

TITLE: POLYPHASIC TAXONOMY OF ISOLATES FROM NODULES OF *Chamaecrista fasciculata* NATIVE OF USA AND DESCRIPTION OF NOVEL *Bradyrhizobium* SPECIES

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ABSTRACT:

Plant growth is highly related to the nitrogen availability, as this nutrient is required for structural, metabolic and genetic processes. Biological nitrogen fixation is the main process of N₂ incorporation into terrestrial environments, and the enzymatic conversion of atmospheric nitrogen provides assimilable forms of this nutrient to plants. The process is restricted to a specific group of prokaryotes, mainly bacteria, and with an emphasis on rhizobia, which establish symbiotic associations with plants of the Fabaceae family, through a specialized structure on the roots and occasionally on stem, called nodule. Recent studies suggest the reclassification of the Fabaceae family into six subfamilies, but nodulation is restricted to the Papilionoideae and Caesalpinioideae. To date, the *Bradyrhizobium* genus has been reported as the major microsymbiont of the Caesalpinioideae; however, the diversity of rhizobia associated with this subfamily is poorly known. In this study a polyphasic analysis of rhizobia isolated from nodules of *Chamaecrista fasciculata*, a native Caesalpinioideae broadly found in USA was performed. Based on the 16S rRNA analysis, three CNPSO strains shared 100% of nucleotide identity (NI) to each other and with *B. diazoefficientes*, *B. betae*, *B. shewense* and *B. ottawaense*. The intergenic transcribed spacer (ITS) phylogeny grouped the CNPSO strains in an individual clade, with *B. japonicum* as the most related species. The MLSA (*multilocus sequencing analysis*) with six housekeeping genes -*atpD*, *dnaK*, *glnII*, *gyrB*, *recA*, *rpoB*-indicated *B. diazoefficiens* as the closest species, but sharing only 83 % of similarity. The *nodC* and *nifH* phylogenies grouped the strains with *B. arachidis*, *B. forestalis* and *B. cajanus*. The genome of CNPSO 3448 strain was compared by ANI (average nucleotide identity) and dDDH (digital DNA-DNA hybridization) with the genomes of the closest *Bradyrhizobium* species. All values were lower than 93.3 % in ANI and 53.50 % in dDDH. The BOX-PCR profiles confirmed high intraespecific diversity between the strains. The most relevant differences in phenotypic features were on the source C assimilation and antibiotics resistance. The results support the description of the CNPSO strains as a novel species, with the suggested nomenclature of *Bradyrhizobium niftali* sp. nov., with CNPSO 3448^T (=USDA 10051^T = U687^T =CL 40^T) designated as the type strain.

Keywords: Polyphasic taxonomy, *Bradyrhizobium*, Nodulation, Caesalpinioideae.

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