



Complete Genome Sequence of *Bradyrhizobium ottawaense* OO99^T, an Efficient Nitrogen-Fixing Symbiont of Soybean

Hai D. T. Nguyen,^a Sylvie Cloutier,^a Eden S. P. Bromfield^a

^aOttawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada

ABSTRACT We present the complete genome sequence of *Bradyrhizobium ottawaense* strain OO99^T, a nitrogen-fixing bacterium from root nodules of soybean. The genome consists of a single 8.6-Mb chromosome and includes a symbiosis island. Genes involved in symbiotic nitrogen fixation, stress response, resistance to antibiotics, and toxic compounds were detected.

Some species of soil bacteria belonging to the genus *Bradyrhizobium* have the ability to form nitrogen-fixing symbioses with economically important legumes, including soybean (*Glycine max*). In previous studies (1, 2), strains of symbiotic bacteria from root nodules of soybean grown in Ottawa, Canada, were characterized and described as the species *Bradyrhizobium ottawaense*. The type strain of this species, OO99 (= LMG 26739^T = HAMBI 3284^T), is highly efficient in symbiotic nitrogen fixation with soybean (1).

A draft genome sequence of *B. ottawaense* OO99^T was previously obtained with Illumina technology, but the assembly was fragmented (150 contigs; GenBank accession number [NPNY000000000](#)) (3). In this study, we resequenced strain OO99^T using PacBio technology. Bacteria from a pure culture were grown on yeast extract mannitol agar medium (2). Genomic DNA was extracted using the Promega Wizard SV genomic DNA purification system and cleaned with a Qiagen DNeasy PowerClean Pro kit. Sequencing was done at the Genome Quebec Innovation Centre in Montreal, Canada, with the Pacific Biosciences (PacBio) RS II single-molecule real-time (SMRT) platform (4). A total of 108,802 polymerase reads with an average read length of 13,248 bp were generated; estimated genome coverage was 141-fold. Reads were *de novo* assembled with the hierarchical genome assembly process (HGAP) (5). In the preassembly step, quality control was performed by aligning short subreads on long subreads with BLASR (6); assembly was performed with Celera Assembler (7) and polished with Quiver (5).

The complete genome of *Bradyrhizobium ottawaense* OO99^T comprises a single circular chromosome of 8,606,328 bp with an average G+C content of 63.8%. A total of 8,238 genes, 8,180 coding sequences, 51 tRNAs, and a single rRNA operon were found with the NCBI Prokaryotic Genome Annotation Pipeline v. 4 (PGAP-4) (8, 9). Analyses performed with the PATRIC v. 3.5.26 platform (10) indicated that the most abundant genes were those involved in metabolism (1,084 genes), energy (391 genes), membrane transport (245 genes), and protein processing (238 genes). Genes implicated in motility, chemotaxis, siderophore production, polysaccharide biosynthesis, and stress response (heat, cold, and osmotic shock) were found. We also identified 196 genes involved in resistance to antibiotics and toxic compounds, including arsenic and chromium.

A total of 25 putative genomic islands were detected based on Island Path-DIMOB prediction implemented in the IslandViewer 4 platform (11). A symbiosis island (about 527 kb) flanked by two integrase genes was found. The symbiosis island carries genes required for nodulation and nitrogen fixation. The *nodZ* gene and the regulatory genes *nodD1* and *nodD2* that are implicated in host specificity were present (12). Type I, II,

Received 26 October 2018 **Accepted** 2 November 2018 **Published** 29 November 2018

Citation Nguyen HDT, Cloutier S, Bromfield ESP. 2018. Complete genome sequence of *Bradyrhizobium ottawaense* OO99^T, an efficient nitrogen-fixing symbiont of soybean. *Microbiol Resour Announc* 7:e01477-18. <https://doi.org/10.1128/MRA.01477-18>.

Editor Frank J. Stewart, Georgia Institute of Technology

© Crown copyright 2018. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](#).

Address correspondence to Hai D. T. Nguyen, hai.nguyen2@canada.ca, or Eden S. P. Bromfield, Eden.Bromfield@canada.ca.

II/IV, and III secretion systems genes and those required for conjugative transfer were also detected.

Further analysis of the OO99^T genome will contribute to the knowledge of economically important symbiotic bacteria and will be useful for evolutionary and taxonomic studies of the genus *Bradyrhizobium*.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [CP029425](https://doi.org/10.1093/nar/gkw569). Raw PacBio data have been deposited in the NCBI Sequence Read Archive under the BioProject accession number [PRJNA470945](https://doi.org/10.1093/nar/gkw569).

ACKNOWLEDGMENT

Funding by Agriculture and Agri-Food Canada is gratefully acknowledged.

REFERENCES

1. Tang J, Bromfield ESP, Rodrigue N, Cloutier S, Tambong JT. 2012. Microevolution of symbiotic *Bradyrhizobium* populations associated with soybeans in east North America. *Ecol Evol* 2:2943–2961. <https://doi.org/10.1002/ece3.404>.
2. Yu X, Cloutier S, Tambong JT, Bromfield ESP. 2014. *Bradyrhizobium ottawaense* sp. nov., a symbiotic nitrogen fixing bacterium from root nodules of soybeans in Canada. *Int J Syst Evol Microbiol* 64:3202–3207. <https://doi.org/10.1099/ijs.0.065540-0>.
3. Bromfield ESP, Cloutier S, Tambong JT, Thi TVT. 2017. Corrigendum to “soybeans inoculated with root zone soils of Canadian native legumes harbour diverse and novel *Bradyrhizobium* spp. that possess agricultural potential.” *Syst Appl Microbiol* 40:517. <https://doi.org/10.1016/j.syapm.2017.10.003>.
4. Nguyen HDT, Lewis CT, Lévesque CA, Gräfenhan T. 2016. Draft genome sequence of *Alternaria alternata* ATCC 34957. *Genome Announc* 4:e01554-15. <https://doi.org/10.1128/genomeA.01554-15>.
5. Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
6. Chaisson MJ, Tesler G. 2012. Mapping single molecule sequencing reads using Basic Local Alignment with Successive Refinement (BLASR): application and theory. *BMC Bioinformatics* 13:238. <https://doi.org/10.1186/1471-2105-13-238>.
7. Myers EW, Sutton GG, Delcher AL, Dew IM, Fasulo DP, Flanigan MJ, Kravitz SA, Mobarry CM, Reinert KNH, Remington KA, Anson EL, Bolanos RA, Chou H-H, Jordan CM, Halpern AL, Lonardi S, Beasley EM, Brandon RC, Chen L, Dunn PJ, Lai Z, Liang Y, Nusskern DR, Zhan M, Zhang Q, Zheng X, Rubin GM, Adams MD, Venter JC. 2000. A whole-genome assembly of *Drosophila*. *Science* 287:2196–2204. <https://doi.org/10.1126/science.287.5461.2196>.
8. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44: 6614–6624. <https://doi.org/10.1093/nar/gkw569>.
9. Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Res* 46:D851–D860. <https://doi.org/10.1093/nar/gkx1068>.
10. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. *Nucleic Acids Res* 45: D535–D542. <https://doi.org/10.1093/nar/gkw1017>.
11. Bertelli C, Laird MR, Williams KP, Simon Fraser University Research Computing Group, Lau BY, Hoard G, Winsor GL, Brinkman FSL. 2017. IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. *Nucleic Acids Res* 45:W30–W35. <https://doi.org/10.1093/nar/gkx343>.
12. Menna P, Hungria M. 2011. Phylogeny of nodulation and nitrogen fixation genes in *Bradyrhizobium*: supporting evidence for the theory of monophyletic origin, and spread and maintenance by both horizontal and vertical transfer. *Int J Syst Evol Microbiol* 61:3052–3057. <https://doi.org/10.1099/ijs.0.028803-0>.