



Draft Genome Sequence of *Desulfurobacterium* sp. Strain AV08, a Thermophilic Chemolithoautotroph Isolated from a Deep-Sea Hydrothermal Vent

Emilie J. Skoog,^a  Julie A. Huber,^b Margrethe H. Serres,^b Alice Levesque,^c Lisa Zeigler Allen^{c,d}

^aDepartment of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

^bDepartment of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA

^cMicrobial and Environmental Genomics, J. Craig Venter Institute, San Diego, California, USA

^dMarine Biology Research Division, Scripps Institution of Oceanography, La Jolla, California, USA

ABSTRACT A thermophilic chemolithoautotrophic bacterium was isolated from vent fluids at Axial Seamount, an active deep-sea volcano in the northeast Pacific Ocean. We present the draft genome sequence of *Desulfurobacterium* sp. strain AV08.

Members of the *Desulfurobacterium* genus have been identified globally in deep-sea vents (1–6). *Desulfurobacterium* sp. strain AV08 was isolated from Bag City, a low-temperature (23°C) diffuse flow vent at Axial Seamount located on the Juan de Fuca Ridge (7). Fluid sampling was conducted using the hydrothermal fluid particle sampler (8) aboard the ROV *ROPOS*, at a depth of 1,520 m. Samples were immediately cultured anaerobically in Balch tubes by inoculation of 1 ml of fluid sample into 7 ml of artificial seawater medium with 0.001% yeast extract (9). Culture tubes were reduced with 0.05% Na₂S and given a headspace of 80% H₂/20% CO₂. Cultures were incubated at 70°C. AV08 was then isolated by serial dilution to extinction (10) at 70°C.

After isolation, AV08 was grown in the laboratory at 70°C in *Sulfurimonas parvolinella* medium (DSMZ 1053 medium) reduced with 0.05% Na₂S before inoculation and topped off with a headspace of 80% H₂/20% CO₂ after inoculation. Genomic DNA was extracted from a single culture using the cetyltrimethylammonium bromide (CTAB) phenol-chloroform-based extraction method and sequenced using both long-read (Oxford Nanopore Technologies [ONT]) and short-read (Illumina MiSeq) (PE 250 bp) sequencing technologies. Postprocessing, assembly, annotation, and bioinformatic analyses used default parameters for all software unless otherwise specified. The collective reads were assembled using a SPAdes v3.5.0 hybrid assembly (11) (Table 1). This resulted in 38 total contigs, with an N_{50} value of 354,053 bp, a maximum contig length of 815,718 bp, and a total length of 1,732,745 bp. CheckM v1.0.18 was used to determine genome completion and contamination values of 99.58% and 1.27%, respectively (12). QUAST v4.4 was used to identify a G+C content of 39.33% (13).

The genome was annotated with the Joint Genome Institute (JGI) Integrated Microbial Genomes (IMG) Annotation Pipeline v5.0.20 (14, 15), resulting in 1,763 protein-coding genes, 1 complete rRNA gene, 46 tRNA genes, and 4 CRISPR loci (Table 1). *Desulfurobacterium* sp. strain AV08 has genes for the reductive tricarboxylic acid (rTCA) cycle for CO₂ fixation, sulfur reduction, and denitrification, as well as genes for hydrogen oxidation and formate utilization. The JGI IMG pipeline-annotated 16S rRNA gene was used to identify homologous sequences within the NCBI GenBank nonredundant database (16). Based on 16S rRNA gene analysis, AV08 is most similar to *Desulfurobacterium crinifex* NE1206 (GenBank accession number [AJ507320.2](https://doi.org/10.1128/MRA.00615-21)) (3) (99.4%) and *Desulfurobacterium thermolithotrophum* DSM 11699 (GenBank accession number [NR_075040.1](https://doi.org/10.1128/MRA.00615-21)) (6) (97.3%). A phylogenomic tree constructed with GToTree v1.4.5 (17) indicated that AV08 is most closely related to *D. thermolithotrophum*

Citation Skoog EJ, Huber JA, Serres MH, Levesque A, Zeigler Allen L. 2021. Draft genome sequence of *Desulfurobacterium* sp. strain AV08, a thermophilic chemolithoautotroph isolated from a deep-sea hydrothermal vent. *Microbiol Resour Announc* 10:e00615-21. <https://doi.org/10.1128/MRA.00615-21>.

Editor J. Cameron Thrash, University of Southern California

Copyright © 2021 Skoog 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 Lisa Zeigler Allen, lzeigler@ucsd.edu.

Received 15 June 2021

Accepted 27 July 2021

Published 26 August 2021

TABLE 1 Organism isolation, growth, and sequencing data

Parameter ^a	Data
Environmental data	
Geographic location	
Region	Juan de Fuca Ridge, northeast Pacific Ocean
Volcano	Axial Seamount
Geographic coordinates	45°58' N, 130°00' W
Collection date	July 1999
Marine biome	Hydrothermal vent
Vent name	Bag City
Vent temp (°C)	23
Sampling depth (m)	1,520
Sampling method	ROV-based fluid sampler
Growth conditions	DSMZ 1053 medium, anaerobically
Sequencing	
RNA/DNA quantification instrument	Nanodrop UV/visible spectrophotometer (Thermo Fisher Scientific)
Sample purity	
260 nm/280 nm	~1.7
260 nm/230 nm	~1.3
Nanopore library	ONT rapid PCR-free kit
Illumina library	Accel-NGS 2S Plus DNA library preparation kit/ MiSeq nanokit v2
Sequencing technologies	ONT plus Illumina MiSeq (hybrid) ^b
No. of Nanopore reads	5,475
Nanopore read N_{50} (bp)	2,092
Longest Nanopore read length (bp)	80,194
No. of Illumina reads	736,493
Nanopore base calling and postprocessing	Guppy
Nanopore flow cell	ONT MinION R9.4.1
Sequencing center	J. Craig Venter Institute (La Jolla, CA)
Assembler	SPAdes v3.5.0
No. of contigs	38
Largest contig (bp)	815,718
Contig N_{50} (bp)	354,053
ORF caller	JGI IMG Annotation Pipeline
Genomic features	
Genome size (bp)	1,732,745
G+C content (mol%)	0.3933
No. of genes	1,824
No. of protein-coding genes	1,763
No. of genes with COG identity	1,472
No. of RNA genes	51
No. of rRNA genes	3
No. of 5S rRNA genes	1
No. of 16S rRNA genes	1
No. of 23S rRNA genes	1
No. of tRNA genes	46
No. of other RNA genes	2
No. of CRISPR loci	4

^aORF, open reading frame; COG, Clusters of Orthologous Groups of proteins.

^bThe same DNA extracted from a single culture was used for both library preparations and sequencing technologies.

(Fig. 1). Currently, no genomic data are available for *D. crinifex*. Overall genome relatedness index values of 81.95 and 79.99 were determined using average nucleotide identity analyses within GTDB-Tk v1.1.0 (18) and the JGI IMG system, respectively. These values fall well below the 95% to 96% cutoff values for species determination.

Data availability. The complete genome sequence and metadata are publicly available at JGI IMG under analysis project number [Ga0466268](#). Raw sequence reads are available at NCBI under BioProject accession number [PRJNA731061](#) and SRA accession numbers [SRR14730166](#) and [SRR14730165](#).

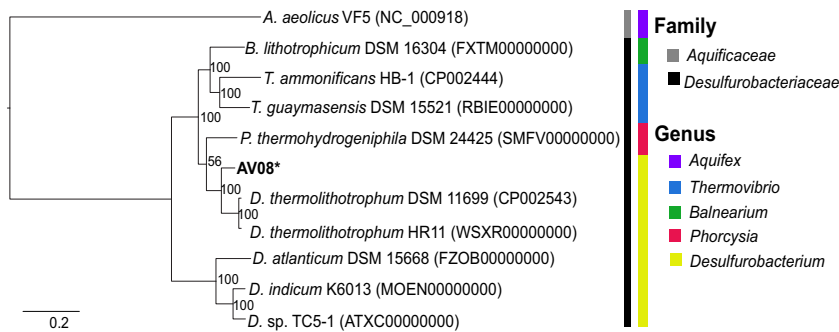


FIG 1 Phylogenomic tree of *Desulfurobacterium* sp. strain AV08 (marked with an asterisk) and related *Desulfurobacteriaceae* and *Aquificaceae* (outgroup) family strains. All available *Desulfurobacteriaceae* genomes with >97% genome completeness are included. Families and genera are indicated by color.

ACKNOWLEDGMENTS

This research was supported by the National Aeronautics and Space Administration Exobiology Program (grant 80NSSC18K1076 to L.Z.A. and J.A.H.). This study was also partially supported by the NSF Center for Dark Energy Biosphere Investigations (C-DEBI) (grant OCE-0939564 to J.A.H.).

REFERENCES

- Reysenbach AL, Longnecker K, Kirshtein J. 2000. Novel bacterial and archaeal lineages from an in situ growth chamber deployed at a Mid-Atlantic Ridge hydrothermal vent. *Appl Environ Microbiol* 66:3798–3806. <https://doi.org/10.1128/AEM.66.9.3798-3806.2000>.
- Huber JA, Butterfield DA, Baross JA. 2003. Bacterial diversity in a subseafloor habitat following a deep-sea volcanic eruption. *FEMS Microbiol Ecol* 43:393–409. <https://doi.org/10.1111/j.1574-6941.2003.tb01080.x>.
- Alain K, Rolland S, Crassous P, Lesongeur F, Zbinden M, Le Gall C, Godfroy A, Page A, Juniper SK, Cambon-Bonavita MA, Duchiron F, Querellou J. 2003. *Desulfurobacterium crinifex* sp. nov., a novel thermophilic, pinkish-streamer forming, chemolithoautotrophic bacterium isolated from a Juan de Fuca Ridge hydrothermal vent and amendment of the genus *Desulfurobacterium*. *Extremophiles* 7:361–370. <https://doi.org/10.1007/s00792-003-0329-4>.
- L'Haridon S, Reysenbach AL, Tindall BJ, Schonheit P, Banta A, Johnsen U, Schumann P, Gambacorta A, Stackebrandt E, Jeanthon C. 2006. *Desulfurobacterium atlanticum* sp. nov., *Desulfurobacterium pacificum* sp. nov. and *Thermovibrio guaymasensis* sp. nov., three thermophilic members of the *Desulfurobacteriaceae* fam. nov., a deep branching lineage within the *Bacteria*. *Int J Syst Evol Microbiol* 56:2843–2852. <https://doi.org/10.1099/ijs.0.63994-0>.
- Holden JF, Bardwell CP, Kashyap S. 2020. Draft genome sequence of *Desulfurobacterium thermolithotrophum* strain HR11, a novel thermophilic autotrophic subspecies from a deep-sea hydrothermal vent. *Microbiol Resour Announc* 9:e00167-20. <https://doi.org/10.1128/MRA.00167-20>.
- L'Haridon S, Cilia V, Messner P, Raguene G, Gambacorta A, Sleytr UB, Prieur D, Jeanthon C. 1998. *Desulfurobacterium thermolithotrophum* gen. nov., sp. nov., a novel autotrophic, sulphur-reducing bacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Bacteriol* 48:701–711. <https://doi.org/10.1099/00207713-48-3-701>.
- Chadwick WW, Butterfield DA, Embley RW, Tunnicliffe V, Huber JA, Nooner SL, Clague DA. 2010. Axial Seamount. *Oceanography* 23:38–39. <https://doi.org/10.5670/oceanog.2010.73>.
- Butterfield DA, Lilley MD, Huber JA, Roe KK, Embley RW, Baross JA, Massoth GJ. 2004. Mixing, reaction, and microbial activity in sub-seafloor hydrothermal upflow zones: evidence from diffuse flow outcrops across the 1998 Axial Volcano sea-floor eruption area through time, p 269–289. *In* Wilcock W, DeLong EF, Kelley DS, Baross JA, Cary SC (ed), *The subseafloor biosphere at Mid-Ocean Ridges*. American Geophysical Union Press, Washington, DC.
- Holden JF, Summit M, Baross JA. 1998. Thermophilic and hyperthermophilic microorganisms in 3–30°C hydrothermal fluids following a deep-sea volcanic eruption. *FEMS Microbiol Ecol* 25:33–41. [https://doi.org/10.1016/S0168-6496\(97\)00081-0](https://doi.org/10.1016/S0168-6496(97)00081-0).
- Baross JA. 2018. Isolation and cultivation of hyperthermophilic bacteria from marine and freshwater habitats, p 21–30. *In* Kemp PF, Sherr BF, Sherr EB, Cole JJ (ed), *Handbook of methods in aquatic microbial ecology*. CRC Press, Boca Raton, FL.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Chen IA, Chu K, Palaniappan K, Ratner A, Huang J, Huntemann M, Hajek P, Ritter S, Varghese N, Seshadri R, Roux S, Woyke T, Eloe-Fadrosh EA, Ivanova NN, Kyrpides NC. 2021. The IMG/M data management and analysis system v.6.0: new tools and advanced capabilities. *Nucleic Acids Res* 49:D751–D763. <https://doi.org/10.1093/nar/gkaa939>.
- Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Palaniappan K, Szeto E, Pillay M, Chen IM, Pati A, Nielsen T, Markowitz VM, Kyrpides NC. 2015. The standard operating procedure of the DOE-JGI Microbial Genome Annotation Pipeline (MGAP v.4). *Stand Genomic Sci* 10: 86. <https://doi.org/10.1186/s40793-015-0077-y>.
- Sayers EW, Cavanaugh M, Clark K, Pruitt KD, Schoch CL, Sherry ST, Karsch-Mizrachi I. 2021. GenBank. *Nucleic Acids Res* 49:D92–D96. <https://doi.org/10.1093/nar/gkaa1023>.
- Lee MD. 2019. GToTree: a user-friendly workflow for phylogenomics. *Bioinformatics* 35:4162–4164. <https://doi.org/10.1093/bioinformatics/btz188>.
- Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* 36:1925–1927. <https://doi.org/10.1093/bioinformatics/btz848>.