

Title: GENETIC DIVERSITY OF BACTERIA IN ROOT NODULES OF BRACATINGA (*Mimosa scabrella*).

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Abstract:

Bracatinga (*Mimosa scabrella* Benth.) is a leguminous tree native to southern Brazil with high economic potential as a source of timber and biomass. Like other leguminous species, *M. scabrella* forms associations with N₂ fixing bacteria in root nodules. However, despite reports that different N₂ fixing and endophytic bacteria occur in nodules of *Mimosa* sp., little is known about such bacterial diversity. We isolated 288 bacterial colonies from root nodules collected from *M. scabrella* in 61 locations across its native range. Strains were characterized according to morphological traits: size (<1mm or > 1 mm), shape (circular or punctiform), color (white or other), transparency (transparent or opaque), quantity of mucus (little, medium, much), colony edge (circular or irregular), elevation (flat or high), time to appearance of isolated colonies (rapid: up to 3 days; intermediate: 4 to 5 days and slow: over 6 days) and changes in pH (acid, neutral and alkaline). Using the above traits, isolated strains were grouped into 30 different morphotypes at 100% similarity. The 30 morphotypes were analyzed using a similarity matrix. According to the Jaccard index and a dendrogram based on the UPGMA grouping method at a 70% cutoff we found 11 distinct groups. Approximately one out of eight strains, was randomly selected across all distinct morphological groups and, subsequently, 16S rRNA sequencing. Sequences were identified using the BLAST tool on GenBank, aligned with related sequences using Muscle and subjected to phylogenetic analysis with software MEGA. Among the strains, *Burkholderia* was the most common genus, represented in 25 strains. On the basis of 16S rRNA gene sequence similarities, most strains were closely related to *Burkholderia nodosa*. However, strains related to *B. mimosarum* and *B. phytofirmans* were also present. Two strains corresponded to bacteria belonging to the *Cupriavidus* genus and one to *Rhizobium* genus. Ten strains of non-nodulating endophytic bacteria were detected inhabiting the nodules. Endophytic bacteria detected in the nodules corresponded to the genera *Pseudomonas*, *Pantoea*, and, for the first time, *Paenibacillus*, *Brevibacillus*, *Serratia* and *Arthrobacter*. In conclusion, our data shows that α -rhizobia and different β - rhizobia associate with *M. scabrella* nodules and that several endophytic bacteria are also present in the nodules.

Keywords: symbiosis, forestry, nitrogen fixation.

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