



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.

Rhizobium 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 CaCl₂) 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

Citation Servín-Garcidueñas LE, Guerrero G, Rogel-Hernández MA, Martínez-Romero E. 2019. Genome sequence of *Rhizobium jaguaris* CCGE525^T, a strain isolated from *Calliandra grandiflora* nodules from a rain forest in Mexico. *Microbiol Resour Announc* 8:e01584-18. <https://doi.org/10.1128/MRA.01584-18>.

Editor Julia A. Maresca, University of Delaware

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Received 19 November 2018

Accepted 23 January 2019

Published 28 February 2019

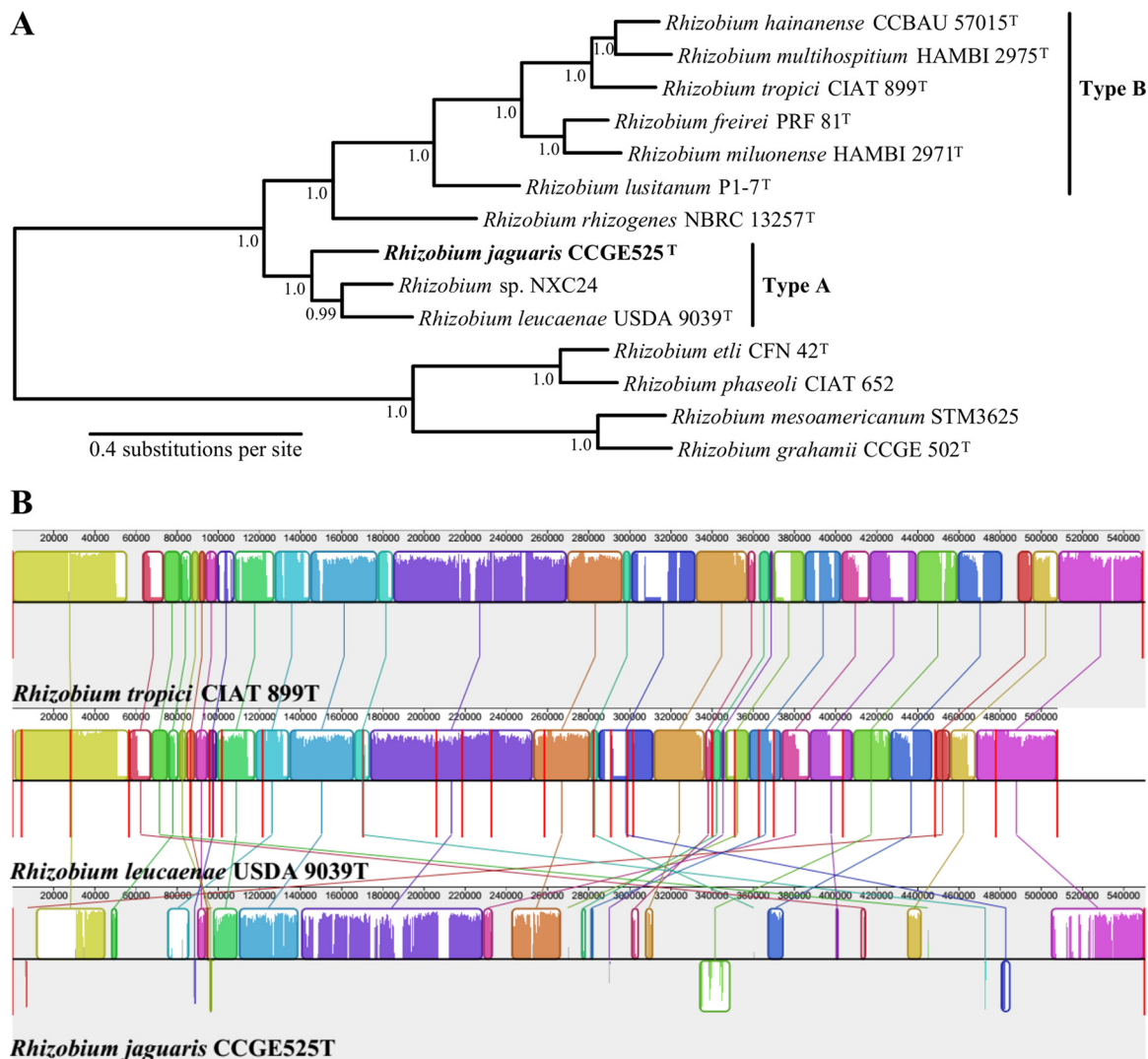


FIG 1 (A) Phylogenomic analysis showing the evolutionary relationships between sequenced *Rhizobium* strains belonging to the *R. tropici* type A and type B groups. The phylogeny is based on 373 marker proteins. Branch labels correspond to Shimodaira-Hasegawa-like support values. (B) Genome alignment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

ACKNOWLEDGMENTS

This research was supported by CONACyT 253116 and PAPIIT IN207718 from UNAM.

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