

C9. Diversity and Ecology of Rhizobial Strains Used in Commercial Inoculants in Brazil

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With more than 30 million doses of rhizobial inoculants marketed per year, it is probable that Brazilian agriculture benefits more than that of any other country from symbiotic N₂ fixation. Selection programs are mandatory to guarantee the maximization of the contribution of the biological process and have identified strains that are recommended for use in commercial inoculants for almost one-hundred leguminous crops. We have analyzed ribosomal and host keeping genes and rep-PCR profiles of 122 of those strains. Polyphasic approaches have detected a very high level of genetic diversity among those strains, but apparently with no relation between phylogeny of rhizobia and of the legume host. Multilocus sequence analysis (MLSA) of housekeeping genes (*atpD*, *dnaK*, *glnII*, *gltA*, *recA* and *rpoA*) has proven to be a reliable way of providing information on phylogenetic relationships and taxonomy, being also very useful in the identification of rhizobial strains potentially representative of new species.

More than one-hundred experiments performed by our group have proven that a massive inoculation with selected strains of *Bradyrhizobium japonicum* and *B. elkanii* can significantly increase grain yields even in soils with high populations of established soybean bradyrhizobia. However, in a series of studies in which we have re-isolated soybean rhizobia from areas previously inoculated with exotic *B. japonicum* and *B. elkanii* strains, we found a high level of genetic diversity in comparison to the putative parental strains. In one of those experiments genetic variability was such that even within a genetically more stable group, such as that of CPAC 15, only 6.4% of the isolates showed high similarity to the inoculant strain, whereas none was similar to CPAC 7. High rates of horizontal transfer of the symbiotic island from inoculant strains to indigenous rhizobia have also been observed, and included transference of genes to bacteria belonging to other genera. The results highlight the strategies that bacteria may commonly use to obtain ecological advantages, as the acquisition of genes to establish effective symbioses with an exotic host legume.

New insights in the ecology of soybean and common bean (*Phaseolus*

vulgaris L.) inoculant strains have also been achieved by means of genomic and proteomic approaches, with an emphasis on comparative studies of natural variant strains differing in competitiveness or in the capacity of fixing N₂. Genomic drafts were obtained and others are in progress with common bean and soybean inoculant strains, and highlight several putative genes that might be related to the high competitive and saprophytic capacity of these strains. Functionality of some of those genes has been confirmed by proteomics and RT-qPCR, and highlight the role of some interesting genes, e.g. *nopP* in *B. japonicum* strain CPAC 15 and *nodG* in *R. tropici* strain PRF 81. Most interesting are several genes that play key roles and might explain the broad capacity of adaptation of these strains to a variety of soils and environmental conditions.