

Preliminary results of studies of symbionts of *Lotus* and their association with solubilising of phosphate bacteria

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INTRODUCTION. Nitrogen is the nutrient that mostly determines the growth and performance of the cultivations in the soils of the Region Pampeana, followed in importance by phosphorus. An option for the use of fertilizers would be the utilization of plant growth promoting bacteria (PGPB). The action of the PGPB is manifested in different mechanisms such as; control of deleterious organisms; atmospheric nitrogen fixation; and mechanisms for moving and solubilising minerals like phosphorus and sulphur and arrange them for the plant growth. The action of the PGPB microorganisms is determined by biotic and abiotic factors, in this case it is important the salinity, sodicity, and flood conditions.

OBJECTIVE. To study the development of new alternatives of application of FBN in soils with constraints such as; salinity, and/or salinity-sodicity, and with low available phosphorus content.

MATERIALS AND METHODS. *Isolation of native rhizobia:* It was carried out in those rhizosphere soils corresponding to the region of the depressed Pampa of the Salado (soil 3: pH=7.35, soil 5: pH =7.05, soil 8: pH =7.87, soil 10: pH =9.50), by means of the trap plant method. Isolations were preserved in tubes with specific medium for the growth of *Mesorhizobium loti*. *Isolation of phosphorus solubilizing bacteria:* It was carried out of the rhizosphere of *Melilotus* utilized in the recovery of flooded fields in the province of La Pampa. The visual detection and the estimation of the solubilising capacity of the bacteria were determined by observing translucent areas (halos) around those colonies grown in specific media for phosphorus solubilising bacteria. *Choice of the isolations of native strains of rizobios to be utilized in the assay of effectiveness and infectivity:* it was done by means of the electrophoresis of isoenzymes technique (M.L.E.E) revealing for α and β -esterases. *Assay in climatized chamber:* design totally random with 10 repetitions, in tubes (20x2,5cm), with Jensen media, and light control (16-8 hours) and temperature (25-19°C). The assay finished when senescence could be seen in the treatment test (6 weeks). *Treatments:* Treatment test without inoculation and plus N; Inoculated with strain LL-32 alone and coinoculated with SP21 and Spp; Inoculated with strain S83 alone and coinoculated with SP21 and Spp; Inoculated with strain S38 alone and coinoculated with SP21 and Spp. *Evaluated parameters:* Dry weight; Nitrogen content. *Statistical analysis:* Analysis of variance-ANAVA; Comparison between treatments (Tukey)

Biotechnological parameters. Determination of growth velocity and generation time of the chosen rhizobia: strains were developed in liquid media. The cell concentration was

measured every 3 hours for a period of 51 hours, by means of the turbidimetric method at 600 nm, the values obtained were expressed as units of optical density (U.D.O).

Quantification of the phosphorus solubilised by the bacteria: by means of the Fiske technique and modified by Subbarow (colorimetric). The bacterial biomass was developed in liquid media. The concentration of solubilised phosphorus was established at different times, in two media, with pH control.

RESULTS AND DISCUSSION. Isolation of native rhizobia. Eight isolations were obtained, Soil 3: S38, S35, S36, Soil 5: S54, Soil 8: S83, Soil 9: S95, S96, S92.

Choice of the isolation of native strains of rhizobia to be utilized in the effectiveness and infectivity assay. we chose to work with native strains S83 and S38 and with the strain pattern LL-32, because they present a greater α and β esterase activity and because they correspond to soils with different pH, pH=7.35 and pH=8.08.

Isolation of phosphorus solubilising bacteria. it was obtained an isolation that was designed as Spp.

Assay in climatized chamber with *Lotus tenuis* (=L.glaber)

Dry weight. There are significant differences between inoculated and coinoculated treatments with the treatment test without inoculation (T). There are no significant differences among the treatments S83, S38, TN. There are significant differences among S83, S38, and LL-32, being the dry weight values obtained in the treatment with LL-32 significantly lower than the dry weight values obtained in the treatments S38 and S83. The dry weight values obtained in all coinoculated treatments with strain pattern SP21 are significantly lower than the ones obtained by inoculating with native strain Spp.

Nitrogen content. Nitrogen content is greater in inoculated and coinoculated plants than in the treatment test without inoculation (T). The coinoculation with the phosphorus solubilising bacteria Spp increases the percentages in comparison with the treatments only inoculated with the native rhizobio. It can be observed the negative effect of the coinoculation with the solubilising strain pattern (SP21).

Growth of rizobios strains. Isolations present UDO values higher than 7, which would correspond to a cell concentration of 10^9 - 10^{10} viable cells/ml, adequate values if we think of a future transfer.

Quantification of the property of solubilizing phosphorus. The bacterium Spp solubilises a greater quantity of phosphorus than the pattern one, this is improved if the culture media utilized is NBRIP. Bacteria reduce the pH of the media in the first hours of their growth, the mechanism utilized for solubilising phosphorus would be the release of acids.

Conclusions. The isolations of *Mezorhizobium loti* studied possess a high infectivity and effectivity in vitro, higher than the strain pattern. Regarding biotechnological parameters in the study of the isolations of native rizobios it is concluded that they are bacteria with a high aptitude for their possible technological transfer. The native phosphorus solubilising bacterium shows and efficient solubilising power both in the quantifications done in vitro compared to the strain pattern, and in the assay in acclimatized chamber, where it was

inoculated with the isolations of *Mezorhizobium loti*, showing in this case an increase of the parameters studied in comparison with simple inoculations.