and CAT activities, in YM, reveal a pattern compatible with the growth stage, as higher levels occured in log phase, due to the formation of reactive oxigen species (ROS) by the aerobic metabolism of the bacteria. In the genome of CIAT 899, we found two genes that codify SOD, sodC and sodM. In this study, we only detected the activity of Mn-SOD, product of *sodM* gene, which expression is positively regulated when the superoxide radical is exogenous. The highest activity of this enzyme in 25h support the hypothesis that the partial degradation of atrazine generate even more toxic compounds. The activity of CAT is an evidence for the toxicity of atrazine to the bacteria, since it was superior in YM+A in all the times tested. Also, in R. etli CFN 42, the main role of CAT in the maintenance of exponencial growth was determined. The activities of SOD and CAT enzymes may be related to CIAT 899 ability of toleration and degradation of atrazine, as well as the high tolerance capacity to cope with a variety of stress conditions.

Keywords: biological nitrogen fixation, adaptation, superoxide dismutase, catalase, bioremediation.

Funding agencies: Capes, CNPq