GENOME SEQUENCES





Draft Genomes of *Methanocalculus taiwanensis* P2F9704a^T and *Methanocalculus chunghsingensis* K1F9705b^T, Hydrogenotrophic Methanogens Belonging to the Family *Methanocalculaceae*

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ABSTRACT The family *Methanocalculaceae* comprises hydrogen- and formate-utilizing methanogens. Here, we report two additional draft genome sequences of *Methanocalculaceae*, those of *Methanocalculus taiwanensis* P2F9704a^T (equivalent to BCRC 16182^T and DSM 14663^T) and *Methanocalculus chunghsingensis* K1F9705b^T (equivalent to DSM 14646^T and OCM 772^T), which were selected for further species delineation and comparative genomic analyses.

The members of the genus *Methanocalculus* belong to the *Methanocalculaceae* family. *Methanocalculus* spp. use only formate and hydrogen as electron donors for methanogenesis and require acetate as a carbon source (1). To date (August 2022), the genus *Methanocalculus* comprises six species with validly published names: *Methanocalculus halotolerans* (2), *Methanocalculus pumilus* (3), *Methanocalculus taiwanensis* (4), *Methanocalculus chunghsingensis* (5), *Methanocalculus natronophilus* (6), and *Methanocalculus alkaliphilus* (7). *Methanocalculus* isolates inhabit a wide range of environments, such as an estuary, a marine water aquaculture fishpond, the leachate of a sea-based site for solid waste disposal, a saline oil reservoir, soda lakes, and anaerobic digesters (2–9). Here, we report two draft genomes of *M. taiwanensis* P2F9704a^T and *M. chunghsingensis* K1F9705b^T that provide further understanding about species delineation and microbial adaptation to various environments.

Strains P2F9704a^T and K1F9705b^T were isolated from an estuary of Erlin River and a marine water aquaculture fishpond near Wong-Gong, Taiwan, respectively (4, 5). Both strains were grown in anaerobic MB/W medium with 100 mM sodium formate and 5 mM sodium acetate and incubated at 37°C, according to methods described previously (10–12). Genomic DNA of each strain was isolated and purified using a modification of the methods of Johnson (13) and of Jarrell et al. (14). Briefly, cells of a 500-mL culture were collected and then lysed with sodium dodecyl sulfate (1% [wt/vol]). After phenol-chloroform extraction and isopropanol precipitation, the quantity and quality of the extracted DNA samples were checked by UV-visible spectrophotometry.

The genomes of both strains were sequenced at the Genomics BioSci & Tech Co., Ltd. (Taiwan) using the MiSeq platform (Illumina). For both strains, genomic DNA was sheared randomly, and a paired-end DNA library of 300 bp was constructed by using the TruSeq Nano DNA HT library prep kit and TruSeq DNA with 96 CD indexes (Illumina). The constructed library was sequenced using a MiSeq reagent kit v3 (600 cycles), and 6,537,788 and 4,735,720 reads were generated for strains P2F9704a^T and K1F9705b^T, respectively. All generated reads were quality trimmed to obtain high-quality reads using Trimmomatic

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Received 2 August 2022 Accepted 25 August 2022 Published 6 September 2022 v0.36 (15). For the genome *de novo* assembly, the genome assembler of Shovill v1.0.4 (16) was applied. The sequencing protocol generated ~425-fold and ~547-fold mean coverage for the genomes of strains P2F9704a^T and K1F9705b^T, respectively. Genes of the genome were identified using the Prokaryotic Genome Annotation pipeline (PGAP) of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) (17). For the draft genome of strain P2F9704a^T, a total of 69 contigs were obtained with an N_{50} value of 72,685 bp and a total size of 2,342,634 bp with a 50.41% GC content. The genome was predicted to harbor 2,348 genes. For strain K1F9705b^T, a total of 51 contigs were obtained with an N_{50} value of 84,108 bp and total size of 2,134,545 bp with a 52.22% GC content. The genome was predicted to harbor 2,253 genes. No plasmid was identified in either genome using PlasmidFinder (18). Default parameters were used for all software unless otherwise specified.

Data availability. These whole-genome shotgun projects for strains P2F9704a^T and K1F9705b^T have been deposited in GenBank under the accession numbers VOTZ0000000 and JWHL00000000, respectively. The versions for each genome described in this paper are the respective first versions. The BioProject accession numbers for strains P2F9704a^T and K1F9705b^T are PRJNA559048 and PRJNA224116, respectively. The raw sequence reads for strains P2F9704a^T and K1F9705b^T have been deposited in the Sequence Read Archive under accession numbers SRR18085627 and SRR20645508, respectively.

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REFERENCES

- Fardeau M-L, Cayol J-L, Ollivier B. 2019. Methanocalculus. *In* Whitman WB (ed), Bergey's manual of systematics of Archaea and Bacteria, 1st ed. John Wiley & Sons, Hoboken, NJ, USA.
- Ollivier B, Fardeau ML, Cayol JL, Magot M, Patel BK, Prensier G, Garcia JL. 1998. *Methanocalculus halotolerans* gen. nov., sp. nov., isolated from an oil-producing well. Int J Syst Evol Microbiol 48:821–828. https://doi.org/ 10.1099/00207713-48-3-821.
- Mori K, Yamamoto H, Kamagata Y, Hatsu M, Takamizawa K. 2000. Methanocalculus pumilus sp. nov., a heavy-metal-tolerant methanogen isolated from a waste-disposal site. Int J Syst Evol Microbiol 50:1723–1729. https:// doi.org/10.1099/00207713-50-5-1723.
- Lai M-C, Chen S-C, Shu C-M, Chiou M-S, Wang C-C, Chuang M-J, Hong T-Y, Liu C-C, Lai L-J, Hua JJ. 2002. *Methanocalculus taiwanensis* sp. nov., isolated from an estuarine environment. Int J Syst Evol Microbiol 52: 1799–1806. https://doi.org/10.1099/00207713-52-5-1799.
- Lai MC, Lin CC, Yu PH, Huang YF, Chen SC. 2004. *Methanocalculus chunghsingensis* sp. nov., isolated from an estuary and a marine fishpond in Taiwan. Int J Syst Evol Microbiol 54:183–189. https://doi.org/10.1099/ijs.0 .02761-0.
- Zhilina T, Zavarzina D, Kevbrin V, Kolganova T. 2013. Methanocalculus natronophilus sp. nov., a new alkaliphilic hydrogenotrophic methanogenic archaeon from a soda lake, and proposal of the new family Methanocalculaceae Microbiology 82:698–706. https://doi.org/10.1134/S0026261713060131.
- Sorokin DY, Abbas B, Merkel AY, Rijpstra WIC, Damsté JSS, Sukhacheva MV, van Loosdrecht MCM. 2015. *Methanosalsum natronophilum* sp. nov., and *Methanocalculus alkaliphilus* sp. nov., haloalkaliphilic methanogens

from hypersaline soda lakes. Int J Syst Evol Microbiol 65:3739–3745. https://doi.org/10.1099/ijsem.0.000488.

- Chen S, He Q. 2015. Enrichment and specific quantification of *Methano-calculus* in anaerobic digestion. J Biosci Bioeng 120:677–683. https://doi .org/10.1016/j.jbiosc.2015.04.007.
- Surakasi VP, Wani AA, Shouche YS, Ranade DR. 2007. Phylogenetic analysis of methanogenic enrichment cultures obtained from Lonar Lake in India: isolation of *Methanocalculus* sp. and *Methanoculleus* sp. Microb Ecol 54:697–704. https://doi.org/10.1007/s00248-007-9228-z.
- Weng C-Y, Chen S-C, Lai M-C, Wu S-Y, Lin S, Yang TF, Chen P-C. 2015. *Methanoculleus taiwanensis* sp. nov., a methanogen isolated from deep marine sediment at the deformation front area near Taiwan. Int J Syst Evol Microbiol 65:1044–1049. https://doi.org/10.1099/ijs.0.000062.
- Chen S-C, Chen M-F, Lai M-C, Weng C-Y, Wu S-Y, Lin S, Yang TF, Chen P-C. 2015. *Methanoculleus sediminis* sp. nov., a methanogen from sediments near a submarine mud volcano. Int J Syst Evol Microbiol 65:2141–2147. https://doi.org/10.1099/ijs.0.000233.
- 12. Wu SY, Chen SC, Lai MC. 2005. *Methanofollis formosanus* sp. nov., isolated from a fish pond. Int J Syst Evol Microbiol 55:837–842. https://doi.org/10 .1099/ijs.0.63475-0.
- Johnson JL. 1985. DNA reassociation and DNA hybridization of bacterial nucleic acids. Methods Microbiol 18:33–74. https://doi.org/10.1016/S0580 -9517(08)70471-9.
- Jarrell KF, Faguy D, Hebert AM, Kalmokoff ML. 1992. A general method of isolating high molecular weight DNA from methanogenic archaea (archaebacteria). Can J Microbiol 38:65–68. https://doi.org/10.1139/ m92-010.

- 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.
- 16. Seemann T. 2017. Shovill: faster SPAdes assembly of Illumina reads (v0.9.0). https://github.com/tseemann/shovill.
- 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.

 Carattoli A, Zankari E, Garcia-Fernandez A, Larsen MV, Lund O, Villa L, Aarestrup FM, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/ AAC.02412-14.