AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequences of Two Gammaproteobacterial Methanotrophs Isolated from a Mercury-Contaminated Stream

Christina S. Kang-Yun,[a] Jin Chang,[a] Scott C. Brooks,[b] Baohua Gu,[b] Jeremy D. Semrau[a]

[a]Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, Michigan, USA
[b]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

ABSTRACT The genomes of *Methylomonas* sp. strain EFPC1 and *Methylococcus* sp. strain EFPC2, isolated from a mercury-contaminated stream in Oak Ridge, Tennessee, were sequenced.

Two methanotrophs of the *Gammaproteobacteria* class, *Methylomonas* sp. strain EFPC1 and *Methylococcus* sp. strain EFPC2, were isolated from the mercury-contaminated East Fork Poplar Creek (EFPC) in Oak Ridge, Tennessee, from biofilm samples collected in July, 2020 (specific sampling locations, N35.990385°, W84.317983° and N35.992482°, W84.315327° for *Methylomonas* sp. EFPC1 and *Methylococcus* sp. EFPC2, respectively). Biofilm samples were first inoculated in nitrate mineral salts medium (1) in liquid culture at 30°C with methane as the sole carbon and energy source to enrich for methanotrophs. After visible growth on methane, samples were then streaked onto NMS agar plates as described earlier (2). After repeated streaking onto NMS plates with purity confirmed via microscopy, 16S rRNA gene sequencing and negative growth on nutrient agar plates (3), a single colony of each strain was then grown in NMS liquid medium with methane. DNA from 50-ml and 200-ml cultures were extracted using phenol-chloroform extraction (4) and Qiagen Genomic-tip 500/G (Qiagen, Hilden, Germany) for Illumina and GridION Nanopore sequencing, respectively. Libraries for Illumina sequencing were prepared using a NEBNext Ultra II FS DNA Library Prep Kit (New England Biolabs, Inc., Ipswich, MA) with 15-min fragmentation and size selected for 275 to 475 bp. Libraries for GridION Nanopore sequencing were prepared using ligation sequencing and native barcoding expansion kits (SQK-LSK109 and EXP-NBD104; Oxford Nanopore Technologies, Littlemore, UK) following the manufacturers' protocols. Genomic DNA (gDNA) was sequenced using separate Nano flow cells and 500 cycle V2 kits on a MiSeq sequencer (Illumina, Inc., San Diego, CA) at the University of Michigan Advanced Genomics Core (AGC). Long-read sequencing was performed on the GridION X5 platform at the University of Michigan AGC (Oxford Nanopore Technologies, Littlemore, UK). Basecalling was performed using Guppy (v.4.2.3) (5). Sequence quality was assessed using FastQC (v0.11.9) (6) before and after trimming. The short and long reads were trimmed using Trimmomatic (v0.39) (7) and Porechop (v0.2.4) (8), respectively, and then were assembled using Unicycler (v0.4.9b) with no correction (9). Assembly completeness was assessed via BUSCO (v4.1.4) (10) and also visually confirmed using Bandage (v0.8.1) (11). The final contigs were annotated using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (v5.1) (12). The annotated 16S rRNA sequences were used as queries in search of the most similar organism using the Basic Local Alignment Search Tool (BLAST; v2.11.0) (13). Default parameters were used for all software unless otherwise specified.

Methylomonas sp. strain EFPC1 and *Methylococcus* sp. strain EFPC2 genomes were 4.99 Mbp and 4.56 Mbp (96% and 95.2% completion), consisting of either 1 chromosome and 1 plasmid (for *Methylomonas* sp. strain EFPC1) or 1 chromosome and 2 plasmids (for *Methylococcus* sp. strain EFPC2). All chromosomes and plasmids were circularized and then rotated according to the starting gene via Unicycler and were visually inspected using

Citation Kang-Yun CS, Chang J, Brooks SC, Gu B, Semrau JD. 2021. Complete genome sequences of two gammaproteobacterial methanotrophs isolated from a mercury-contaminated stream. Microbiol Resour Announc 10:e00181-21. https://doi.org/10.1128/MRA.00181-21.

Editor J. Cameron Thrash, University of Southern California

Address correspondence to Jeremy D. Semrau, jsemrau@umich.edu.

Received 24 February 2021
Accepted 14 April 2021
Published 20 May 2021

TABLE 1 General features of the *Methylomonas* sp. strain EFPC1 and *Methylococcus* sp. strain EFPC2 genomes

Strain	Complete genome size (bp)	No. of plasmids	G+C content (%)	Total no. of coding sequences	No. of rRNA genes (16S, 23S, and 5S)	No. of tRNA genes	Methane monooxygenase(s) present	No. of Illumina reads (accession no.)	No. of GridION reads (accession no.)	N_{50} of GridION reads (bp)	Annotated genome sequence accession no.	Closest neighbor based on 16S rRNA sequence	16S rRNA similarity with closest neighbor (%)	ANI[a] with closest neighbor (%)
Methylomonas sp. strain EFPC1	4,993,755	1	51.8	4,488	9	47	pMMO, pXMO, sMMO	2,230,538 (SRX10121820)	232,890 (SRX10121821)	51,070	CP070494, CP070495	*Methylomonas* sp. LW13	99.87	95.20
Methylococcus sp. strain EFPC2	4,558,902	2	61.2	3,891	9	50	pMMO	2,379,682 (SRX10121822)	135,052 (SRX10121823)	50,620	CP070491, CP070492, CP070493	*Methylococcus geothermalis* IM1^T	96.30	72.70

[a] Average nucleotide identity.

Bandage. 16S rRNA sequence analyses of *Methylomonas* sp. strain EFPC1 indicated that it was phylogenetically similar to *Methylomonas* sp. LW13 (14), and *Methylococcus* sp. strain EFPC2 was most similar to *Methylococcus geothermalis* IM1^T (15). Average nucleotide identity (ANI) values between *Methylomonas* sp. strain EFPC1 and *Methylomonas* sp. LW13 and between *Methylococcus* sp. strain EFPC2 and *Methylococcus* sp. IM1^T were ~95% and 73%, respectively (16). Genes for particulate methane monooxygenase (pMMO) were found in both *Methylomonas* sp. strain EFPC1 and *Methylococcus* sp. strain EFPC2, while evidence of a divergent form of pMMO (pXMO) and soluble methane monooxygenase was found in only *Methylomonas* sp. strain EFPC1. These results are summarized in Table 1.

Data availability. Accession numbers for the annotated sequences and raw reads are posted in Table 1.

ACKNOWLEDGMENTS

This study was supported by grants from the National Science Foundation (1724430 and 1724744) as well as the United States Department of Energy (DOE) (DE-SC0018059). Oak Ridge National Laboratory is managed by UT-Battelle, LLC under contract number DE-AC05-00OR22725 with DOE. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

REFERENCES

1. Whittenbury R, Phillips KC, Wilkinson JF. 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. J Gen Microbiol 61:205–218. https://doi.org/10.1099/00221287-61-2-205.
2. Gu W, Semrau JD. 2017. Copper and cerium-regulated gene expression in *Methylosinus trichosporium* OB3b. Appl Microbiol Biotechnol 101:8499–8516. https://doi.org/10.1007/s00253-017-8572-2.
3. Im J, Lee S-W, Yoon S, DiSpirito AA, Semrau JD. 2011. Characterization of a novel *Methylocystis* species capable of growth on methane, acetate and ethanol. Environ Microbiol Rep 3:174–181. https://doi.org/10.1111/j.1758-2229.2010.00204.x.
4. Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ. 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol 66:5488–5491. https://doi.org/10.1128/AEM.66.12.5488-5491.2000.
5. Wick RR, Judd LM, Holt KE. 2019. Performance of neural network basecalling tools for Oxford Nanopore sequencing. Genome Biol 20:129. https://doi.org/10.1186/s13059-019-1727-y.
6. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
7. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
8. Wick RR. 2018. Porechop: an adapter trimmer for Oxford Nanopore reads. https://github.com/rrwick/Porechop.
9. 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.
10. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210–3212. https://doi.org/10.1093/bioinformatics/btv351.
11. Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualisation of de novo genome assemblies. Bioinformatics 31:3350–3352. https://doi.org/10.1093/bioinformatics/btv383.
12. 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.
13. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic Local Alignment Search Tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
14. Kalyuzhnaya MG, Lamb AE, McTaggert TL, Oshkin IY, Shapiro N, Woyke T, Chistoserdova L. 2015. Draft genome sequences of gammproteobacterial methanotrophs isolated from Lake Washington sediment. Genome Announc 3:e00103-15. https://doi.org/10.1128/genomeA.00103-15.
15. Awala SI, Bellosillo LA, Gwak J-H, Nguyen N-L, Kim S-J, Lee B-H, Rhee S-K. 2020. *Methylococcus geothermalis* sp. nov., a methanotroph isolated from a geothermal field in the Republic of Korea. Int J Syst Evol Microbiol 70:5520–5530. https://doi.org/10.1099/ijsem.0.004442.
16. Yoon S-H, Ha S-M, 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.