TITLE: *Bradyrhizobium frederickii* sp. nov., A NITROGEN-FIXING LINEAGE ISOLATED FROM NODULES OF CAESALPINIOID SPECIES *Chamaecrista fasciculata* AND ADAPTED TO HIGH TEMPERATURE

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ABSTRACT:

The symbioses between legumes and nitrogen-fixing bacteria, collectively referred as rhizobia, give the greatest contribution to the global nitrogen input via the process of biological nitrogen fixation (BNF). *Bradyrhizobium* species stands out due to their slow growth rate *in vitro* at 28 ^{o}C, broad geographic distribution and wide host range, including basal legumes of the subfamily Caesalpinioideae. However, there are still few studies concerning the symbionts of this legume subfamily, including the genus *Chamaecrista*. We performed a polyphasic study with eleven strains isolated from root nodules of *Chamaecrista fasciculata*, an annual multi-functional herbaceous native to the midwest, eastern and southern states of the USA. Based on the 16S rRNA gene phylogeny, the strains were clustered in the *B. japonicum* superclade, showing 97-99.8 % similarity with other described *Bradyrhizobium* species. The analysis of the intergenic transcribed spacer (ITS) indicated 93.7-100 % similarity between the CNPSo strains and less than 89.7 % with other *Bradyrhizobium* species. Multilocus sequence analysis (MLSA) with four housekeeping genes (*glnII*, *gyrB*, *recA* and *rpoB*) corroborated with the classification of the CNPSo strains as a new group, sharing less than 95.2 % nucleotide identity with other *Bradyrhizobium* species, being most closely related to *B. liaoningense*. Noteworthy, high genetic diversity among the strains was confirmed in the analyses of ITS, MLSA and BOX-PCR. Average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) analyses showed values below the threshold when compared to the described *Bradyrhizobiu*m species, of 89.4 % and 38.7 %, respectively. In the *nifH* and *nodC* phylogenies, strains were clustered together, but with indication of horizontal gene transfer, showing higher similarity with *B. arachidis* and *B. forestalis*. In addition, other phenotypic, genotypic and symbiotic properties were evaluated. However, it is important to highlight the unusual fast growth by all 11 strains in three days at 37 °C, suggesting a very efficient mechanism of tolerance of high temperature. The data obtained in this study support the description of the CNPSo strains as representatives of a new species, suggested as *Bradyrhizobium frederickii* sp. nov., with CNPSo 3426^{T} as the type strain.

Keywords: MLSA, Caesalpinioideae, biological nitrogen fixation, rhizobia

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