Genome Sequence of Bradyrhizobium stylosanthis Strain BR 446T, a Nitrogen-Fixing Symbiont of the Legume Pasture Stylosanthes guianensis

Jakeline Renata Marçon Delamuta,a,b Renan Augusto Ribeiro,c Douglas Fabiano Gomes,a,b Renata Carolini Souza,a,c Ligia Maria Oliveira Chueire,c Mariangela Hungriaa,b

Embrapa Soja, Soil Biotechnology, Londrina, Paraná, Brazil; CAPES, SBN, Edifício Capes, Brasília, Federal District, Brazil; CNPq, SHIS Qi 1 Conjunto B, Lago Sul, Brasília, Federal District, Brazil

Bradyrhizobium stylosanthis BR 446T is a nitrogen-fixing symbiont of the tropical legume pasture Stylosanthes guianensis. Its draft genome contains 8,801,717 bp and 8,239 coding sequences (CDSs). Several putative genes that might confer high competitiveness and saprophytic capacity under the stressful conditions of tropical soils were identified in the genome.

Received 12 May 2016 Accepted 17 May 2016 Published 30 June 2016

Several legumes stand out as economically important crops, however, legume forages are receiving increasing attention, especially in tropical regions, because of their potential in improving soil fertility. Benefits of using legumes rely mainly on their capacity of fixing atmospheric nitrogen with bacteria collectively known as rhizobia (1, 2).

Bradyrhizobium encompasses several bacteria highly effective in fixing nitrogen, but although the genus represents the most important group of rhizobia in the tropics, our knowledge about their diversity is still poor (1,3–5). Our group has reported several studies on the diversity of Bradyrhizobium strains in Brazil (3,4,6), and some of them derived in the description of new species (7–9). Recently, we have described the new species Bradyrhizobium stylosanthis, including strains isolated from Stylosanthes spp., an important perennial tropical forage legume (10). Now we report the draft genome of the type strain of this species, BR 446T (=CNPSo 2823T =HAMBI 3668T =H-87T).

To access the bacterial genome sequence, total DNA was extracted using the DNeasy blood and tissue kit (Qiagen) and processed on the MiSeq platform (Illumina) at Embrapa Soja, Londrina, Brazil. Shotgun sequencing generated 613,771 paired-end reads (2 × 300 bp), corresponding to approximately a 20.45-fold coverage. The FASTQ files were de novo assembled by A5-miseq pipeline, which performs read trimming, contig assembly, misassembly correction, and final scaffolding (11). The genome analyses revealed that strain BR 446T has one circular chromosome and a G+C content of 63.88 mol%. Sequences were submitted to RAST (12) and the genome is estimated to be 8,801,717 bp, assembled in 22 contigs, with 8,239 predicted coding sequences (CDSs).

The analysis using the SEED system (12) allowed the classification of 40% of the CDSs in 512 subsystems. In the symbiosis, nodulation is a key process and the genes that might confer higher or lower competitiveness to one strain are still not well understood. Interestingly, in BR 446T, 123 CDSs fit into the category of virulence, disease, and defense, including 13 beta-lactamase, bacteriocins, and a whole Mycobacterium virulence operon. There were also 27 CDSs related to iron acquisition and metabolism and 218 CDSs related to motility and chemotaxis, including several putative genes that might help with higher competitiveness. In relation to the nodulation and nitrogen fixation genes, they are in a putative symbiotic island, but we found only one copy of the regulatory nodD. In addition, nif operons were more conserved compared to other Bradyrhizobium strains than the nod operons. A variety of CDSs related to stress response (205) might be implicated in the high saprophytic capacity of the strain under the stressful conditions of tropical soils. The information obtained with the genome of B. stylosanthis contributes to the knowledge of the diversity of tropical rhizobia, as well as to studies on taxonomy and phylogeny of rhizobia.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number LVEM00000000, SUBID SUB1384295, BioProject PRJNA315153, BioSample SAMN04549886. The version described in this paper is LVEM01000000.

Funding information. This work, including the efforts of Mariangela Hungria, was funded by Embrapa (02.13.08.001.000). This work, including the efforts of Mariangela Hungria, was funded by MCTI/CNPq/CAPES (INCT-MPCPAgro).

References

1. Hungria M, Menna P, Delamuta JRM. 2015. Bradyrhizobium, the ancestor of all rhizobia: phylogeny of housekeeping and nitrogen-fxation genes, p. 191–202. In de Bruijn F (ed), Biological nitrogen fxation, vol. 2. John Wiley & Sons, Hoboken, NJ. http://dx.doi.org/10.1002/9781119053095.ch18

2. Ormeño-Orrillo E, Hungria M, Martínez-Romero E. 2013. Dinitrogen-fixing prokaryotes, p. 427–451. In Rosenberg E, de Long EF, Lory S, Stackebrandt E, Thompson F (ed), The prokaryotes—prokaryotic physiology and biochemistry. Springer-Verlag, Berlin. http://dx.doi.org/10.1007/978-3-642-30141-4_72.
3. Menna P, Barcellos FG, Hungria M. 2009. Phylogeny and taxonomy of a diverse collection of Bradyrhizobium strains based on multilocus sequence analysis of the 16S rRNA, ITS region and glnII, recA, atpD and dnaK genes. Int J Syst Evol Microbiol 59:2934–2950. http://dx.doi.org/10.1099/ijs.0.009779-0.

4. Delamuta JRM, Ribeiro RA, Menna P, Bangel EV, Hungria M. 2012. Multilocus sequence analysis (MLSA) of Bradyrhizobium strains: revealing high diversity of tropical diazotrophic symbiotic bacteria. Braz J Microbiol 43:698–710. http://dx.doi.org/10.1590/S1517-83822012000200035.

5. Parker MA. 2015. The spread of Bradyrhizobium lineages across host legume clades: from Abarema to Zygia. Microb Ecol 69:630–640. http://dx.doi.org/10.1007/s00248-014-0503-5.

6. Germano MG, Menna P, Mostasso FL, Hungria M. 2006. RFLP analysis of the RNA operon of a Brazilian collection of bradyrhizobial strains from thirty-three legume species. Int J Syst Evol Microbiol 56:217–229. http://dx.doi.org/10.1099/ijs.0.02917-0.

7. Delamuta JRM, Ribeiro RA, Ormeño-Orrillo E, Melo IS, Martinez-Romero E, Hungria M. 2013. Polyphasic evidence supporting the reclassification of Bradyrhizobium japonicum group Ia strains as Bradyrhizobium diazoefficiens sp. nov. Int J Syst Evol Microbiol 63:3342–3351. http://dx.doi.org/10.1099/ijs.0.049130-0.

8. Delamuta JRM, Ribeiro RA, Ormeño-Orrillo E, Parma MM, Melo IS, Martinez-Romero E, Hungria M. 2015. Bradyrhizobium tropiciagri sp. nov. and Bradyrhizobium embrapense sp. nov., nitrogen-fixing symbionts of tropical forage legumes. Int J Syst Evol Microbiol 65:4424–4433. http://dx.doi.org/10.1099/ijsem.0.000592.

9. Helene LCF, Delamuta JRM, Ribeiro RA, Ormeño-Orrillo E, Rogel MA, Martinez-Romero E, Hungria M. 2015. Bradyrhizobium viridiflagi sp. nov., encompassing nitrogen-fixing symbionts of legumes used for green manure and environmental services. Int J Syst Evol Microbiol 65:4441–4448. http://dx.doi.org/10.1099/ijsem.0.000591.

10. Delamuta JRM, Ribeiro RA, Araújo JLS, Rouws LFM, Zilli JE, Parma MM, Melo IS, Hungria M. 11 May 2016. Bradyrhizobium stylosanthes sp. nov., comprising nitrogen-fixing symbionts isolated from nodules of the tropical legume forage Stylosanthes spp. Int J Syst Evol Microbiol http://dx.doi.org/10.1099/ijsem.0.001148.

11. Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. http://dx.doi.org/10.1093/bioinformatics/btu661.

12. Aziz RK, Bartels D, Best AA, DeLong M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/1471-2164-9-75.