

Draft Genome Sequence of Bradyrhizobium elkanii Strain SEMIA 938, Used in Commercial Inoculants for Lupinus spp. in Brazil

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ABSTRACT Due to its high capacity for nitrogen fixation, strain SEMIA 938 is used in commercial inoculants for lupins in Brazil. Its genome was estimated at 8,780,064 bp and indicates that it belongs to the Bradyrhizobium elkanii species, while the analysis of nodulation genes classifies the strain in the symbiovar sojae.

**Biological nitrogen fixation (BNF) is a key process for global N balance and N input
B**in agriculture [\(1\)](#page-1-0). Outstanding rates of BNF have been cited for grain crops such as soybean (Glycine max) [\(2\)](#page-1-1); however, contributions of forage legumes and green manures also impact agriculture sustainability and mitigation of greenhouse gases [\(1,](#page-1-0) [3\)](#page-1-2). The genus Lupinus comprises more than 200 species of subtropical and temperate annual herb legumes used for grain consumption, green manure, and pastures; the majority of the species are indigenous to the Americas. In Brazil, the area with lupins is still modest, cropped mainly with European species in the southern states. For the past 3 decades, strain SEMIA 938, originally isolated from a nodule of Lupinus albus inoculated with a commercial inoculant for Lupinus from the United States (Hansen), has been officially authorized by Brazilian legislation for use in commercial inoculants for L. albus and Lupinus angustifolius [\(4\)](#page-1-3). However, few studies have been performed with strain SEMIA 938, and now we describe its sequenced genome.

Strain CNPSo 938 (= CNPSo 1004) was grown in modified yeast mannitol (YM) medium at 28°C for 5 days [\(5\)](#page-1-4), and then total DNA was extracted using the DNeasy blood and tissue kit (Qiagen) and processed on the MiSeq platform (Illumina) at Embrapa Soja. Genome sequencing resulted in 871,756,030 bp with assemblage and quality control performed with the A5-MiSeq pipeline (de novo) v.20140604. Sequencing allowed 99.29-fold genome coverage in 80 contigs with an N_{50} value of 279,572 bp. Using QUAST v.2.0 [\(6\)](#page-1-5) with default parameters, the genome was estimated at 8,780,064 bp with a $G-C$ content of 63.87%. A total of 8,821 coding DNA sequences (CDSs) were identified using Rapid Annotations using Subsystems Technology (RAST) [\(7\)](#page-1-6), with 49% classified in 381 subsystems, and the annotation is available in the GenBank database.

Average nucleotide identity (ANI) was successfully used for taxonomic purposes, with cutoff values of 95 to 96% corresponding to 70% DNA-DNA hybridization (DDH), the threshold for species delineation [\(8\)](#page-1-7). Using the ANI calculator [\(9\)](#page-1-8) for comparison with type strains of all Bradyrhizobium species, the highest identity (95.94%) of SEMIA 938 was with Bradyrhizobium elkanii USDA 76T, while digital DDH (dDDH), evaluated with the Genome-to-Genome Distance Calculator (GGDC) v.2.1 using the recommended "formula 2" [\(10\)](#page-1-9), resulted in a value of 65.50%. Therefore, although SEMIA 938 shows the highest relatedness with B. elkanii, it might represent a new species.

Nodulation (nod) genes of Bradyrhizobium species are key for the BNF process with legumes [\(11,](#page-1-10) [12\)](#page-1-11). In SEMIA 938, nod genes are organized as in Bradyrhizobium diazoef-

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ficiens strain USDA 110^T, with the regulatory nodD1 and nodD2 genes followed by the operon nodABC. The genome also carries nodZ, an unusual nod gene related to host specificity with constitutive expression independent of nodD [\(11,](#page-1-10) [13\)](#page-1-12). To determine the symbiovar, or biological variant of symbiotic genes [\(14\)](#page-1-13), of SEMIA 938, nodC, nodY/K, and nifH were analyzed, confirming that the strain belongs to the symbiovar sojae, recently described by our research group [\(12\)](#page-1-11). Information available from this genome helps to elucidate the phylogenetics of conserved and nitrogen-fixation genes of the Bradyrhizobium genus.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the GenBank accession number [SZZP00000000,](https://www.ncbi.nlm.nih.gov/nuccore/SZZP00000000) BioProject number [PRJNA541436,](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA541436) and BioSample number [SAMN11585296.](https://www.ncbi.nlm.nih.gov/biosample/SAMN11585296) The version described in this paper is SZZP01000000.

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REFERENCES

- 1. Ormeño-Orrillo E, Hungria M, Martínez-Romero E. 2013. Dinitrogenfixing 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, Germany. [https://doi](https://doi.org/10.1007/978-3-642-30141-4_72) [.org/10.1007/978-3-642-30141-4_72.](https://doi.org/10.1007/978-3-642-30141-4_72)
- 2. Hungria M, Mendes IC. 2015. Nitrogen fixation with soybean: the perfect symbiosis?, p 1009 –1023. In de Bruijn F (ed), Biological nitrogen fixation, vol 2. John Wiley & Sons, Inc., Hoboken, NJ. [https://doi.org/10.1002/](https://doi.org/10.1002/9781119053095.ch99) [9781119053095.ch99.](https://doi.org/10.1002/9781119053095.ch99)
- 3. Helene LCF, Delamuta JRM, Ribeiro RA, Ormeño-Orrillo E, Rogel MA, Martínez-Romero E, Hungria M. 2015. Bradyrhizobium viridifuturi sp. nov., encompassing nitrogen-fixing symbionts of legumes used for green manure and environmental services. Int J Syst Evol Microbiol 65: 4441– 4448. [https://doi.org/10.1099/ijsem.0.000591.](https://doi.org/10.1099/ijsem.0.000591)
- 4. MAPA (Ministério da Agricultura, Pecuária e Abastecimento). 2011. Instrução Normativa SDA no. 13, 24 March 2011. Available at [http://www](http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-agricolas/fertilizantes/legislacao/in-sda-13-de-24-03-2011-inoculantes.pdf) [.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-agricolas/](http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-agricolas/fertilizantes/legislacao/in-sda-13-de-24-03-2011-inoculantes.pdf) [fertilizantes/legislacao/in-sda-13-de-24-03-2011-inoculantes.pdf.](http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-agricolas/fertilizantes/legislacao/in-sda-13-de-24-03-2011-inoculantes.pdf) Accessed 6 May 2019.
- 5. Hungria M, O'Hara GW, Zilli JE, Araujo RS, Deaker R, Howieson JG. 2016. Isolation and growth of rhizobia, p 39-60. In Howieson JG, Dilworth JG (ed), Working with rhizobia. ACIAR, Canberra, Australia.
- 6. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072-1075. [https://](https://doi.org/10.1093/bioinformatics/btt086) [doi.org/10.1093/bioinformatics/btt086.](https://doi.org/10.1093/bioinformatics/btt086)
- 7. Aziz RK, Bartels D, Best AA, DeJongh 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. [https://doi.org/10.1186/1471-2164-9-75.](https://doi.org/10.1186/1471-2164-9-75)
- 8. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME. 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461– 466. [https://doi.org/10.1099/ijsem.0](https://doi.org/10.1099/ijsem.0.002516) [.002516.](https://doi.org/10.1099/ijsem.0.002516)
- 9. Rodriguez-R LM, Konstantinidis KT. 2016. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. PeerJ PrePrints 4:e1900v1. [https://doi.org/10.7287/peerj.preprints.1900v1.](https://doi.org/10.7287/peerj.preprints.1900v1)
- 10. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. 2013. Genome sequencebased species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. [https://doi.org/10.1186/](https://doi.org/10.1186/1471-2105-14-60) [1471-2105-14-60.](https://doi.org/10.1186/1471-2105-14-60)
- 11. Menna P, Hungria M. 2011. Phylogeny of nodulation and nitrogen fixation genes in Bradyrhizobium: support for the theory of monophyletic origin and spread and maintenance by both horizontal and vertical transference. Int J Syst Evol Microbiol 61:3052–3057. [https://doi.org/10](https://doi.org/10.1099/ijs.0.028803-0) [.1099/ijs.0.028803-0.](https://doi.org/10.1099/ijs.0.028803-0)
- 12. Delamuta JRM, Menna P, Ribeiro RA, Hungria M. 2017. Phylogenies of symbiotic genes of Bradyrhizobium symbionts of legumes of economic and environmental importance in Brazil support the definition of new symbiovars pachrhizi and sojae. Syst Appl Microbiol 40:254 –265. [https://](https://doi.org/10.1016/j.syapm.2017.04.005) [doi.org/10.1016/j.syapm.2017.04.005.](https://doi.org/10.1016/j.syapm.2017.04.005)
- 13. López-Lara IM, Blok-Tip L, Quinto C, Garcia ML, Stacey G, Bloemberg GV, Lamers GE, Lugtenberg BJ, Thomas-Oates JE, Spaink HP. 1996. NodZ of Bradyrhizobium extends the nodulation host range of Rhizobium by adding a fucosyl residue to nodulation signals. Mol Microbiol 21:397– 408. [https://doi.org/10.1046/j.1365-2958.1996.00644.x.](https://doi.org/10.1046/j.1365-2958.1996.00644.x)
- 14. Rogel MA, Ormeño-Orrillo E, Romero EM. 2011. Symbiovars in rhizobia reflect bacterial adaptation to legumes. Syst Appl Microbiol 34:96 –104. [https://doi.org/10.1016/j.syapm.2010.11.015.](https://doi.org/10.1016/j.syapm.2010.11.015)