Title: Evaluation of phosphate-solubilizing bacteria associated to pastures of *Bracharia* from acid soils

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Abstract

Rhizobia have been widely known by their capacity to form a symbiotic relationship with legumes and fix atmospheric nitrogen. Recently, however, rhizobia have shown to associate with plants in different botanical families. In this study, we aimed at elucidating the diversity of rhizobia associated to grasses, and determine their capabilities to solubilize phosphate in both lab and greenhouse experiments. Isolation of rhizobia was performed using rhizosphere from *Brachiaria brizantha* and *B. decumbens* and a promiscuous legume trap plant (i.e. *Vigna unguiculata*). Thirty days after inoculation of the trap plant, rhizobia were isolated from nodules using the conventional protocol, classified in basis on their phenotypic features, and molecularly grouped using Amplified Ribosomal DNA Restriction Analysis (ARDRA). Finally, phosphate solubilization assays and greenhouse experiments were carried out on representatives of each ARDRA cluster. The results showed that the diversity of rhizobia varied between both plant species, which suggests that plant exudates significantly determine the composition of the plant microbiome. Surprisingly, most of the isolated associated to *B. brizantha* rhizosphere exhibited typical attributes of slow-growing rhizobia, whereas rhizobia from *B. decumbens* displayed a mixed diversity including slow-, intermediate-, and fast-growing rhizobia. Sequencing of 16S rRNA of ARDRA representatives showed that most of the rhizobia isolated from *B. brizantha* belonged to the *Mesorhizobium* and *Bradyrhizobium* genera, while those isolated from *B. decumbens* were phylogenetically clustered into *Rhizobium* and *Bradyrhizobium*. The capability of the isolates to solubilize phosphate was studied using iron and calcium phosphate. We observed that overall Bradyrhizobium exhibited the highest ability to solubilize iron phosphate; by contrast, calcium phosphate was similarly solubilized within representatives of the three genera. In greenhouse experiments, we found that plants inoculated with isolated BT53, BD17 and BD21 exhibited a significantly higher content of phosphorus ($p \leq 0.05$). Additionally, dry weight was significantly higher in

the treatment inoculated with BT16 isolate ($p \leq 0.05$). We conclude that 1) rhizobia is found associated with grasses, 2) plant genotype determines rhizobia diversity 3) rhizobia are able to solubilize phosphorus, and 4) they might be used to promote plant in different plant families. We further believe that further studies will reveal the true role of those old-known legume symbionts in development and growth of other important crops.

Keywords: *Brachiaria* sp., phosphate-solubilizing bacteria, acid soils.

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