



Draft Genome Sequences of *Pseudomonas* sp. Strains MWU13-2100 and MWU13-2105 Isolated from Wild Cranberry Bog Soil in the Cape Cod National Seashore

^DBrian Mayer,^a ^{Scott} Soby^{a,b}

^aBiomedical Sciences, College of Graduate Studies, Midwestern University, Glendale, Arizona, USA ^bCollege of Veterinary Medicine, Midwestern University, Glendale, Arizona, USA

ABSTRACT *Pseudomonas* sp. strains MWU13-2100 and MWU13-2105 were isolated from a wild cranberry bog with Pipestone loamy coarse sand soil in Truro, Massachusetts, and taxonomically assigned based on whole-genome sequences. The draft genomes are most closely related to *P. batumici* (41.4% and 41.8% dDDH_{d4}), but with only 50.8 dDDH_{d4} to each other.

ike other wetlands bog soils, cranberry bog soil is a rich microbial environment, yet little is known about interactions between microbes, or with their host plants. Pseudomonas spp. have been implicated in suppression of fungal diseases and maintaining a beneficial environment for plant growth (1-8). Understanding the properties of these bacteria could have potential benefits that could be translated to increasing crop yields and quality. Thus, identifying soil bacteria and their products is important. Pseudomonas sp. strains MWU13-2100 and MWU13-2105 were isolated from wild cranberry bog soil in the Cape Cod National Seashore (42.064742, -70.117562) by briefly vortexing a \approx 2g sample from the top 5 cm of the soil profile in sterile water. The supernatant was plated on King's medium B (KMB) agar containing $50 \,\mu \text{g mL}^{-1}$ each of cycloheximide and ampicillin, incubated at 26°C for 48 h, colony-purified $3\times$, and stored in 34% glycerol at -80° C. Isolates were recovered from frozen storage on fresh KMB, and populations were inoculated into overnight KMB broth cultures. DNeasy blood and tissue kits (Qiagen) were used for gDNA isolation, and the genome was sequenced at the Arizona State University Genomics Core facility. Illumina-compatible genomic DNA (gDNA) libraries were generated with a Kapa Biosystem Hyperplus library preparation kit (KK8514). DNA was enzymatically sheared to \approx 500bp fragments, end repaired, and A-tailed. Illuminacompatible adapters with unique indexes (IDT 00989130v2) were individually ligated to each sample, cleaned using pure beads (Kapa Biosciences; KK8002), and amplified with Kapa HiFi enzyme (KK2502). Each library was analyzed for fragment size (Agilent Tapestation) and quantified by gPCR (Kapa library quantification kit, KK4835; Thermo Fisher Scientific, Quantstudio 5) before multiplex-pooling and sequencing on a Illumina MiSeq 2 \times 250bp flow cell. Unicycler v0.4.8 (9) was used to assemble the raw reads and Pilon v1.23 (10) was used to polish them in the PATRIC Comprehensive Genome Analysis pipeline v3.6.12. Default parameters were used for all software except for Trim in PATRIC, which was set to "true" (11). Trim Galore v0.4.0 and QUAST v5.1 (12, 13) were used for adapter trimming and quality control Table 1. Genome sequences were annotated using RASTtk v1.073 (14) as part of the PATRIC pipeline. MWU13-2100 and MWU13-2105 were placed taxonomically using the Type (Strain) Genome Server v342 (TYGS; https://tygs.dsmz.de/) (15). The isolates were most similar to P. batumici UCM B- 321^{T} (JXDG00000000.1) but with only 41.8 and 41.4% dDDH_{d4} values, respectively.

Data availability. This whole-genome sequence project has been deposited at DDBJ/EMBL/GenBank under BioProject PRJNA691338 with the accession numbers JALLIX000000000 for MWU13-2100, and JALLIY000000000 for MWU13-2105. The versions described in this paper are JALLIX000000000.1 and JALLIY000000000.1, respectively. Sequence

Editor Leighton Pritchard, SIPBS, University of Strathclyde

Copyright © 2022 Mayer and Soby. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Scott Soby, ssobyx@midwestern.edu.

The authors declare no conflict of interest.

Received 31 May 2022 Accepted 9 September 2022 Published 22 September 2022

	TABLE 1 Data summa	v for Pseudomonas sp	. MWU13-2100 and MWU13-210
--	--------------------	----------------------	----------------------------

lsolate	Biosample	Genome size (bp)	no. of Contigs	% GC content	Coverage (x)	Total reads (x10 ⁶)	Total read length (bp)	Read length (bp)	N ₅₀
MWU13-2100	SAMN27107747	6,589,945	178	61.33	105	3.01	692,910,330	229	114,002
MWU13-2105	SAMN27107502	7,183,010	102	61.51	91	2.72	650,339,742	238	281,724

Read Archive (SRA) are available from GenBank under the accession numbers SRR18662642 for MWU13-2100 and SRR18662649 for MWU13-2105. RASTtk annotations are available under open license at Zenodo (https://zenodo.org/record/6399230#.YpZNMqDMKUk and https://zenodo.org/record/6399253#.YpZNCqDMKUk).

ACKNOWLEDGMENTS

We thank Alisha Harrison for curation of the cranberry bog bacterial collection and for isolation of gDNA from MWU13-2100 and MWU13-2105. This research was supported by the Office of Research and Sponsored Programs, and Biomedical Sciences Program, College of Graduate Studies, Midwestern University. Library construction and Illumina sequencing were performed at the Arizona State University Genomics Core Facility. We gratefully acknowledge the generous cooperation of the Cape Cod National Seashore National Park under the US National Parks Service permit CACO-2012-SCI-0024. This manuscript fulfills a course requirement for B.M.

REFERENCES

- Kuzmanovic N, et al. 2018. Analysis of the genome sequence of plant beneficial strain Pseudomonas sp RU47. J Biotechnology 281:183–192. https://doi .org/10.1016/j.jbiotec.2018.07.023.
- Li HB, Singh RK, Singh P, Song QQ, Xing YX, Yang LT, Li YR. 2017. Genetic diversity of nitrogen-fixing and plant growth promoting pseudomonas species isolated from sugarcane rhizosphere. Front Microbiol 8:1268. https://doi.org/10.3389/fmicb.2017.01268.
- Cheng X, Cordovez V, Etalo DW, van der Voort M, Raaijmakers JM. 2016. Role of the GacS sensor kinase in the regulation of volatile production by plant growth-promoting pseudomonas fluorescens SBW25. Front Plant Sci 7:1706–1706. https://doi.org/10.3389/fpls.2016.01706.
- Lugtenberg B, Kamilova F. 2009. Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556. https://doi.org/10.1146/annurev.micro .62.081307.162918.
- Passarelli-Araujo H, Franco GR, Venancio TM. 2022. Network analysis of ten thousand genomes shed light on Pseudomonas diversity and classification. Microbiol Res 254:126919. https://doi.org/10.1016/j.micres.2021.126919.
- Mülner P, Bergna A, Wagner P, Sarajlić D, Gstöttenmayr B, Dietel K, Grosch R, Cernava T, Berg G. 2019. Microbiota associated with sclerotia of soilborne fungal pathogens—a novel source of biocontrol agents producing bioactive volatiles. Phytobiomes J 3:125–136. https://doi.org/10.1094/PBIOMES-11-18-0051-R.
- Zhang Y, Li T, Liu Y, Li X, Zhang C, Feng Z, Peng X, Li Z, Qin S, Xing K. 2019. Volatile organic compounds produced by pseudomonas chlororaphis subsp. aureofaciens sps-41 as biological fumigants to control ceratocystis fimbriata in postharvest sweet potatoes. J Agric Food Chem 67:3702–3710. https://doi.org/ 10.1021/acs.jafc.9b00289.
- Mannaa M, Kim KD. 2018. Biocontrol activity of volatile-producing bacillus megaterium and Pseudomonas protegens against Aspergillus and Penicillium spp. predominant in stored rice grains: study II. Mycobiology 46: 52–63. https://doi.org/10.1080/12298093.2018.1454015.

- 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.
- 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.
- 11. Wattam ARDJJ, 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.
- Krueger F. Trim Galore: a wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files, with some extra functionality for Mspl-digested RRBS-type (Reduced Representation Bisufite-Seq) libraries. 2014. https://www.bioinformatics.babraham.ac.uk/ projects/trim_galore/.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https:// doi.org/10.1093/bioinformatics/btt086.
- 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.
- 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.