



# Revealing the Draft Genome Sequence of *Bradyrhizobium* sp. Strain USDA 3458, an Effective Symbiotic Diazotroph Isolated from Cowpea (*Vigna unguiculata*) Genotype IT82E-16

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**ABSTRACT** Pairing plants with plant growth-promoting bacteria is critical to the future of agriculture. *Bradyrhizobium* sp. strain USDA 3458 isolated from *Vigna unguiculata* (cowpea) paired with cowpea genotype IT82E-16 represents a novel combination in arid regions. Here, we report the draft genome sequence of strain USDA 3458.

The rhizosphere microbiome is both numerically large and highly diverse (1–3). Critical members of the rhizosphere microbiome are symbiotic nitrogen fixers (SNF) (4). SNF account for ~80% of all fixed nitrogen (5, 6), and SNF ability is selected for by the plant host, resulting in fitness alignment between the host and symbiont (7). Finding plant growth-promoting rhizobacteria (PGPR) has been a focus of the U.S. Department of Agriculture (USDA) for over 100 years (8, 9).

*Bradyrhizobium* sp. strain USDA 3458 was isolated from a *Vigna unguiculata* (cowpea) nodule from Nigeria in 1975 (<https://data.nal.usda.gov/dataset/usda-ars-national-rhizobium-germplasm-collection>). In both the greenhouse and field, experimental strain USDA 3458 formed the most effective symbiosis with the cowpea genotype IT82E-26, which is resistant to cowpea aphid (*Aphis craccivora* Koch) (10, 11); further inoculation with strain USDA 3458 may provide useful plant growth promotion in semiarid tropical regions (e.g., Africa).

A lyophilized culture of *Bradyrhizobium* sp. strain USDA 3458 was obtained from the Agricultural Research Service at the U.S. Department of Agriculture (USDA) National Rhizobium Germplasm Collection. The bacterial culture (single colony) was inoculated in AG broth (12) at 30°C at 200 rpm to obtain biomass for DNA extraction (12, 13).

DNA extraction, purification, and quality control are described in reference 13. Sequencing libraries were prepared using the SeqOnce RhinoSeq kit, following the manufacturer's protocols. Sequencing was performed on a HiSeq 4000 instrument with 150-bp paired-end read format at the Michigan State University Research Technology Support Facility (RTSF).

Default parameters were used for all software, unless otherwise specified. Sequencing data were quality filtered and decontaminated using ATLAS (version 1.0) (14). The quality-controlled sequencing data (2,233,194 paired-end reads) were then assembled with Unicycler (version 0.4.7), using default Illumina assembly parameters (15).

The genome assembly contains 226 contigs, with a genome size of 8,601,019 bp, a G+C content of 63.93%, and an  $N_{50}$  value of 59,884 bp. CheckM (version 1.0.12) calculated strain USDA 3458 completeness to be 97.84%, with 1.04% contamination

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(16). We removed the genome contamination using RefineM (genomic properties setting, version 0.0.25) (17).

Prokka was used for annotation (version 1.13.3 with -rfam flag), which predicted 56 tRNAs, 1 transfer-messenger RNA (tmRNA), 37 noncoding RNAs (misc\_RNA), 1 copy of a 5S-16S-23S operon, 0 CRISPRs, and 8,018 coding genes (18).

We provide this high-quality draft genome sequence as a template for further metabolic engineering and synthetic biology applications, including plant growth-promoting symbiosis in vector-resistant crops.

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [VIDV0000000](https://doi.org/10.1093/nar/gkz000). The version described in this paper is version VIDV01000000. Raw data, contigs, and annotations for this genome can be found at <https://osf.io/7t4j8/>. The code used to generate the assembly can be found at [https://github.com/friesenlab/Bradyrhizobium\\_USDA3458](https://github.com/friesenlab/Bradyrhizobium_USDA3458).

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## REFERENCES

- White RA, III, Rivas-Ubach A, Borkum MI, Köberl M, Bilbao A, Colby SM, Hoyt DW, Bingol K, Kim YM, Wendler JP, Hixson KK, Jansson C. 2017. The state of rhizospheric science in the era of multi-omics: a practical guide to omics technologies. *Rhizosphere* 3:212–221. <https://doi.org/10.1016/j.rhisph.2017.05.003>.
- Berendsen RL, Pieterse CM, Bakker PA. 2012. The rhizosphere microbiome and plant health. *Trends Plant Sci* 17:478–486. <https://doi.org/10.1016/j.tplants.2012.04.001>.
- White RA, III, Borkum MI, Rivas-Ubach A, Bilbao A, Wendler JP, Colby SM, Köberl M, Jansson C. 2017. From data to knowledge: the future of multi-omics data analysis for the rhizosphere. *Rhizosphere* 3:222–229. <https://doi.org/10.1016/j.rhisph.2017.05.001>.
- Masson-Boivin C, Giraud E, Perret X, Batut J. 2009. Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? *Trends Microbiol* 17:458–466. <https://doi.org/10.1016/j.tim.2009.07.004>.
- Adesemoye AO, Torbert HA, Kloepper JW. 2009. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microb Ecol* 58:921–929. <https://doi.org/10.1007/s00248-009-9531-y>.
- Werner GD, Cornwell WK, Sprent JI, Kattge J, Kiers ET. 2014. A single evolutionary innovation drives the deep evolution of symbiotic N<sub>2</sub> fixation in angiosperms. *Nat Commun* 5:4087. <https://doi.org/10.1038/ncomms5087>.
- Friesen ML. 2012. Widespread fitness alignment in the legume-rhizobium symbiosis. *New Phytol* 194:1096–1111. <https://doi.org/10.1111/j.1469-8137.2012.04099.x>.
- Ahkami AH, White RA, III, Handakumbura PP, Jansson C. 2017. Rhizosphere engineering: enhancing sustainable plant ecosystem productivity. *Rhizosphere* 3:233–243. <https://doi.org/10.1016/j.rhisph.2017.04.012>.
- Busby PE, Soman C, Wagner MR, Friesen ML, Kremer J, Bennett A, Morsy M, Eisen JA, Leach JE, Dangl JL. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biol* 15:e2001793. <https://doi.org/10.1371/journal.pbio.2001793>.
- Marsh LE, Baptiste R, Marsh DB, Trinklein D, Kremer RJ. 2006. Temperature effects on Bradyrhizobium spp. growth and symbiotic effectiveness with pigeon pea and cowpea. *J Plant Nutr* 29:331–346. <https://doi.org/10.1080/01904160500476921>.
- Benchasri S, Nualsri C, Santipracha Q, Ngampongsai A. 2006. Evaluation of aphid (*Aphis craccivora* Koch) resistance in 24 accessions of yardlong bean and cowpea, p 215–222. *In Proceedings of the 1st Joint PSU-UNS International Conference on BioScience: Food, Agriculture, and the Environment*, Songkhla, Thailand.
- Sadowsky MJ, Tully RE, Cregan PB, Keyser HH. 1987. Genetic diversity in *Bradyrhizobium japonicum* serogroup 123 and its relation to genotype-specific nodulation of soybean. *Appl Environ Microbiol* 53:2624–2630.
- White RA, III, Norman J, Mclachlan EE, Dunham JP, Garoutte A, Friesen ML. 2019. Elucidation of the genome of *Bradyrhizobium* sp. strain USDA 3456, a historic agricultural diazotroph from cowpea (*Vigna unguiculata*). *Microbiol Resour Announc* 8:e00812-19. <https://doi.org/10.1128/MRA.00812-19>.
- White RA, III, Brown J, Colby S, Overall CC, Lee J, Zucker J, Glaesemann KR, Jansson C, Jansson JK. 2017. ATLAS (Automatic Tool for Local Assembly Structures)—a comprehensive infrastructure for assembly, annotation, and genomic binning of metagenomic and metatranscriptomic data. *PeerJ Prepr* 5:e2843v1. <https://doi.org/10.7287/peerj.preprints.2843v1>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
- Parks DH, Rinke C, Chuvochina M, Chaumeil PA, Woodcroft BJ, Evans PN, Hugenholtz P, Tyson GW. 2017. Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. *Nat Microbiol* 2:1533–1542. <https://doi.org/10.1038/s41564-017-0012-7>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.