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Mesorhizobium loti- Lotus spp. interaction: basic and applied research

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Rhizobia-legume interactions lead to the establishment of a symbiosis in which atmospheric-nitrogen fixation occurs. This symbiosis allows the plant to grow in soils with limited nitrogen source. Nodule formation requires a sequence of highly regulated and coordinated events which began with the signal exchange between the host plant and the bacterial symbiont. In our country, the improvement of *Lotus glaber* cultivars have had a great influence on the expansion of cultivated areas to the marginal ones, such as the Basin of Salado River in Buenos Aires. *Mesorhizobium loti* strains inoculated at field must to compete with the indigenous populations of rhizobia that could nodulate but generally will conduce to a poor nitrogen fixation process. Nodulation competitiveness between bacteria could occur in any step of the process from root colonization to the release into the nodule cells. A knowledge of the molecular bases that are involved in the *M. loti*-Lotus interaction and of which are the molecules, components or systems which presence improve or negatively affect this process is necessary if our aim is in an early future create and use organisms more competitive and efficient for nodulation and nitrogen fixation.

Our objective is to explore the symbiosis between *M. loti* and *Lotus* spp. combining the characterization of different bacterial mutants with the study of their nodulation phenotype and the effect of this mutation on the plant transcriptional profile during the interaction. We had analyzed the effect of the mutation of lipopolysaccharide and the cyclic glucan biosynthesis of the bacterium, in the process of nodulation with Lotus. The mutant affected in the cyclic glucan (*cgs* mutant) induced the formation of nodules empty of bacteria (pseudonodules), and the mutant affected in the lipopolysaccharide (*lpsβ2* mutant) presented lower nodulation competitiveness than the wild type strain. To study how the nodulation process is regulated at the transcriptional level we compared, using the macroarray technology, the transcriptional profile of *Lotus japonicus* plant roots inoculated with the wild type bacterial strain with those inoculated with each of the characterized mutants. We identified hundred of genes which expression is upon the bacterial infection. Several of the differentially expressed genes are involved in the plant defense response. This is in concordance with the idea that in spite the beneficial effect of the nodulation on both symbiosis partners, it resembles in some aspects a pathogen-host interaction. Analysis results of the relative expression level for some selected genes at different post inoculation times, suggested the existence of a control of the plant defense response induced during the symbiosis. Type three secretion system (T3SS) and the effectors proteins translocated through it into the host cell, were described as one of the bacterial components involved in the modulation of the plant defense. *M. loti* has a T3SS. We found that mutation of *M. loti*

T3SS affected its competitiveness on *Lotus glaber*. Through a bioinformatics approach we identified other genes induced by the presence of NodD and the specific flavonoids such as were the T3SS components. Two of the new identified genes were able to be secreted to the external media through the T3SS. The determination of the specific role of each of the putative T3SS effectors that were identified is remaining.

In addition with our basic research, in our laboratory we are also working to create *M. loti* modified strains that could be more competitive in the process of nodulation with *Lotus* spp. As a result of biotic and abiotic stress (flooding, light, drought) plant ethylene levels increase. Ethylene has a negative effect on the nodulation. *M. loti* has the *acdS* gene that codifies for ACC deaminase, enzyme that degrades an ethylene biosynthesis precursor. Its expression only occurs in the bacteroid, inside the nodule. We have obtained a modified *M. loti* strain that expresses the *acdS* gene also in the free-living state. The modified strain presented higher competitiveness in nodulation assays carried out in stress conditions.