



Draft Genome Sequence of *Pseudomonas* sp. Strain MWU13-2517, Isolated from a Wild Cranberry Bog in Provincetown, MA

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ABSTRACT *Pseudomonas* spp. are dominant in many soils, but little is known about how they interact with other members of the soil microbiome. *Pseudomonas* sp. strain MWU13-2516, isolated from a wild cranberry bog in Massachusetts, has predicted genes for hemolysins, usually associated with pathogens, and type 6 secretion systems.

The ecosystems of lower Cape Cod are endangered by sea level rise, erosion, and climate change. These fragile ecosystems are supported by soil microbe interactions, but little is known about how these microbes interact with each other or with the biological or geophysical environment. As part of a larger microbiome project, we sampled bacteria from wild cranberry bogs in the Cape Cod National Seashore to better understand these bacterial populations. *Pseudomonas* sp. strain MWU13-2517 was isolated from a Pipestone loamy coarse sand soil (<https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>) (1) (42.070624 N, –70.210548 W) by vortexing in sterile water, which was then plated onto King's medium B (KMB) agar amended with 50 $\mu\text{g mL}^{-1}$ each of cycloheximide and ampicillin, incubated at 26°C for 48 h, colony-purified 3 \times , and stored at –80°C in 34% glycerol. Isolates from frozen storage were recovered on KMB agar and then grown overnight in KMB broth for genomic DNA (gDNA) isolation using a DNeasy blood and tissue kit (Qiagen). Illumina-compatible gDNA libraries were constructed using a HyperPlus library preparation kit (KK8514; Kapa Biosystems, Roche, USA). DNA was enzymatically sheared to \approx 500-bp fragments, end-repaired, and A-tailed. Illumina-compatible adapters with unique indexes (IDT 00989130v2) were ligated to each sample, cleaned using Kapa Biosystems pure beads (KK8002), and amplified with Kapa HiFi enzyme (KK2502). The libraries were analyzed for fragment size using the Agilent TapeStation system and quantified by quantitative PCR (qPCR) with a Kapa library quantification kit (KK4835; Thermo Fisher Scientific; QuantStudio 5). The libraries were then multiplex-pooled and sequenced using an Illumina MiSeq device on a 2 \times 250-bp flow cell. The raw reads were assembled using Unicycler v0.4.8 (2) and polished with Pilon v1.23 (3) within the PATRIC Comprehensive Genome Analysis pipeline v3.6.12 (<http://patricbrc.org>) using default settings, except for the trim setting, which was set to “true” (4). The PATRIC pipeline includes quality control with QUAST (5) and Trim Galore v0.4.0 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) (6) and annotation with RASTtk (7). MWU13-2517 was placed with high confidence within the genus *Pseudomonas* by a GBDP phylogenetic tree constructed using the Type (Strain) Genome Server (<https://tygs.dsmz.de/>) (8), but it does not cluster with any named species. Its closest relative was the plant growth-promoting species *Pseudomonas palleroniana* (digital DNA–DNA hybridization [dDDH_{aa}] score = 45.7%).

Pseudomonas sp. MWU13-2517 was assembled into 47 contigs with a genome size of 6,067,066 bp and 60.2% G+C content from 1,375,575 reads and a total of 650,504,622 bases sequenced, providing 107 \times coverage with an N_{50} value of 296,837 bp. The genome contained 5,520 protein-coding, 56 tRNA, and 2 rRNA genes. The presence of predicted genes for

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hemolysins, which have not been observed in the genus outside the *P. aeruginosa* and *P. syringae* groups, and type VI secretion systems may be involved in maintaining their specific niche within the diverse microbiome of the soil (9).

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under accession number [JALMFA000000000](#), BioProject accession number [PRJNA691338](#), and BioSample accession number [SAMN26899419](#). The version described in this paper is version [JALMFA010000000](#). The SRA accession number is [SRR18508977](#). The RASTtk annotations are available under open license at Zenodo (<https://zenodo.org/record/6416124#.YI3d45PMK3I>).

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REFERENCES

1. Soil Survey Staff. 1999. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys, 2nd ed. U.S. Department of Agriculture handbook 436. Natural Resources Conservation Service, Washington, DC.
2. 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>.
3. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
4. 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>.
5. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–5. <https://doi.org/10.1093/bioinformatics/btt086>.
6. Krueger F. 2015. Trim Galore: a wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files, with some extra functionality for MspI-digested RRBS-type (reduced representation bisulfite-seq) libraries. https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/.
7. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep* 5:8365. <https://doi.org/10.1038/srep08365>.
8. Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 10:2182. <https://doi.org/10.1038/s41467-019-10210-3>.
9. Decoin V, Gallique M, Barbey C, Le Mauff F, Poc CD, Feuilloley MGJ, Orange N, Merieau A. 2015. A *Pseudomonas fluorescens* type 6 secretion system is related to mucoidy, motility and bacterial competition. *BMC Microbiol* 15:72. <https://doi.org/10.1186/s12866-015-0405-9>.