



Complete Genome Sequence of *Halomonas hydrothermalis* Strain Slthf2, a Halophilic Bacterium Isolated from a Deep-Sea Hydrothermal-Vent Environment

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ABSTRACT *Halomonas hydrothermalis* strain Slthf2 is a Gram-negative bacterium isolated from low-temperature hydrothermal fluids in South Pacific Ocean vent fields located at 2,580-m depth. Here, we report the complete genome sequence of this strain, which has a genome size of 4.12 Mb, with a GC content of 53.2%.

Halomonas hydrothermalis strain Slthf2 (ATCC BAA-800, CECT 5814, DSM 15725) is a Gram-negative, psychrotolerant, and moderately halophilic bacterium classified in the phylum *Proteobacteria*, order *Oceanospirillales*, and family *Halomonadaceae*. It was first isolated by Kaye et al. from low-temperature hydrothermal fluid at 2,580-m depth in the South Pacific Ocean near Easter Island (1). Some *Halomonas* strains produce polyhydroxybutyrate (PHB), which can be used as a substitute for petroleum plastics (2–4). In addition, a wide variety of carbon sources can be used for growth, including waste glycerol from the biodiesel manufacturing process, as well as PHB production (5). Therefore, here, we report the complete genome sequence of *H. hydrothermalis* strain Slthf2 to better understand the potential for industrial use of *Halomonas* species as PHB producers.

We picked one colony of *H. hydrothermalis* strain Slthf2 (obtained from J. Z. Kaye) and cultured it overnight at 37°C in SW10 culture solution, which had the following composition (% [wt/vol]): NaCl (8.1), MgCl₂ (0.7), MgSO₄ (0.96), CaCl₂ (0.036), KCl (0.2), NaHCO₃ (0.006), NaBr (0.0026), proteose peptone (Difco; 0.5), yeast extract (Difco; 1.0), glucose (0.1), and agar (1.5). The genomic DNA was extracted using Genomic-tip 20/G (Qiagen) according to the manufacturer's protocol. The long-read sequence libraries were prepared using a rapid barcoding kit (SQK-RBK004; Oxford Nanopore Technologies) and were sequenced using the GridION device with a FLO-MIN106 flow cell (Oxford Nanopore Technologies). Illumina sequencing was performed for error correction by using a KAPA HyperPlus kit (Kapa Biosystems) for library preparation and a NextSeq 500 sequencer for sequencing using high-output mode and a run of 75 cycles (Illumina). Reads with at least 40,000 bp were used for the *de novo* assembly (71-fold coverage; 5,165 out of 266,015 sequenced reads) using Canu v1.8 (6), and the assembled single contig was manually circularized by eliminating an overlapping end. Contigs obtained from the assembly were further polished using Pilon v1.23 (7) with the short reads. The assembly completeness was assessed by BUSCO v1 (8) on the gVolante server (9), and the resulting genome sequence was functionally annotated using the DDBJ Fast Annotation and Submission Tool (DFAST) pipeline (10). The assembled genome consists of one circular chromosome of 4,120,823 bp having 53.2% GC content, including 3,761 coding sequences (CDSs), 60 tRNAs, and 18 rRNAs. All software programs were used with the default settings.

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According to the annotation results, the genome of *H. hydrothermalis* strain Slthf2 encodes genes related to PHB production (LOCUS_04570), which is in agreement with previous reports (5). This information will be useful for revealing the production of PHB mediated by *H. hydrothermalis* and for contributing to the field of metabolic engineering.

Data availability. The chromosome sequence reported here was deposited in DDBJ under accession number [AP022843](https://www.ncbi.nlm.nih.gov/nuclseq/AB022843), and the raw reads were deposited in the Sequence Read Archive (SRA) under BioProject accession number [PRJNA606145](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA606145).

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REFERENCES

1. Kaye JZ, Márquez MC, Ventosa A, Baross JA. 2004. *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* sp. nov., *Halomonas axialensis* sp. nov. and *Halomonas hydrothermalis* sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. *Int J Syst Evol Microbiol* 54:499–511. <https://doi.org/10.1099/ijs.0.02799-0>.
2. Kawata Y, Kawasaki K, Shigeri Y. 2012. Efficient secreted production of (R)-3-hydroxybutyric acid from living *Halomonas* sp. KM-1 under successive aerobic and microaerobic conditions. *Appl Microbiol Biotechnol* 96:913–920. <https://doi.org/10.1007/s00253-012-4218-6>.
3. Quillaguamán J, Hashim S, Bento F, Mattiasson B, Hatti-Kaul R. 2005. Poly(β -hydroxybutyrate) production by a moderate halophile, *Halomonas boliviensis* LC1 using starch hydrolysate as substrate. *J Appl Microbiol* 99:151–157. <https://doi.org/10.1111/j.1365-2672.2005.02589.x>.
4. Tan D, Xue Y-S, Aibaidula G, Chen G-Q. 2011. Unsterile and continuous production of polyhydroxybutyrate by *Halomonas* TD01. *Bioresour Technol* 102:8130–8136. <https://doi.org/10.1016/j.biortech.2011.05.068>.
5. Shrivastav A, Mishra SK, Shethia B, Pancha I, Jain D, Mishra S. 2010. Isolation of promising bacterial strains from soil and marine environment for polyhydroxyalkanoates (PHAs) production utilizing *Jatropha* biodiesel byproduct. *Int J Biol Macromol* 47:283–287. <https://doi.org/10.1016/j.ijbiomac.2010.04.007>.
6. 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>.
7. 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>.
8. 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>.
9. Nishimura O, Hara Y, Kuraku S. 2017. gVolante for standardizing completeness assessment of genome and transcriptome assemblies. *Bioinformatics* 33:3635–3637. <https://doi.org/10.1093/bioinformatics/btx445>.
10. Tanizawa Y, Fujisawa T, Kaminuma E, Nakamura Y, Arita M. 2016. DFAST and DAGA: Web-based integrated genome annotation tools and resources. *Biosci Microbiota Food Health* 35:173–184. <https://doi.org/10.12938/bmfh.16-003>.