



Complete Genome Sequence of *Sulfurospirillum* sp. Strain ACS_{DCE}, an Anaerobic Bacterium That Respires Tetrachloroethene under Acidic pH Conditions

Yi Yang,^a Leitao Huo,^{a,b} Xiuying Li,^a Jun Yan,^a Frank E. Löffler^{c,d,e,f,g,h}

^aKey Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, Liaoning, China

^bUniversity of Chinese Academy of Sciences, Beijing, China

^cCenter for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee, USA

^dDepartment of Microbiology, University of Tennessee, Knoxville, Tennessee, USA

^eDepartment of Civil and Environmental Engineering, University of Tennessee, Knoxville, Tennessee, USA

^fDepartment of Biosystems Engineering & Soil Science, University of Tennessee, Knoxville, Tennessee, USA

^gBiosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

^hJoint Institute for Biological Sciences (JIBS), Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

ABSTRACT *Sulfurospirillum* sp. strain ACS_{DCE} couples growth with reductive dechlorination of tetrachloroethene to *cis*-1,2-dichloroethene at pH values as low as 5.5. The genome sequence of strain ACS_{DCE} consists of a circular 2,737,849-bp chromosome and a 39,868-bp plasmid and carries 2,737 protein-coding sequences, including two reductive dehalogenase genes.

Bioremediation of chlorinated contaminants in acidic groundwater is challenging because most organohalide-respiring bacteria cease growth under acidic conditions (1–3). *Sulfurospirillum* strains that grow via tetrachloroethene (PCE) reductive dechlorination under acidic conditions are candidates for bioremediation of chlorinated contaminants in low-pH groundwater (2, 4). *Sulfurospirillum* sp. strain ACS_{DCE} was isolated from a PCE-dechlorinating microcosm established with contaminated soil collected at the Axton Cross Superfund site (Holliston, MA, USA) (2). Strain ACS_{DCE} couples growth with PCE-to-*cis*-1,2-dichloroethene (cDCE) reductive dechlorination at pH values as low as 5.5 (2) and shares 99.3% and 98.5% 16S rRNA gene sequence identities with the PCE-to-trichloroethene dechlorinating *Sulfurospirillum* sp. strain ACS_{TCE} (GenBank accession number CP045453.2) (5) and the PCE-to-cDCE dechlorinating *Sulfurospirillum multivorans* strain DSM 12446 (CP007201.1) (4), respectively.

Strain ACS_{DCE} was grown in anoxic, 2-(*N*-morpholino)-ethanesulfonic acid-buffered mineral salt medium (pH 5.5) with acetate as the carbon source, hydrogen as the electron donor, and PCE as the electron acceptor (2). Cells were collected from a 2-liter culture by centrifugation, and DNA was extracted using the cetyltrimethylammonium bromide method (6). Genome sequencing used a hybrid approach incorporating the Illumina and PacBio platforms. For Illumina sequencing, a library with an average insert size of 350 bp was constructed using the TruSeq DNA sample preparation kit (Illumina, San Diego, CA, USA), and paired-end sequencing (2 × 150 bp) was performed on an Illumina HiSeq 2000 instrument. Adaptors and low-quality sequences were removed from the raw reads using Cutadapt version 1.9.1 (7). For PacBio sequencing (PacBio RS II/Sequel SMRT), genomic DNA was sheared using g-TUBEs (Covaris, Inc., Woburn, WA, USA) to generate 10-kb fragments, followed by ligation with universal hairpin adaptors using the SMRTbell template prep kit version 1.0 (Pacific Biosciences, Menlo Park, CA, USA) (8). The PacBio raw read N_{50} value was 3,932 bp. Genome assembly with 273,566 PacBio raw long reads (coverage, 300×) using Flye version 2.6 (9) was refined with

Citation Yang Y, Huo L, Li X, Yan J, Löffler FE. 2021. Complete genome sequence of *Sulfurospirillum* sp. strain ACS_{DCE}, an anaerobic bacterium that respire tetrachloroethene under acidic pH conditions. Microbiol Resour Announc 10:e01360-20. <https://doi.org/10.1128/MRA.01360-20>.

Editor J. Cameron Thrash, University of Southern California

Copyright © 2021 Yang et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Jun Yan, junyan@iae.ac.cn, or Frank E. Löffler, frank.loeffler@utk.edu.

Received 30 November 2020

Accepted 21 December 2020

Published 14 January 2021

25,162,740 Illumina short reads (coverage, 1,387×) using Pilon version 1.20.1 (10). The assembly resulted in a 2,737,849-bp circular chromosome with a G+C content of 38.8% and a 39,868-bp circular plasmid with a G+C content of 35.6%. Circlator version 1.5.1 (11) was used to remove the overlapping ends, circularize the genome, and rotate the chromosomal origin of replication to the starting position of the *dnaA* gene. Gene identification and functional annotation were performed using the NCBI Prokaryotic Genome Annotation Pipeline version 4.12 (12). Default parameters were used for all software unless otherwise specified.

The strain ACS_{DCE} chromosome contains 2,737 protein-coding sequences, 47 tRNAs, and 3 rRNA operons organized in the order 16S, 23S, 5S. The chromosome harbors two putative reductive dehalogenase (*rdh*) A genes (FA584_00035 and FA584_00060), both adjacent to a downstream *rdhB* gene encoding a membrane anchor protein. The strain ACS_{DCE} chromosome harbors a fumarate reductase gene cluster (FA584_09715, FA584_09720, and FA584_09725) and a gene (FA584_11415) coding for the catalytic subunit NapA of periplasmic nitrate reductase, suggesting that nitrate and fumarate are alternate electron acceptors. The plasmid harbors 47 genes, including genes encoding transposases (four), DNA polymerase IV (two), and subunits of type II toxin-antitoxin systems (six). The results expand the *Sulfurospirillum* pangenome and identify biomarkers for monitoring reductive dechlorination activity in low-pH groundwater.

Data availability. The genome project and sample are indexed at GenBank under BioProject [PRJNA534159](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA534159) and BioSample [SAMN11478956](https://www.ncbi.nlm.nih.gov/biosample/SAMN11478956). The GenBank accession numbers of the chromosomal and plasmid sequences are [CP039734](https://www.ncbi.nlm.nih.gov/nuccore/CP039734) and [CP059996](https://www.ncbi.nlm.nih.gov/nuccore/CP059996), respectively. The raw sequencing reads have been deposited in the Sequence Read Archive (SRA) under accession numbers [SRR12322185](https://www.ncbi.nlm.nih.gov/sra/SRR12322185) (Illumina) and [SRR12322184](https://www.ncbi.nlm.nih.gov/sra/SRR12322184) (PacBio).

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (41673126, 41977295, and 41907287), the Key Research Program of Frontier Science of the Chinese Academy of Sciences (ZDBS-LY-DQC038), and the Liaoning Revitalization Talents Program (XLYC1807139). F.E.L. acknowledges support from the Strategic Environmental Research and Development Program (SERDP) (project ER-2312). We acknowledge the kind support from members of Galaxy Australia, Galaxy Europe, and the Galaxy Project (13).

REFERENCES

- Henterly RW, Harms WD. 2015. Optimal treatment zone moves during enhanced reductive dechlorination in fractured bedrock. *Remediation* 25:81–88. <https://doi.org/10.1002/rem.21441>.
- Yang Y, Cápiro NL, Marcet TE, Yan J, Pennell KD, Löffler FE. 2017. Organohalide respiration with chlorinated ethenes under low pH conditions. *Environ Sci Technol* 51:8579–8588. <https://doi.org/10.1021/acs.est.7b01510>.
- Yang Y, Cápiro NL, Yan J, Marcet TF, Pennell KD, Löffler FE. 2017. Resilience and recovery of *Dehalococcoides mccartyi* following low pH exposure. *FEMS Microbiol Ecol* 93:fix130. <https://doi.org/10.1093/femsec/fix130>.
- Scholz-Muramatsu H, Neumann A, Meßmer M, Moore E, Diekert G. 1995. Isolation and characterization of *Dehalospirillum multivorans* gen. nov., sp. nov., a tetrachloroethene-utilizing, strictly anaerobic bacterium. *Arch Microbiol* 163:48–56. <https://doi.org/10.1007/BF00262203>.
- Huo L, Yang Y, Lv Y, Li X, Löffler FE, Yan J. 2020. Complete genome sequence of *Sulfurospirillum* strain ACS_{TCE}, a tetrachloroethene-respiring anaerobe isolated from contaminated soil. *Microbiol Resour Announc* 9:e00941-20. <https://doi.org/10.1128/MRA.00941-20>.
- Joint Genome Institute. 2012. Bacterial genomic DNA isolation using CTAB. <http://jgi.doe.gov/wp-content/uploads/2014/02/JGI-Bacterial-DNA-isolation-CTAB-Protocol-2012.pdf>.
- Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* 17:10–12. <https://doi.org/10.14806/ej.17.1.200>.
- McCarthy A. 2010. Third generation DNA sequencing: Pacific Biosciences' single molecule real time technology. *Chem Biol* 17:675–676. <https://doi.org/10.1016/j.chembiol.2010.07.004>.
- Lin Y, Yuan J, Kolmogorov M, Shen MW, Chaisson M, Pevzner PA. 2016. Assembly of long error-prone reads using de Bruijn graphs. *Proc Natl Acad Sci U S A* 113:E8396–E8405. <https://doi.org/10.1073/pnas.1604560113>.
- 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>.
- Hunt M, De Silva N, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. *Genome Biol* 16:294. <https://doi.org/10.1186/s13059-015-0849-0>.
- 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>.
- Jalili V, Afgan E, Gu Q, Clements D, Blankenberg D, Goecks J, Taylor J, Nekrutenko A. 2020. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2020 update. *Nucleic Acids Res* 48:W395–W402. <https://doi.org/10.1093/nar/gkaa434>.