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Complete genome of a fluorescent *Pseudomonas* sp. isolated from a PFAS groundwater treatment site

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ABSTRACT *Pseudomonas* sp. CBR-F is a bacterial species isolated from a water treatment plant targeting for per- and poly-fluoroalkyl substances, a difficult-to-degrade family of anthropogenic compounds. Here, we report a complete genome for *Pseudomonas* sp. CBR-F, sequenced with Oxford Nanopore Technology. The genome consists of one 6.91 Mbp chromosome.

KEYWORDS environmental microbiology, PFAS

Per- and poly-fluoroalkyl substances (PFAS) are a class of organofluorine compounds that are resistant to degradation and have accumulated to high levels in soils (1) and water (2). Global efforts are under way to assess the impact of PFAS on health, ecosystems, and the microbial capacity for bioremediation. Several *Pseudomonas* spp. have been implicated in PFAS breakdown (3), and other members of this environmentally ubiquitous genus are thought to hold promise for PFAS remediation efforts (4). We isolated a *Pseudomonas* sp.—strain CBR-F—from a PFAS-impacted environment; here, we describe its complete genome.

Spent granular activated carbon (GAC) from a PFAS groundwater treatment plant in the Mid-Atlantic United States was used to pack a recirculating, continuous-flow, aerobic bioreactor containing a medium composed of 25% PFAS-contaminated groundwater, 74.9% PFAS-free groundwater, and 0.1% fresh cheese whey. The medium was recirculated through the column from a 200 mL reservoir, a portion of which was periodically replaced.

After 50 days, an aliquot from the column media overlying the GAC was swabbed onto a Reasoner's 2 Agar (R2A) plate with 10 ppm perfluorooctanoic acid (PFOA) and incubated under aerobic conditions at 30°C overnight. One colony, surrounded by a fluorescent green halo, was restruck on a fresh R2A plate with 10 ppm PFOA. One colony from this plate was inoculated into PFOA-spiked R2A broth. After overnight growth, the culture appeared green and fluoresced under UV light. An aliquot of this culture was swabbed onto a PFOA R2A plate. An isolated colony was selected and grown in liquid R2A. After overnight incubation, this culture was preserved in a 25% glycerol stock and stored at −80°C.

DNA was extracted from a 0.5 mL culture grown in 10 ppm PFOA R2A for 16 h with shaking at 30°C using the MasterPure Complete DNA and RNA Purification Kit (Lucigen). Sequencing libraries were prepared with the Oxford Nanopore Technologies' Rapid Barcoding Kit (SQK-RBK004), and long-read sequencing was performed using the MinION Mk-1C with a FLO-MIN106D flow cell ONT (Oxford Nanopore Technologies).

Sequences were basecalled and demultiplexed using Guppy (version 6.4.6, ONT) (5), and a total of 10,871 reads were obtained with an average length of 32,990 bp and N_{50} of 34,046 bp. The genome was assembled and polished with Flye (version 2.9.2 [6]), with an estimated 51× coverage. Completeness was estimated at 99.35% by CheckM (version 1.2.1 [7]). The assembled genome is a circular 6,912,633bp-long contig with 61.1% GC

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content. All software was run with default parameters, including chimeric read detection and low-quality read removal by Flye.

The genome was annotated with the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (version 6.5) (8), which detected 6,305 coding sequences, 66 tRNA sequences, and 16 rRNAs. Notably, this genome encodes multiple siderophore synthesis pathways, two of which are predicted to make the fluorescent siderophore pyoverdine (9), which may underpin the fluorescence observed in culture. The genome has a high proportion of frameshifted pseudogenes, which is common in non-hybrid, Nanopore-based genome assemblies and may indicate a higher error rate. However, this genome can still be a resource in efforts to understand how PFAS affects biological evolution and how these adaptations can be leveraged for remediation efforts.

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DATA AVAILABILITY

The genome sequence has been deposited in GenBank under accession number CP124779.1 as part of National Center for Biotechnology Information (NCBI) BioProject PRJNA956891. MinION raw data are available in the NCBI Sequence Read Archive under accession number SRX25696260.

REFERENCES

1. Brusseau ML, Anderson RH, Guo B. 2020. PFAS concentrations in soils: background levels versus contaminated sites. Sci Total Environ 740:140017. https://doi.org/10.1016/j.scitotenv.2020.140017
2. Xu B, Liu S, Zhou JL, Zheng C, Weifeng J, Chen B, Zhang T, Qiu W. 2021. PFAS and their substitutes in groundwater: occurrence, transformation and remediation. J Hazard Mater 412:125159. https://doi.org/10.1016/j.jhazmat.2021.125159
3. Smorada CM, Sima MW, Jaffé PR. 2024. Bacterial degradation of perfluoroalkyl acids. Curr Opin Biotechnol 88:103170. https://doi.org/10.1016/j.copbio.2024.103170
4. Wackett LP. 2022. *Pseudomonas*: versatile biocatalysts for PFAS. Environ Microbiol 24:2882–2889. https://doi.org/10.1111/1462-2920.15990
5. 2024. Guppy repository. Available from: https://github.com/nanoporetech/pyguppyclient
6. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol 37:540–546. https://doi.org/10.1038/s41587-019-0072-8
7. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/%2010.1101/gr.186072.114
8. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569
9. Ringel MT, Brüser T. 2018. The biosynthesis of pyoverdines. Microb Cell 5:424–437. https://doi.org/10.15698/mic2018.10.649