AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Whole-Genome Sequence of *Pseudomonas* sp. Strain MM211, Isolated from Soil in Langenfeld, Germany

Donat Wulf,[a,b] Jonas Arndt,[c] Isabell E. Bleile,[c] Charlotte Bramers,[d] Pia L. Gülpen,[e] Bart Verwaaijen[a]

[a]Computational Biology, Faculty of Biology, Bielefeld University, Bielefeld, Germany
[b]Graduate School DILS, Bielefeld Institute for Bioinformatics Infrastructure, Bielefeld University, Bielefeld, Germany
[c]Molecular Biotechnology, Bielefeld University, Bielefeld, Germany
[d]Biology, Bielefeld University, Bielefeld, Germany
[e]Molecular Biology, Bielefeld University, Bielefeld, Germany

ABSTRACT Here, we present the genome sequence of *Pseudomonas* sp. strain MM211, which was isolated from garden soil. The complete circular genome consists of a 5,281,862-bp chromosome, with a GC content of 61.5%.

The Gram-negative rod-shaped bacterial genus *Pseudomonas* lives in diverse habitats (1–3) and is well characterized (4). Currently, 258 validated species are published (5), including human, animal, and plant pathogens (6). In addition, some species interact with plants and can promote plant growth and influence resistance against plant diseases (7, 8). Some *Pseudomonas* species are able to grow in association with other organisms in highly polluted environments and degrade various substances (9). Because of these many different properties, the organisms of this genus have great potential to be some of the most influential bacteria in research and development (10).

We isolated *Pseudomonas* sp. strain MM211 from a soil sample obtained in Langenfeld, North Rhine-Westphalia, Germany (51°06′31.1″N, 6°56′40.2″E), from dark humus at a depth of 10 cm. The sample was diluted with 0.9 NaCl, filtered (431015; Macherey-Nagel, Düren, Germany), plated (1.5% agar, 1% peptone from soy, 0.3% NaCl, 0.1% sucrose, 0.1% cellulose, 0.1% xylan, 0.1% chitin, and 0.05% Tris-HCl), and incubated at 28°C until colonies were observed. DNA was isolated from a single colony with a NucleoSpin microbial DNA minikit (Macherey-Nagel) with RNA digestion. DNA was barcoded with the native barcoding kit (Oxford Nanopore Technologies, Oxford, UK) and sequenced on a GridION system with a R9.4.1 flow cell (Oxford Nanopore Technologies). Sequences were called using the super accuracy base-calling model in MinKNOW (v1.4.3; Oxford Nanopore Technologies). Adapters were trimmed using Porechop (v0.2.4) (11). The genome was assembled with Canu (v2.1.1) (12) set to a genome size of 8 Mb and was polished with Racon (v1.4.20) (13) in combination with BWA (v0.7.17) (14) and Medaka (v1.4.3; Oxford Nanopore Technologies). Completeness was examined with Benchmarking Universal Single-Copy Orthologs (BUSCO) (v5.1.2) (15) set to genome, with the lineage set to pseudomonadales_odb10. The final single-contig assembly was circularized and oriented with berokka (v0.2.3) (https://github.com/tseemann/berokka) and uploaded to NCBI. Default settings were used for all tools unless stated otherwise. All relevant assembly statistics, including BUSCO results, are listed in Table 1.

The genome sequence of *Pseudomonas* sp. strain MM211 presented here has *Pseudomonas flavescens* LMG 18387 (GenBank accession number GCA_900100535.1) (16) and *Pseudomonas seleniipraecipitans* LMG 25475 (GenBank accession number

Editor Catherine Putonti, Loyola University Chicago

Address correspondence to Bart Verwaaijen, bverwaai@cebitec.uni-bielefeld.de.

The authors declare no conflict of interest.

Received 26 October 2021
Accepted 19 January 2022
Published 3 February 2022

TABLE 1 Sequencing and assembly statistics for *Pseudomonas* sp. strain MM211

Parameter[a]	Finding
Raw read sequencing	
No. of reads	168,644
N_{50} (bp)	13,834
Total length (bp)	1,579,810,087
Assembly	
Coverage (×)	286
GC content (%)	61.5
Length (bp)	5,281,862
Annotation	
Total no. of genes	4,853
No. of coding genes	4,645
BUSCO results (%)	
Complete	98.8
Single copy	98.3
Duplicated	0.5
Fragmented	0.4
Missing	0.8

[a] Coverage was based on mapping of the trimmed reads to the assembly with SAMtools (v1.12) (25). Annotation was based on NCBI PGAP (v5.3) annotation of GCA_020386635.1 on 15 November 2021 (26). BUSCO values represent complete, single copy, duplicated, fragmented, and missing single-copy orthologue genes.

GCA_900102335.1) (17) as its closest relatives (Fig. 1). The digital DNA-DNA hybridization (dDDH) shows values of 41.8% with *P. flavescens* LMG 18387 and 36.4% with *P. seleniipraecipitans* LMG 25475, both well below the 70% cutoff value for dDDH (18). A carotenoid biosynthetic gene cluster was identified using the antiSMASH server (19, 20). A KEGG analysis showed that *Pseudomonas* sp. strain MM211 is likely able to grow a flagellum (21). Furthermore, MM211 may be auxotrophic for biotin. *P. flavescens*, the most closely related species, is also capable of producing a flagellum and pigments (16).

Data availability. The MM211 assembly, RefSeq annotation, and reads are available at NCBI GenBank under accession numbers GCA_020386635.1, CP081942.1, and SRR15526917, respectively.

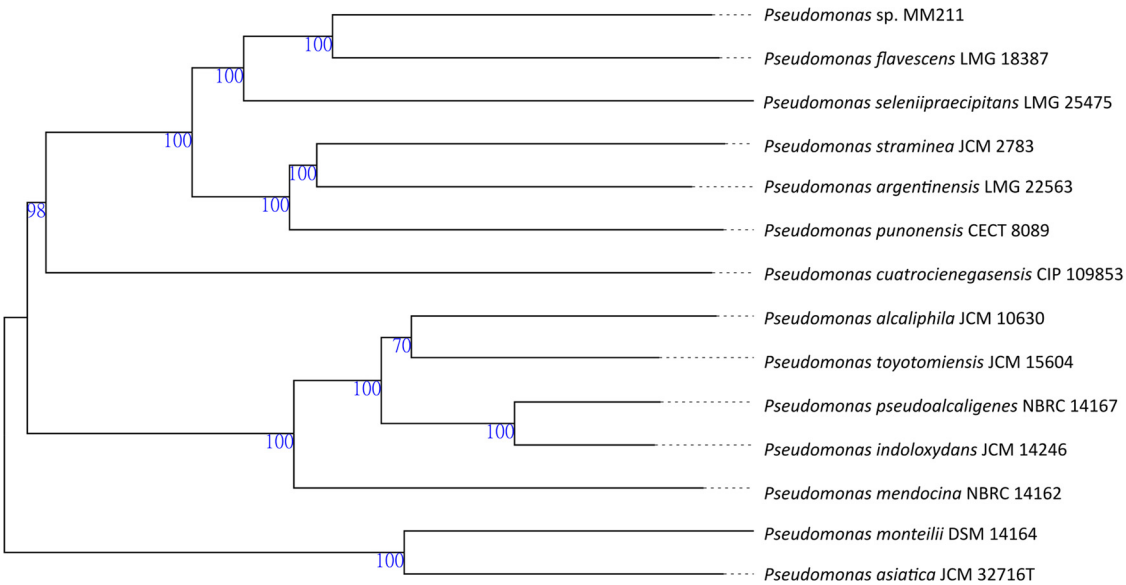

FIG 1 Genome BLAST Distance Phylogeny (GBDP) tree. The phylogenetic tree was created with the Type (Strain) Genome Server (TYGS) (22). The tree was inferred with FastME (v2.1.6.1) (23) from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5. The numbers at the branches are GBDP pseudo-bootstrap support values of >60% from 100 replications, with an average branch support of 100.0%. The tree was rooted at the midpoint (24).

ACKNOWLEDGMENTS

We thank Kristin Rojek for assistance with isolation of bacteria, Andrea Bräutigam for proofreading, and Jörn Kalinowski for providing the flow cell and sequencing platform.

Sequences were generated, assembled, and analyzed as part of a molecular biology of microorganisms (MM) course at Bielefeld University in 2021.

This work was supported by the BMBF-funded de.NBI Cloud within the German Network for Bioinformatics Infrastructure (de.NBI) (031A532B, 031A533A, 031A533B, 031A534A, 031A535A, 031A537A, 031A537B, 031A537C, 031A537D, 031A538A). Furthermore we acknowledge support for the publication costs by the Open Access Publication Fund of Bielefeld University and the Deutsche Forschungsgemeinschaft (DFG).

REFERENCES

1. Lopes LD, Davis EW, II, Pereira E, Silva MC, Weisberg AJ, Bresciani L, Chang JH, Loper JE, Andreote FD. 2018. Tropical soils are a reservoir for fluorescent *Pseudomonas* spp. Environ Microbiol 20:62–74. https://doi.org/10.1111/1462-2920.13957.
2. Amoozegar MA, Shahinpei A, Sepahy AA, Makhdoumi-Kakhki A, Seyedmahdi SS, Schumann P, Ventosa A. 2014. *Pseudomonas salegens* sp. nov., a halophilic member of the genus *Pseudomonas* isolated from a wetland. Int J Syst Evol Microbiol 64:3565–3570. https://doi.org/10.1099/ijs.0.062935-0.
3. Vyas P, Rahi P, Gulati A. 2009. Stress tolerance and genetic variability of phosphate-solubilizing fluorescent *Pseudomonas* from the cold deserts of the trans-Himalayas. Microb Ecol 58:425–434. https://doi.org/10.1007/s00248-009-9511-2.
4. Lalucat J, Mulet M, Gomila M, García-Valdés E. 2020. Genomics in bacterial taxonomy: impact on the genus *Pseudomonas*. Genes 11:139. https://doi.org/10.3390/genes11020139.
5. LPSN. 2021. Genus *Pseudomonas*. https://lpsn.dsmz.de/genus/pseudomonas. Accessed 22 July 2021.
6. 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.
7. Scales BS, Dickson RP, LiPuma JJ, Huffnagle GB. 2014. Microbiology, genomics, and clinical significance of the *Pseudomonas fluorescens* species complex, an unappreciated colonizer of humans. Clin Microbiol Rev 27:927–948. https://doi.org/10.1128/CMR.00044-14.
8. Duke KA, Becker MG, Girard IJ, Millar JL, Dilantha Fernando WG, Belmonte MF, de Kievit TR. 2017. The biocontrol agent *Pseudomonas chlororaphis* PA23 primes *Brassica napus* defenses through distinct gene networks. BMC Genomics 18:467. https://doi.org/10.1186/s12864-017-3848-6.
9. Roberts C, Edwards S, Vague M, León-Zayas R, Scheffer H, Chan G, Swartz NA, Mellies JL. 2020. Environmental consortium containing *Pseudomonas* and *Bacillus* species synergistically degrades polyethylene terephthalate plastic. mSphere 5:6. https://doi.org/10.1128/mSphere.01151-20.
10. Spiers AJ, Buckling A, Rainey PB. 2000. The causes of *Pseudomonas* diversity. Microbiology (Reading, England) 146:2345–2350. https://doi.org/10.1099/00221287-146-10-2345.
11. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Completing bacterial genome assemblies with multiplex MinION sequencing. Microb Genom 3: e000132. https://doi.org/10.1099/mgen.0.000132.
12. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. Genome Res 27:722–736. https://doi.org/10.1101/gr.215087.116.
13. Vaser R, Sović I, Nagarajan N, Šikić M. 2017. Fast and accurate de novo genome assembly from long uncorrected reads. Genome Res 27:737–746. https://doi.org/10.1101/gr.214270.116.
14. Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 1303.3997v2. https://arxiv.org/abs/1303.3997.
15. Manni M, Berkeley MR, Seppey M, Simao FA, Zdobnov EM. 2021. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol Biol Evol 38:4647–4654. https://doi.org/10.1093/molbev/msab199.
16. Hildebrand DC, Palleroni NJ, Hendson M, Toth J, Johnson JL. 1994. *Pseudomonas flavescens* sp. nov., isolated from walnut blight cankers. Int J Syst Bacteriol 44:410–415. https://doi.org/10.1099/00207713-44-3-410.
17. Hunter WJ. 2014. *Pseudomonas seleniipraecipitans* proteins potentially involved in selenite reduction. Curr Microbiol 69:69–74. https://doi.org/10.1007/s00284-014-0555-2.
18. 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.
19. Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, Weber T. 2021. antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res 49:W29–W35. https://doi.org/10.1093/nar/gkab335.
20. Sedkova N, Tao L, Rouvière PE, Cheng Q. 2005. Diversity of carotenoid synthesis gene clusters from environmental *Enterobacteriaceae* strains. Appl Environ Microbiol 71:8141–8146. https://doi.org/10.1128/AEM.71.12.8141-8146.2005.
21. Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. 2007. KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res 35:W182–W185. https://doi.org/10.1093/nar/gkm321.
22. 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.
23. Lefort V, Desper R, Gascuel O. 2015. FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 32:2798–2800. https://doi.org/10.1093/molbev/msv150.
24. Farris JS. 1972. Estimating phylogenetic trees from distance matrices. Am Nat 106:645–668. https://doi.org/10.1086/282802.
25. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/bioinformatics/btp352.
26. Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvernin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. 2021. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. Nucleic Acids Res 49:D1020–D1028. https://doi.org/10.1093/nar/gkaa1105.