



# Draft Genome Sequence of *Pseudomonas* sp. Strain MWU15-20650, Isolated from Wild Cranberry Fruit in the Cape Cod National Seashore

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**ABSTRACT** *Pseudomonas* sp. strain MWU15-20650 was isolated from wild cranberry fruit surfaces in the Cape Cod National Seashore. The draft genome is 6.2 Mbp, with a G+C content of 59%, and contains predicted genes for type VI secretion systems and an N-acyl-homoserine lactone acylase. The closest known relative is *Pseudomonas haemolytica*.

The wild cranberry bogs of the Cape Cod National Seashore represent an understudied ecosystem. Because little to no fungal disease is apparent in wild bogs, the bacteria associated with healthy wild cranberry flowers and fruits may represent a resource for natural disease suppression that can be translated to cultivated plants (1). A dominant group of these bacteria is *Pseudomonas* spp., some of which can produce secondary metabolites with biological activity against pests and pathogens (2–4). Here, we present the draft genome sequence of *Pseudomonas* sp. strain MWU15-20650, which was isolated as part of a survey of berry and flower surfaces in July 2015 during late flowering to early fruit in wild cranberry bogs in the Cape Cod National Seashore in Provincetown, Massachusetts (42.062065N, 70.118679W). Cranberry fruits were vortexed in sterile water, and the rinsate was plated on King's medium B (KMB) agar containing 50  $\mu\text{g mL}^{-1}$  each of ampicillin and cycloheximide. Fluorescent colonies were streaked for isolation on fresh KMB agar, single colony purified three times, and stored at  $-80^{\circ}\text{C}$  in 34% glycerol. MWU15-20650 was then grown in KMB broth overnight for genomic DNA (gDNA) isolation (DNeasy blood and tissue kit; Qiagen). Libraries were generated with a HyperPlus library preparation kit (KK8514; Kapa Biosystems, Roche, USA). DNA was enzymatically fragmented to  $\sim 500$  bp, end repaired, and A-tailed as described in the Kapa Biosystems protocol. Illumina-compatible adapters with unique indexes (00989130v2; Integrated DNA Technologies) were individually ligated to each sample, the samples were cleaned with KAPA pure beads (KK8002; Kapa Biosystems) and amplified with HiFi enzyme (KK2502; Kapa Biosystems). Fragment sizes were determined on an Agilent TapeStation system and quantified using quantitative PCR (qPCR) (KAPA library quantification kit [KK4835]) on a QuantStudio 5 system (ThermoFisher Scientific). The library was multiplex pooled for sequencing in a  $2 \times 250$ -bp flow cell on the Illumina MiSeq platform. Raw reads were assembled and quality controlled in the PATRIC (<http://patricbrc.org>) Comprehensive Genome Analysis pipeline v3.6.12 (5) using default settings except for the automated trimming function (which was set to true), Unicycler v0.4.8 (6), and two rounds of polishing with Pilon v1.23 (7). The pipeline includes quality control with Trim Galore v0.4.0 ([https://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore](https://www.bioinformatics.babraham.ac.uk/projects/trim_galore)) (8) and annotation with RASTtk (9). Strain MWU15-20650 had a genome size of 6,082,616 bp assembled into 86 contigs from 1,094,993 reads, with a total read length of 530,079,409 bp. The G+C content was 59.92%, and the  $N_{50}$  value was 122,598 bp, with  $87\times$  coverage. The isolate was placed in the genus *Pseudomonas* by genome BLAST distance phylogeny (GBDP) using the TYGS online tool (<https://tygs.dsmz.de>), but it is not a member of any known species, being most closely related to *Pseudomonas haemolytica* (digital DNA-DNA

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hybridization [dDDH<sub>q4</sub>], 35.4%). The genome contains predicted genes for type VI secretion systems and an *N*-acyl-homoserine lactone acylase, indicating that MWU15-20650 may affect members of the microbiome directly by competition (10) and indirectly by interference with their quorum-sensing systems (11).

**Data availability.** This whole-genome shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession number [JALJFG000000000](https://doi.org/10.1128/MRA.01479-18) for *Pseudomonas* MWU15-20650. The version described in this paper is version [JALJFG000000000.1](https://doi.org/10.1128/MRA.01479-18), with BioProject accession number [PRJNA691338](https://doi.org/10.1128/MRA.01479-18) and BioSample accession number [SAMN26725498](https://doi.org/10.1128/MRA.01479-18). The Sequence Read Archive (SRA) accession number is [SRR18508442](https://doi.org/10.1128/MRA.01479-18). RASTtk annotations are available under open license at Zenodo (<https://zenodo.org/record/6392140#.YpFJpqDMKUK>).

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## REFERENCES

1. Ebadzadsahrai G, Soby S. 2019. 16S rRNA amplicon profiling of cranberry (*Vaccinium macrocarpon* Ait.) flower and berry surfaces. *Microbiol Resour Announc* 8:e01479-18. <https://doi.org/10.1128/MRA.01479-18>.
2. Hesse C, Schulz F, Bull CT, Shaffer BT, Yan Q, Shapiro N, Hassan KA, Varghese N, Elbourne LDH, Paulsen IT, Kyrpides N, Woyke T, Loper JE. 2018. Genome-based evolutionary history of *Pseudomonas* spp. *Environ Microbiol* 20:2142–2159. <https://doi.org/10.1111/1462-2920.14130>.
3. Vesga P, Flury P, Vacheron J, Keel C, Croll D, Maurhofer M. 2020. Transcriptome plasticity underlying plant root colonization and insect invasion by *Pseudomonas protegens*. *ISME J* 14:2766–2782. <https://doi.org/10.1038/s41396-020-0729-9>.
4. Ebadzadsahrai G, Higgins Keppler EA, Soby SD, Bean HD. 2020. Inhibition of fungal growth and induction of a novel volatilome in response to *Chromobacterium vaccinii* volatile organic compounds. *Front Microbiol* 11:1035. <https://doi.org/10.3389/fmicb.2020.01035>.
5. 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>.
6. 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>.
7. 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>.
8. Krueger F. 2014. 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](https://www.bioinformatics.babraham.ac.uk/projects/trim_galore).
9. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, 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>.
10. Russell AB, Peterson SB, Mougous JD. 2014. Type VI secretion system effectors: poisons with a purpose. *Nat Rev Microbiol* 12:137–148. <https://doi.org/10.1038/nrmicro3185>.
11. Fetzner S. 2015. Quorum quenching enzymes. *J Biotechnol* 201:2–14. <https://doi.org/10.1016/j.jbiotec.2014.09.001>.