




# Complete Genome Sequence of *Methanothermobacter* sp. Strain THM-1, a Thermophilic and Hydrogenotrophic Methanogen Isolated from an Anaerobic Reactor in South Korea

Byoung-Seung Jeon,<sup>a,b</sup> Hyunjin Kim,<sup>b</sup> Young Wook Go,<sup>b</sup> Hyunook Kim,<sup>c</sup> Okkyoung Choi,<sup>b</sup>  Byoung-In Sang<sup>b</sup>

<sup>a</sup>Convergence Bioceramic Materials Center, Korea Institute of Ceramic Engineering and Technology, Cheongju, Republic of Korea

<sup>b</sup>Department of Chemical Engineering, Hanyang University, Seoul, Republic of Korea

<sup>c</sup>Department of Environmental Engineering, University of Seoul, Seoul, Republic of Korea

Byoung-Seung Jeon and Hyunjin Kim contributed equally to this work. Author order was determined by order of increasing seniority.

**ABSTRACT** *Methanothermobacter* sp. strain THM-1, a thermophilic and hydrogenotrophic methanogen, was isolated from an anaerobic reactor enriched with thermophilic methanogens. The genome of THM-1 shares 98.81% of its sequence with *Methanothermobacter wolf-eii* isolate SIV6 and consists of 1,724,502 bp with 1,665 protein-coding genes, 50 noncoding RNAs, and a GC content of 48.6%.

Thermophilic and hydrogenotrophic methanogens (THMs) have been suggested to produce methane from carbon dioxide and hydrogen in power-to-gas and biogas upgrading processes (1).

Here, we report the complete genome sequence of the newly isolated methanogen *Methanothermobacter* sp. strain THM-1 (hereinafter THM-1). THM-1 was isolated from an anaerobic reactor integrated with a bioelectrochemical system, which produced methane and acetate from carbon dioxide using electricity (2). Less than 10  $\mu$ l of the reactor sample was injected into 50 ml of basic anaerobic agar (1.5% [wt/vol] agar added to basic anaerobic medium [2]) reinforced with sodium bicarbonate. The agar solidified after homogenization at approximately 60°C (3, 4). The anaerobic agar medium containing the bacteria was incubated at 60°C under atmospheric conditions that included CO<sub>2</sub> and H<sub>2</sub> at a ratio of 1:4. The samples were cultivated until 1- to 2-mm colonies were visible inside the agar medium. The agar, including a single colony, was then diced into 0.125-cm<sup>3</sup> pieces and sequentially subcultured. Identification by amplification and sequencing of the 16S rRNA gene was conducted using the primers from a previous study (5). THM-1 was classified as a member of the genus *Methanothermobacter* by phylogenetic comparison of the 16S rRNA genes using MEGA7 (6, 7).

The DNA of THM-1 was taken from cells purely cultivated, which were enriched in basic anaerobic medium at 60°C under a pressurization system of 2 bars. In this system, CO<sub>2</sub> and H<sub>2</sub> were used as the sole carbon and energy sources, respectively. Genomic DNA was extracted using the DNeasy UltraClean microbial kit (Qiagen Korea Ltd., South Korea) (8) and sequenced using the PacBio RS II platform (PacBio single-molecule real-time sequencing) at Macrogen Co. Ltd. (Seoul, South Korea). The library was prepared using the SMRTbell template prep kit (9). The preassembly read quality check (QC) was performed using a BLAST v2.7.1 search of the subreads with the NCBI nucleotide database, excluding the phage sequences (10). *De novo* assembly was performed using Falcon-integrate v2.1.4 (11). Default parameters were used for all software unless otherwise specified. After the whole genome was assembled, the gene functions were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (12).

The filtered data included a total of 1,283,479,478 bases, and the number of reads was 139,486. The mean subread and  $N_{50}$  read lengths were 9,201 bases and 14,093 bases,

**Citation** Jeon B-S, Kim H, Go YW, Kim H, Choi O, Sang B-I. 2021. Complete genome sequence of *Methanothermobacter* sp. strain THM-1, a thermophilic and hydrogenotrophic methanogen isolated from an anaerobic reactor in South Korea. *Microbiol Resour Annu* 10:e00587-21. <https://doi.org/10.1128/MRA.00587-21>.

**Editor** J. Cameron Thrash, University of Southern California

**Copyright** © 2021 Jeon 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 Okkyoung Choi, okgii77@hanyang.ac.kr, or Byoung-In Sang, biosang@hanyang.ac.kr.

**Received** 8 June 2021

**Accepted** 23 August 2021

**Published** 23 September 2021

respectively. The genome comprised a single chromosome (1,724,502 bp) with an average reference coverage of 533×. One contig was generated, and both ends of the contig overlapped, indicating that the genome is circular. The genes from the assembled genome were annotated, and a total of 1,808 genes were identified, of which 1,665 were predicted to contain coding sequences (CDSs); 39 were tRNAs, and 9 were rRNAs. The GC content was 48.59%. The orthologous average nucleotide index (OrthoANI) of THM-1 was 98.81% of the nucleotide sequence of *Methanothermobacter wolfeii* isolate SIV6 (13, 14). The OrthoANI was measured using OAT v0.93 (15). The shuttle-vector system for *Methanothermobacter* species was recently developed (16). The genetic resources for THMs obtained via genome sequencing could be applied to develop highly effective methanogenic archaea to utilize CO<sub>2</sub> and convert it into energy. This resource is expected to accelerate global efforts to reduce carbon dioxide.

**Data availability.** This whole-genome project (in GenBank format) and the corresponding raw data files (fastq format) have been deposited under the GenBank accession number [CP044013](#) and the SRA accession number [SRR10567007](#), respectively. The 16S rRNA gene was deposited under the GenBank accession number [MH605439](#).

## ACKNOWLEDGMENTS

This work was partially supported by a Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Ministry of Trade, Industry and Energy (MOTIE) (number 20202020800330) and by a Graduate School of Post-Plastic Specialization grant of the Korea Environmental Industry and Technology Institute, funded by the Ministry of Environment, Republic of Korea. This work was also supported in part by a grant funded by Hanyang University, Republic of Korea (HY-20110000000233-N).

We declare no financial or nonfinancial competing interests.

## REFERENCES

- Vo TTQ, Xia A, Wall DM, Murphy JD. 2017. Use of surplus wind electricity in Ireland to produce compressed renewable gaseous transport fuel through biological power to gas systems. *Renew Energy* 105:495–504. <https://doi.org/10.1016/j.renene.2016.12.084>.
- Song H, Choi O, Pandey A, Kim YG, Joo JS, Sang B-I. 2019. Simultaneous production of methane and acetate by thermophilic mixed culture from carbon dioxide in bioelectrochemical system. *Bioresour Technol* 281:474–479. <https://doi.org/10.1016/j.biortech.2019.02.115>.
- Angelidaki I, Sanders W. 2004. Assessment of the anaerobic biodegradability of macropollutants. *Rev Environ Sci Biotechnol* 3:117–129. <https://doi.org/10.1007/s11157-004-2502-3>.
- Choi O, Kim M, Go Y, Hong M-G, Kim B, Shin Y, Lee S, Kim YG, Joo JS, Jeon BS, Sang B-I. 2019. Selective removal of water generated during hydrogenotrophic methanation from culture medium using membrane distillation. *Energies* 12:4130. <https://doi.org/10.3390/en12214130>.
- Jeon BS, Kim H, Go YW, Kim YG, Joo JS, Choi O, Sang B-I. 2020. Complete genomic sequence of the thermophilic and hydrogenotrophic methanogen *Methanothermobacter* sp. strain KEPCO-1. *Microbiol Resour Announc* 9:e01034-19. <https://doi.org/10.1128/MRA.01034-19>.
- Stackebrandt E, Goebel BM. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* 44:846–849. <https://doi.org/10.1099/00207713-44-4-846>.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>.
- Göttig S, Frank D, Mungo E, Nolte A, Hogardt M, Besier S, Wichelhaus TA. 2019. Emergence of ceftazidime/avibactam resistance in KPC-3-producing *Klebsiella pneumoniae* in vivo. *J Antimicrob Chemother* 74:3211–3216. <https://doi.org/10.1093/jac/dkz330>.
- Rhoads A, Au KF. 2015. PacBio sequencing and its applications. *Genomics Proteomics Bioinformatics* 13:278–289. <https://doi.org/10.1016/j.gpb.2015.08.002>.
- Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* 10:421. <https://doi.org/10.1186/1471-2105-10-421>.
- Chin C-S, Peluso P, Sedlazeck FJ, Nattestad M, Concepcion GT, Clum A, Dunn C, O'Malley R, Figueroa-Balderas R, Morales-Cruz A, Cramer GR, Delledonne M, Luo C, Ecker JR, Cantu D, Rank DR, Schatz MC. 2016. Phased diploid genome assembly with single-molecule real-time sequencing. *Nat Methods* 13:1050–1054. <https://doi.org/10.1038/nmeth.4035>.
- Li W, O'Neill KR, Haft DH, DiCuccio M, Chetverin V, Badretin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwazd M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. 2021. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. *Nucleic Acids Res* 49:D1020–D1028. <https://doi.org/10.1093/nar/gkaa1105>.
- Kim M, Oh H-S, Park S-C, Chun J. 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 64:346–351. <https://doi.org/10.1099/ijs.0.059774-0>.
- Hassa J, Wibberg D, Maus I, Pühler A, Schlüter A. 2020. Genome analyses and genome-centered metatranscriptomics of *Methanothermobacter wolfeii* strain SIV6, isolated from a thermophilic production-scale biogas fermenter. *Microorganisms* 8:13. <https://doi.org/10.3390/microorganisms8010013>.
- Lee I, Kim YO, Park S-C, Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 66:1100–1103. <https://doi.org/10.1099/ijs.0.000760>.
- Fink C, Beblawy S, Enkerlin AM, Mühling L, Angenent LT, Molitor B. 2021. A shuttle-vector system allows heterologous gene expression in the thermophilic methanogen *Methanothermobacter thermautotrophicus* ΔH. bioRxiv <https://doi.org/10.1101/2021.04.20.440605>.