Genome Sequence of *Rhizobium jaguaris* CCGE525[T], a Strain Isolated from *Calliandra grandiflora* Nodules from a Rain Forest in Mexico

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ABSTRACT We present the genome sequence of *Rhizobium jaguaris* CCGE525[T], a nitrogen-fixing bacterium isolated from nodules of *Calliandra grandiflora*. CCGE525[T] belongs to *Rhizobium tropici* group A, represents the symbiovar calliandrae, and forms nitrogen-fixing nodules in *Phaseolus vulgaris*. Genome-based metrics and phylogenomic approaches support *Rhizobium jaguaris* as a novel species.

*R*hizobium jaguaris* CCGE525[T] was isolated from nodules of the medicinal legume *Calliandra grandiflora* growing in a rain forest in Chiapas, Mexico, and was described as related to *Rhizobium tropici* group A (1). *R. tropici* group A was defined by 16S rRNA gene sequences and distinctive phenotypic characteristics (2). We report the genome sequence of strain CCGE525, the type strain of *Rhizobium jaguaris*.

A single colony from a freeze-dried culture sample of *R. jaguaris* CCGE525[T] was incubated on peptone yeast (PY) medium (5 g/liter peptone, 3 g/liter yeast extract, and 0.6 g/liter $CaCl_2$) for 3 days at 30°C. DNA was extracted from 3 ml of culture using a kit for cells and tissues (Roche Applied Science, Germany). A SMRTbell library of 15- to 20-kb insert size was constructed using standard protocols. The library was sequenced on a PacBio RS II sequencer (3) using P6-C4 chemistry, which yielded 3.4 Gb of data. Reads were filtered and assembled *de novo* using Canu v.1.5 (4). Annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) (5). Amino acid sequences served as input to PhyloPhlAn (6) to predict evolutionary relationships. The progressive Mauve tool was used for genome alignments (7). DNA-DNA hybridization (DDH) values were computed using the Genome-to-Genome Distance Calculator v.2.1 (8). Average nucleotide identity (ANI) values were calculated as previously proposed (9) using the ANI calculator from the Konstantinidis Lab (http://enve-omics.ce.gatech.edu/ani/) (10). Default parameters were used for all programs.

The genome of *R. jaguaris* CCGE525[T] (8,025,568 bp, 58.95% G+C content, and ~278-fold coverage) consisted of a chromosome (4,575,315 bp), a chromid (2,584,926 bp), a symbiotic plasmid required for establishing interactions with legumes (550,563 bp), and an additional plasmid (314,764 bp). The genome coded for 8,400 predicted genes.
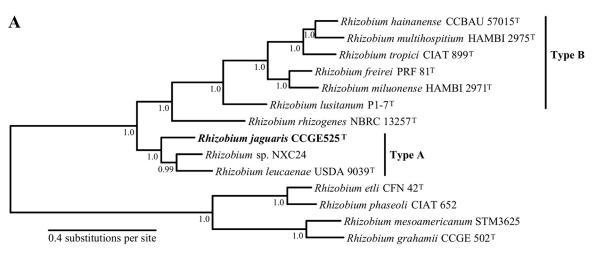
The *R. tropici* group A affiliation of *R. jaguaris* CCGE525[T] was supported by its position in a genome tree (Fig. 1A). This phylogenomic approach increased resolution and confirmed the placement of *R. jaguaris* CCGE525[T] as an isolated branch in the vicinity of *Rhizobium leucaenae* USDA 9039[T].
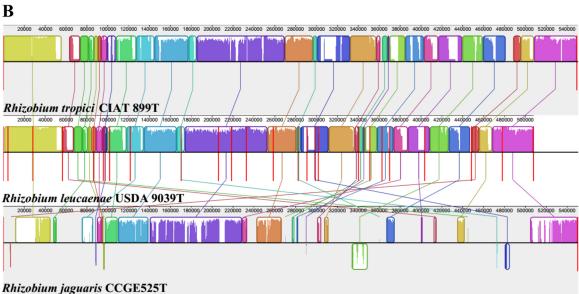
R. jaguaris CCGE525[T] is classified within the symbiovar calliandrae and has the capacity to form nitrogen-fixing nodules with common bean (1). A multiple sequence alignment revealed that the symbiotic plasmid of *R. jaguaris* CCGE525[T] was less

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A

B

FIG 1 (A) Phylogenomic analysis showing the evolutionary relationships between sequenced *Rhizobium* strains belonging to the *R. tropici* type A and type B groups. The phylogeny is based on 373 marker proteins. Branch labels correspond to Shimodaira-Hasegawa-like support values. (B) Genome alignment of symbiotic plasmid sequences of the type strains *R. tropici* CIAT 899, *R. leucaenae* USDA 9039, and *R. jaguaris* CCGE525. Conserved regions are shown in colored blocks.

conserved and presented rearrangements compared to the similar symbiotic plasmids of *Rhizobium tropici* CIAT 899[T] and *R. leucaenae* USDA 9039[T] (Fig. 1B).

Sequence comparisons between the symbiotic plasmid of *R. jaguaris* CCGE525[T] and the counterparts of *R. tropici* CIAT 899[T] and *R. leucaenae* USDA 9039[T] revealed ANI values of 85.40% and 85.48%, respectively. DDH estimates were 29.00% and 29.20% between the corresponding symbiotic plasmids. Thus, the symbiovar calliandrae is further validated.

Full-genome comparisons of *R. jaguaris* CCGE525[T] revealed DDH estimates of 33.90% and 35.00% against *R. leucaenae* USDA 9039[T] and *Rhizobium* sp. strain NXC24, respectively. ANI values of 87.07% and 87.50% were obtained when performing the same comparisons. These DDH and ANI values are below the thresholds for species boundaries of 70% and 95 to 96%, respectively (8, 9, 11–13). Thus, genome-based metrics allowed an accurate taxonomic circumscription of *Rhizobium jaguaris*.

Data availability. The genome sequence was deposited in GenBank under accession numbers CP032694 to CP032697. Raw sequences were submitted to the SRA database under accession number SRP174341.

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