



Draft Genome Sequence of *Pseudomonas* sp. Strain MWU12-2534b, Isolated from a Wild Cranberry Bog in Truro, Massachusetts

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ABSTRACT An unknown *Pseudomonas* sp. most closely related to *Pseudomonas ficuserectae* and *Pseudomonas protegens* was isolated from the rhizospheres of wild cranberry plants in the Cape Cod National Seashore, in the United States. The draft genome of MWU12-2534b is 6.7 Mbp, has 63.32% GC content, and contains multiple potential virulence and antibiotic resistance genes.

Wetland soil microbes are integral in nutrient cycling and biogeochemical processes, but until recently, little was known about the types of organisms present in these ecosystems. *Pseudomonas* sp. strain MWU12-2534b was isolated from the rhizospheres of wild cranberry plants in the Cape Cod National Seashore in Massachusetts during a culture-dependent survey of bacteria in bog soils. MWU12-2534b clustered with *Pseudomonas ficuserectae* and *Pseudomonas protegens* by 16S rRNA phylogeny (Fig. 1), but genomic comparisons indicate that it belongs within neither of these species. MWU12-2534b had OrthoANI values of 77.32% with *P. ficuserectae* (NCBI RefSeq accession number [NZ_LJQJ01000341](https://.ncbi.nlm.nih.gov/nucl/NZ_LJQJ01000341)) and 89.83% with *P. protegens* (GenBank accession number [MAUL01000003](https://ncbi.nlm.nih.gov/nucl/MAUL01000003)), both under the 94% cutoff for species (1, 2); also, MWU12-2534b had digital DNA-DNA hybridization (dDDH) values of 22.5% with *P. ficuserectae* and 39.3% with *P. protegens*, both well below the 70% cutoff for dDDH (3, 4).

Wild cranberry bog soil and root tissue were plated on King's medium B (KMB) agar containing 50 mg ml⁻¹ cycloheximide and ampicillin and grown at 26°C, followed by 3× single-colony purification on KMB agar. Genomic DNA (gDNA) was extracted from overnight KMB broth cultures with a DNeasy blood and tissue kit (Qiagen), and the genome was sequenced at the Arizona State University CLAS Genomics Core facility, first by shearing to ca. 600 bp (Covaris M220 ultrasonicator). Illumina libraries were assembled on an Apollo 384 liquid handler (Wafergen) using a Kapa Biosystems library preparation kit (catalog number KK8201). DNA fragments were end repaired, A tailed, and ligated with combined indexes/adapters (catalog number 520999; Bioo) and then multiplexed into one lane. AMPure beads (catalog number A63883; Agencourt Bioscience/Beckman Coulter, Inc.) were used to clean the adapter-ligated DNA fragments, and then they were amplified with a Kapa HiFi enzyme. An Agilent Bioanalyzer and quantitative PCR (library kit catalog number KK4835; Kapa) were used to assess library quality before pooling and sequencing in 2 × 300- and 2 × 150-bp paired-end flow cells (Illumina MiSeq platform). Read files were then combined, assembled, and annotated on the PATRIC website (<http://patricbrc.org>) using the Comprehensive Genome Analysis pipeline with default parameters (5, 6). The sequence coverage of 183× allowed partial assembly into 35 contigs totaling 6,738,332 bp, with 63.32% G+C

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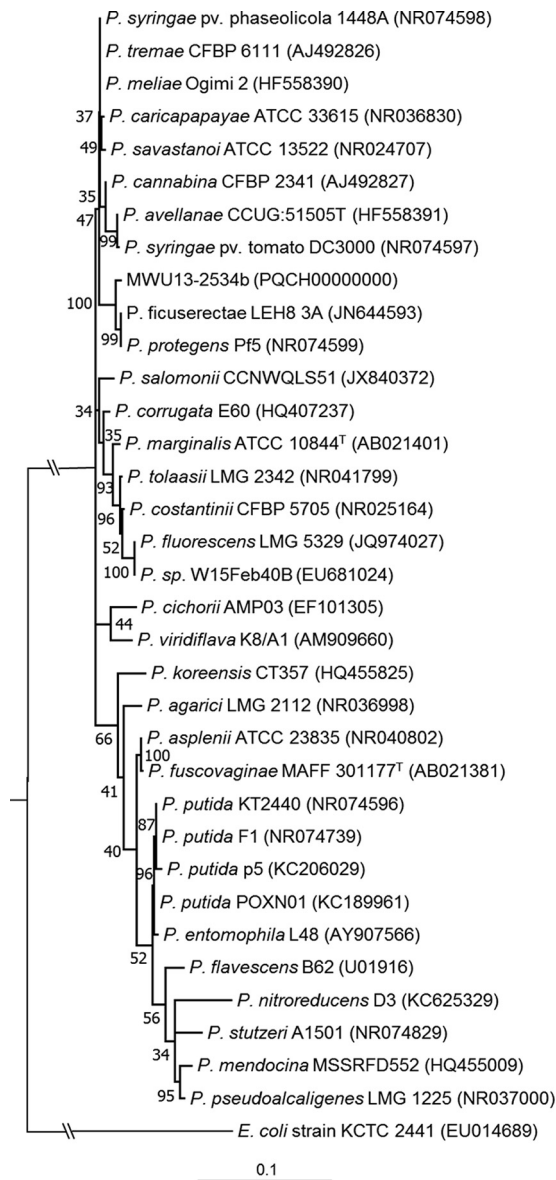


FIG 1 16S rRNA molecular phylogeny of the genus *Pseudomonas*. The evolutionary history of MWU12-2534b within the genus *Pseudomonas* was inferred by maximum likelihood using the Kimura 2-parameter substitution model with complete deletion of gaps and missing data. The tree shown is based on a total of 1,319 positions in the final data set, with the highest log likelihood ($-4,483.66$) shown. The initial tree(s) for the heuristic search was constructed by applying neighbor-joining and BioNJ algorithms to pairwise distances. Gamma distribution was used to model evolutionary rate differences among sites (+G, parameter = 0.1635), allowing for some sites to be evolutionarily invariable ([+I], 58.51% sites). Except for the *E. coli* outgroup, the tree is drawn to scale, with branch lengths measured in the number of substitutions per site. MWU12-2534b forms a clade with *P. ficuserectae* and *P. protegens* but is not a member of either species.

content. The largest contig was 995,949 bp, with an N_{50} value of 817,307 bp. The MWU12-2534b genome contained 6,183 coding sequences (CDSs), plus 74 tRNA and 7 rRNA operons. Predicted virulence factor, antibiotic resistance, and efflux pump genes were identified that may contribute to antifungal activity, including genes for proteases, chitinases, hemolysins, hemolytic phospholipases, and nine resistance-nodulation-division (RND) genes (7, 8). GacA (a system that regulates the expression of extracellular enzymes, toxins, quorum-sensing molecules, and motility) (9), ABC molybdenum transporter, a major facilitator superfamily (MFS)-type efflux system, β -lactamases, aminoglycoside $N(6')$ -acetyltransferase, fluoroquino-

lone resistance, and a type VI secretion system were all found in the genome. *Pseudomonas* MWU12-2534b has the potential to produce secondary metabolites with antibiotic activity, such as colicin V, pyoverdine, and hydrogen cyanide.

Data availability. This whole-genome sequence was deposited at DDBJ/EMBL/GenBank under the accession number [PQCH00000000](https://doi.org/10.1093/nar/gkw1017) for *Pseudomonas* MWU12-2534b. The version described here is version PQCH02000000. The Sequence Read Archive (SRA) is available from GenBank under the accession number [SRP154927](https://doi.org/10.1093/nar/gkw1017).

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REFERENCES

1. Yoon SH, Ha SM, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 110:1281–1286. <https://doi.org/10.1007/s10482-017-0844-4>.
2. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57:81–91. <https://doi.org/10.1099/ijs.0.64483-0>.
3. Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Truper HG. 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 37:463–464. <https://doi.org/10.1099/00207713-37-4-463>.
4. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60. <https://doi.org/10.1186/1471-2105-14-60>.
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. 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>.
7. Ma D, Cook DN, Alberti M, Pon NG, Nikaido H, Hearst JE. 1993. Molecular cloning and characterization of *acrA* and *acrE* genes of *Escherichia coli*. *J Bacteriol* 175:6299–6313. <https://doi.org/10.1128/jb.175.19.6299-6313.1993>.
8. Poole K, Krebs K, McNally C, Neshat S. 1993. Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. *J Bacteriol* 175:7363–7372. <https://doi.org/10.1128/jb.175.22.7363-7372.1993>.
9. Lapouge K, Schubert M, Allain FH-T, Haas D. 2007. Gac/Rsm signal transduction pathway of γ -proteobacteria: from RNA recognition to regulation of social behaviour. *Mol Microbiol* 67:241–253. <https://doi.org/10.1111/j.1365-2958.2007.06042.x>.