STUDY OF THE TRANSFERENCE OF FIXED NITROGEN FROM DIAZOTROPHIC BACTERIA TO SUGARCANE

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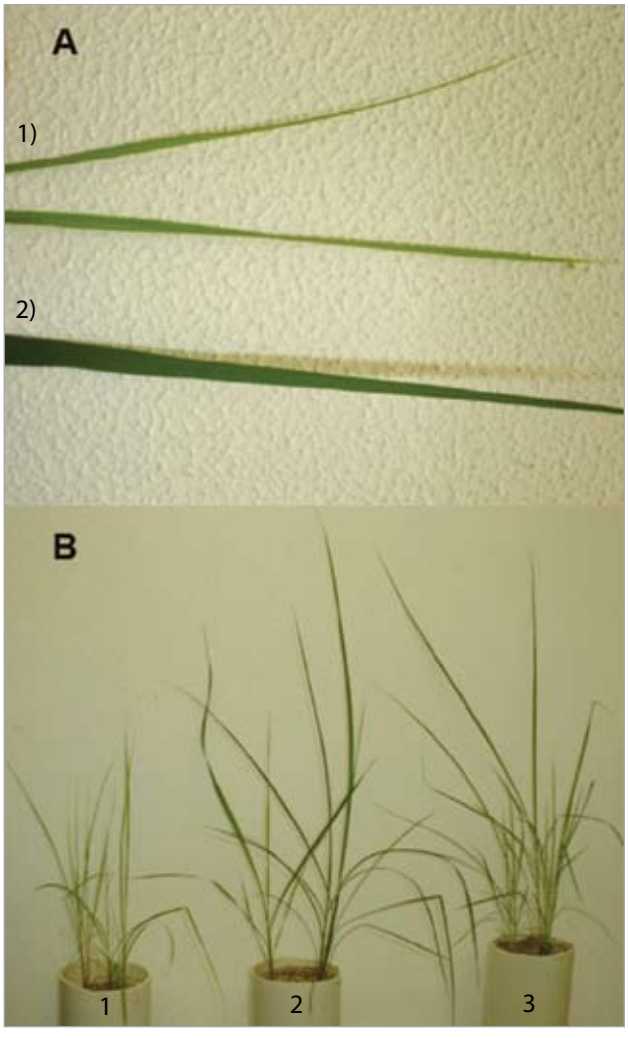


Figure 1. (A) Leaves of sugarcane plantlets treated in absence of combined nitrogen: 1) unfertilized; 2) submitted to organic fertilization. (B) Diferences between leaves lenght of sugarcane plantlets submitted to: 1) no fertilization (control); 2) conventional fertilization and 3) organic fertilization

Sugarcane is one of the most important crops in Brazil. This crop has a low response to nitrogen fertilizer, and even small inputs cause environmental impact. The use of biofertilizer as diazotrophic endophytic bacteria and organic fertilizer can decrease this impact. Endophytic bacteria live inside plant tissues and do not visibly harm the host. The influence of these bacteria on sugarcane is under investigation in this project.

Diazotrophic bacteria may play an important role in the nitrogen nutrition in sugarcane. The aim of the present project is to study the transference of fixed nitrogen from diazotrophic bacteria to sugarcane. To reach this objectives it will be performed: (1) analysis of the protein content and the C/N ratio in plantlets inoculated or not with endophytic diazotrophic bacteria isolated from sugarcane and submitted to different type of treatment: conventional, organic and control and (2) the evaluation of the possible interference of these bacteria on the nitrogen transport mechanism in sugarcane. (3) analysis of sugarcane callus grown in co-cultures with endophytic diazotrophic bacteria to study the bacterial interference on the callus proteins profile. Sugarcane callus can be used to evaluate if mixed diazotrophic bacteria can interact with each other and can also demonstrate the counter effect. The results obtained may contribute with knowledge on cultivation strategies. Thus, biodiversity will be used to the benefit of sustainable cultivation, evaluating the contribution of the nitrogen-fixing bacteria to the sugarcane, as well as the preservation of the soil and for the ecologic equilibrium.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

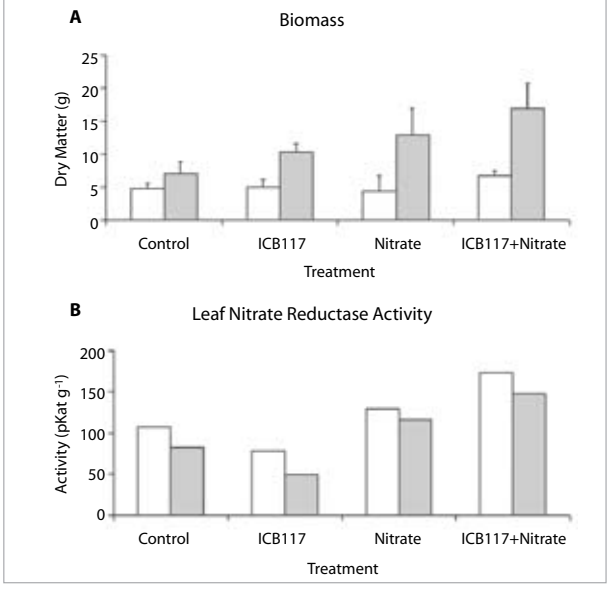
The treatments of sugarcane plantlets, after 60 days of incubation, showed that under organic fertilization, inorganic treatment and in control, respectively:
- a 3.0, 4.7 and 1.7 fold increase in the height of plantlets
- a 15.5, 31.0 and 3.4 fold increase in the fresh weight of plantlets
- a 25.5, 106.0 and 4.3 fold higher dry weight

Sugarcane plants were submitted to 4 treatments: (1) control (no inoculation and no combined nitrogen source); (2) inoculated with *Acinetobacter sp.* (ICB117) in absence of combined nitrogen source; (3) no inoculation, in presence of nitrate and (4) inoculated with ICB117 and in presence of nitrate. Bacterium Inoculated plants showed larger total dry matter, number of leaves and values of CO_2 uptake when compared to uninoculated plants submitted to the same nitrogen treatment. The enzyme nitrate reductase was more active in inoculated plants, in the presence of nitrate; in absence of nitrate, inoculated plants showed lower nitrate reductase activity than control. The endophytic ICB117 population was larger in plants treated without nitrate.

Using co-cultures, its was possible to evaluate that the influence of one bacterial genus on the callus depends on the bacterial strain; a mixture of two genera enhance the nitrogenase activity. Ongoing experiments, carried out in this project, aim to characterise these proteins and verify if there are differences between callus proteins in pure or in co-culture.

Future experiments will be carried out considering the presence or absence of inoculants and type of fertilization.

Figure 2: Dry matter (A) and leaf nitrate reductase activity (B) measured after 30 (white) and 60 (gray) days in plants under different treatments



MAIN PUBLICATIONS

Reinhardt ÉL, Ramos PL, Manfio G P, Barbosa HR, Pavan C, Moreira Filho CA. 2008. Molecular characterization of nitrogen-fixing bacteria isolated from brazilian agricultural plants at São Paulo State. *Braz. J. Microbiol*. **39**:414-422.

Gemelgo MCP, Sant´anna CL, Tucci A, Barbosa HR. 2008. Population dynamics of Cylindropermopsis raciborskii (Woloszynska) Seenayya & Subba Raju, a cyanobacteria toxic species, in water supply reservoirs in São Paulo, Brazil. *Hoehnea*. **35**:297-307.

Chimetto L, Brocchi M, Thompson C, Martins R, Barbosa HR, Thompson F. 2008. Vibrios dominate as culturable nitrogen-fixing bacteria of the Brazilian coral *Mussismilia hispida*. Syst. Appl. *Microbiol*. **31**:312-319.

Liba CM, Ferrara FIS, Manfio GP, Fantinatti-Garboggini F, Albuquerque RC, Pavan C, Ramos PL, Moreira-Filho CA, Barbosa HR. 2006. Vibrios dominate as culturable nitrogen-fixing bacteria of the Brazilian coral *Mussismilia híspida*. *J. Appl Microbiol*. **101**:1076-1086.

Barbosa HR, Thuler DS, Floh E, Handro W. 2003. *Beijerinckia derxii* releases plant growth regulators and amino acids in synthetic media independent of nitrogenase activity. *J. Appl Microbiol.* **95**:799-806.

Barbosa HR, Myiasaka N, Thuler DS, Floh E, Handro W, Toledo MD, Gagioti SM. 2003. During Stationary phase, *Beijerinckia derxii* shows nitrogenase activity concomitant with the release and accumulation of nitrogenated substances. *Microbiol Res*. **158**:309-315.

Barbosa HR, Thuler DS, Floh E, Handro W. 2003. Plant Growth regulators and amino acids released by *Azospirillum sp.* in chemically defined media. *Lett Appl Microbiol.* **37**:174-178.

Barbosa HR, Moretti MA, Thuler DS, Augusto EF. 2002. Nitrogenase activity of *Beijerinckia derxii* is preserved under adverse conditions for its growth. *Braz. J. Microbiol.* **33**:223-229.

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