TITLE: CHARACTERIZATION OF *Bradyrhizobium* STRAINS INDIGENOUS TO WESTERN AUSTRALIA AND SOUTH AFRICA INDICATES REMARKABLE GENETIC DIVERSITY AND REVEALS PUTATIVE NEW SPECIES

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ABSTRACT:

Biological nitrogen fixation (BNF) can highly impact agriculture, and the most relevant organisms involved are the symbiotic diazotrophic bacteria generally known as rhizobia, which can associate with a broad range of legumes forming specific organs called nodules, where the fixation process takes place. The genus *Bradyrhizobium* comprises N_2-fixing microsymbionts with relevant applications in agriculture sustainability. The aim of this study was to investigate the phylogenetic relationships of conserved and symbiotic genes, and to access the genetic diversity of 21 *Bradyrhizobium* strains isolated from nodules of different legume plants in Western Australia and South Africa. The 16S rRNA phylogeny divided the strains in two superclades, of *B. japonicum* and *B. elkanii*, but with low discrimination among the species, due to its high conservation. The multilocus sequence analysis (MLSA) with four protein-coding housekeeping genes (*dnaK*, *glnII*, *gyrB* and *recA*) pointed out seven groups as putative new species, two within the *B. japonicum*, and five within the *B. elkanii* superclades. The remaining eleven strains showed higher similarity with six species, *B. lupini*, *B. liaoningense*, *B. yuanmingense*, *B. subterraneum*, *B. brasilense* and *B. retamae*. The phylogenetic analysis of the *nodC* symbiotic gene clustered 13 strains in three different symbiovars (sv. vignae, sv. genistearum and sv. retamae), while seven other strains might compose new symbiovars; we were unable to amplify the *nodC* of one strain (WSM 1704). The genetic profile of the strains was evaluated by BOX-PCR, and revealed high intra- and interspecific diversity. The results point out the high level of diversity still to be explored within the *Bradyrhizobium* genus. Further studies might confirm the putative new species and symbiovars, also aiming at the elucidation of the symbiotic properties of rhizobial strains, which is of great importance to explore their biotechnological potential.

Keywords: multilocus sequence analysis, phylogenetic diversity, taxonomy, biological nitrogen fixation, nodulation.