



# Draft Genome Sequence of *Pseudomonas* sp. Strains MWU12-2020 and MWU12-3103b, Isolated from Wild and Cultivated Cranberry Bogs in Massachusetts

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**ABSTRACT** Here, we present the draft genome sequences of *Pseudomonas* sp. strains MWU12-2020 and MWU12-3103b, isolated from the rhizospheres of wild and cultivated cranberry bogs in southeastern Massachusetts; these strains are unrelated to known *Pseudomonas* species. The genomes of both isolates exceed 6 Mbp and contain predicted ice nucleation and type VI and III secretion system genes.

Secondary metabolites produced by members of the genus *Pseudomonas* allow them to interact and survive within many different environments (1–5), but little is known about the role these bacteria play in soil dynamics or plant health in critical wetlands ecosystems. *Pseudomonas* sp. strain MWU12-2020 was isolated from wild cranberry bog soil (42.070624 N, 70.210548 W) and strain MWU12-3103b was isolated from cultivated cranberry bog roots (41.766767 N, 70.66842 W) during culture-dependent microbe surveys. Cranberry plants have a network of fine roots supported by ericoid mycorrhizae, which makes it difficult to differentiate between root and fungus and determine where the root zone is (6). In the case of MWU12-2020, there was no visible root or mycelium in the sample, but for MWU12-3103b, soil was attached to the root/mycelial network. The soil and rhizosphere samples were vortexed in sterile water; supernatants were plated onto King's medium B (KMB) supplemented with 50  $\mu\text{g mL}^{-1}$  each of ampicillin and cycloheximide and incubated at 26°C for 48 h. Fluorescent colonies were colony purified 3 $\times$  on KMB and stored at –80°C in 34% glycerol. DNeasy blood and tissue kits (Qiagen, USA) were used to extract genomic DNA (gDNA) from overnight KMB broth cultures. Libraries for genomic sequencing were generated from enzymatically sheared DNA ( $\approx$ 500 bp) using a HyperPlus library preparation kit (Kapa Biosystems KK8514; Roche, USA). The sheared fragments were end repaired and A-tailed at the 3' end. Indexed Illumina-compatible adapters (IDT number 00989130v2) were ligated to the A-tailed fragments; the library was cleaned using KAPA pure beads (KK8002) and then amplified using HiFi enzyme (KAPA KK2502). Fragment sizes were determined using an Agilent TapeStation device and then quantified using a KAPA quantitative PCR (qPCR) library quantification kit (KK4835) on a QuantStudio 5 system (Thermo Fisher, USA) for sequencing on the Illumina MiSeq platform (2  $\times$  250-bp format). Using the Comprehensive Genome Analysis feature of PATRIC v3.6.12 (<https://www.patricbrc.org>) (7), assembly of the raw reads was completed using Unicycler v0.4.8 (8); the reads were polished using Pilon v1.23 (9), with default parameters except for enabling automated end trimming, and annotated using RASTtk (10). QUAST v5.0.2 and Trim Galore v0.4.0 were used within PATRIC (11, 12) for quality control and adapter trimming, respectively. A summary of the assembly and annotation is given in Table 1. Both isolates were placed in the genus *Pseudomonas* by genome BLAST distance phylogeny using TYGS v342 (13), but though most closely related to *Pseudomonas koreensis* DSM 16610<sup>T</sup> (GenBank accession number [JAAQYM000000000](https://www.ncbi.nlm.nih.gov/nuccore/JAAQYM000000000)) (digital DNA-DNA hybridization [ $\text{dDDH}_{\text{d4}}$ ] = 51.2 and 43.7 for MWU12-2020 and MWU12-3103b, respectively), neither can be assigned to an

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**TABLE 1** Genomic data summary

Isolate	BioSample accession no.	GenBank accession no.	SRA accession no.	Genome size (bp)	No. of contigs	$N_{50}$ (bp)	G+C content (%)	Mean read length (bp)	No. of reads	Coverage (×)	No. of CDSs	No. of rRNAs	No. of tRNAs
MWU12-2020	<a href="#">SAMN26879216</a>	<a href="#">JALMEX000000000</a>	<a href="#">SRR18531153</a>	6,326,693	65	542,118	60.3	240.75	3,378,022	128	5,924	2	64
MWU12-3103b	<a href="#">SAMN26879228</a>	<a href="#">JALMEY000000000</a>	<a href="#">SRR18531152</a>	6,275,820	55	276,792	60.2	236.97	3,012,488	114	5,831	2	64

existing species, and they do not belong in the same species ( $dDDH_{d4} = 51.2$ ). Both genomes contain putative *inaA* genes for ice nucleation (14, 15) and type VI and III secretion systems (16–18), all of which have potential implications for interactions with other members of the ecosystem.

**Data availability.** This whole-genome shotgun sequencing project has been deposited at DDBJ/EMBL/GenBank under accession numbers [JALMEX010000000](#) and [JALMEY010000000](#) and BioProject accession number [PRJNA691338](#) (Table 1). The versions represented in the paper are the first versions. The RASTtk annotations are available under open license at Zenodo (<https://zenodo.org/record/6412376#.Yxj1mEfMKUK>).

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## REFERENCES

- Lopes LD, Davis EW, II, Pereira e Silva MDC, Weisberg AJ, Bresciani L, Chang JH, Loper JE, Andreote FD. 2018. Tropical soils are a reservoir for fluorescent *Pseudomonas* spp. biodiversity. *Environ Microbiol* 20:62–74. <https://doi.org/10.1111/1462-2920.13957>.
- Lopes LD, Pereira e Silva MDC, Weisberg AJ, Davis EW, II, Yan Q, Varize CDS, Wu C-F, Chang JH, Loper JE, Andreote FD. 2018. Genome variations between rhizosphere and bulk soil ecotypes of a *Pseudomonas koreensis* population. *Environ Microbiol* 20:4401–4414. <https://doi.org/10.1111/1462-2920.14363>.
- 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>.
- Loper JE, Hassan KA, Mavrodi DV, Davis EW, II, Lim CK, Shaffer BT, Elbourne LDH, Stockwell VO, Hartney SL, Breakwell K, Henkels MD, Tetu SG, Rangel LI, Kidarsa TA, Wilson NL, van de Mortel JE, Song C, Blumhagen R, Radune D, Hostetler JB, Brinkac LM, Durkin AS, Kluepfel DA, Wechter WP, Anderson AJ, Kim YC, Pierson LS, Pierson EA, Lindow SE, Kobayashi DY, Raaijmakers JM, Weller DM, Thomashow LS, Allen AE, Paulsen IT. 2012. Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genet* 8:e1002784. <https://doi.org/10.1371/journal.pgen.1002784>.
- Kim YC, Leveau J, McSpadden Gardener BB, Pierson EA, Pierson LS, Ryu C-M. 2011. The multifactorial basis for plant health promotion by plant-associated bacteria. *Appl Environ Microbiol* 77:1548–1555. <https://doi.org/10.1128/AEM.01867-10>.
- Kosola KR, Workmaster BAA. 2007. Mycorrhizal colonization of cranberry: effects of cultivar, soil type, and leaf litter composition. *J Amer Soc Hort Sci* 132:134–141. <https://doi.org/10.21273/JASHS.132.1.134>.
- 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>.
- 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>.
- 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>.
- 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>.
- 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.
- 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>.
- Failor KC, Schmale DG, Vinatzer BA, Monteil CL. 2017. Ice nucleation active bacteria in precipitation are genetically diverse and nucleate ice by employing different mechanisms. *ISME J* 11:2740–2753. <https://doi.org/10.1038/ismej.2017.124>.
- Lindow SE. 1983. The role of bacterial ice nucleation in frost injury to plants. *Annu Rev Phytopathol* 21:363–384. <https://doi.org/10.1146/annurev.py.21.090183.002051>.
- Deng W, Marshall NC, Rowland JL, McCoy JM, Worrall LJ, Santos AS, Strynadka NCJ, Finlay BB. 2017. Assembly, structure, function and regulation of type III secretion systems. *Nat Rev Microbiol* 15:323–337. <https://doi.org/10.1038/nrmicro.2017.20>.
- 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>.
- 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>.