





## Draft Genome Sequence of Parageobacillus thermoglucosidasius Strain TG4, a Hydrogenogenic Carboxydotrophic Bacterium Isolated from a Marine Sediment

Masao Inoue,<sup>a</sup> Dayumi Tanimura,<sup>a</sup> Yusuke Ogami,<sup>a</sup> Taiki Hino,<sup>a</sup> Suguru Okunishi,<sup>b</sup> Hiroto Maeda,<sup>b</sup> Takashi Yoshida,<sup>a</sup> Yoshihiko Sako<sup>a</sup>

<sup>a</sup>Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan

ABSTRACT Parageobacillus thermoglucosidasius possesses biotechnological potential for fuel generation. Here, we report the draft genome sequence of P. thermoglucosidasius strain TG4, which was first isolated from a marine sediment. The genome sequence provides insight into the plasmid diversity and carbon monoxide-dependent hydrogen production capacity of P. thermoglucosidasius.

arageobacillus thermoglucosidasius is a Gram-positive thermophilic facultatively anaerobic spore-forming bacterium. This species has biotechnological potential for fuel generation from plant biomass through fermentation (1-3). Recently, P. thermoglucosidasius was also identified as a hydrogenogenic carboxydotroph, suggesting its further potential for energetics (4). The genome sequences of nine strains, including DSM 2542<sup>T</sup>, have already been published (5-9). P. thermoglucosidasius is present in various environments, such as soils, hot springs, and milk plants (3, 7-9). Here, we report the draft genome sequence of P. thermoglucosidasius strain TG4, first isolated from a marine sediment.

The sediment was collected from the Aira Caldera in Kagoshima Bay, Japan (31°38′9″N, 130°46′53″E, 83-m depth). Strain TG4 was enriched at 65°C under 100% CO gas in B medium (10) modified to contain 1.8% NaCl, 0.07% KCl, 0.03% NH<sub>4</sub>Cl, and 0.39% MgCl<sub>2</sub> · 6H<sub>2</sub>O. Single-colony isolation was performed aerobically using NBRC 802 agar medium. Cells were grown in B medium supplemented with 0.2% sodium pyruvate under 100% CO gas.

Genomic DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany). A DNA library was prepared using the Nextera mate pair library preparation kit (Illumina, San Diego, CA). Sequencing was performed on the Illumina MiSeq instrument with MiSeq reagent kit v.3 (600 cycles), which generated 6,822,150 paired-end reads. Quality trimming and adapter removal were performed using Trimmomatic v.0.3.6 (11). Mate pair reads were selected and junction adapters were trimmed using NxTrim v.0.4.1 (12). De novo genome assembly was performed with SPAdes v.3.13.0 (13) using the filtered 3,879,002 mate pair reads. The assembled scaffolds were quality controlled using the Burrows-Wheeler Aligner (BWA) v.0.7.17 (14), SAMtools v.0.1.19 (15), and NxRepair v.0.13 (16). Annotation was performed with the DFAST server v.1.0.2 (17). Genomic comparison was performed using the OrthoANIu tool (18) and BLASTn (19).

The draft genome was assembled into 12 scaffolds, including one circular plasmid, with an  $N_{50}$  value of 3,846,774 bp, an average coverage of 288.74 $\times$ , a total length of 3,948,523 bp, and an average G+C content of 43.34%. The numbers of predicted protein-coding genes, rRNAs, and tRNAs were 3,906, 25, and 90, respectively. The orthologous average nucleotide identity with DSM 2542<sup>T</sup> was 98.9%.

Citation Inoue M, Tanimura A, Ogami Y, Hino T, Okunishi S, Maeda H, Yoshida T, Sako Y. 2019. Draft genome sequence of Parageobacillus thermoglucosidasius strain TG4, a hydrogenogenic carboxydotrophic bacterium isolated from a marine sediment. Microbiol Resour Announc 8:e01666-18. https://doi.org/ 10.1128/MRA.01666-18.

Editor Frank J. Stewart, Georgia Institute of

Copyright © 2019 Inoue et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Yoshihiko Sako, sako@kais.kyoto-u.ac.jp.

Received 11 December 2018 Accepted 11 January 2019 Published 31 January 2019

<sup>&</sup>lt;sup>b</sup>Faculty of Fisheries, Kagoshima University, Kagoshima, Japan



A circular plasmid (1,586 bp) exhibited homology (76% query coverage with 85% identity) to plasmid pGTG5 (1,540 bp) from *Geobacillus* sp. strain 1121 (20). We also found two plasmid-like scaffolds (60,265 and 34,404 bp) that exhibited homology (30% and 99% query coverages with 97% and 99% identities, respectively) to plasmid pNCl001 (83,935 bp) from *P. thermoglucosidasius* NCIMB 11955 (5). Moreover, we identified a gene cluster of carbon monoxide dehydrogenase/hydrogen-evolving hydrogenase complex, including 15 genes exhibiting ≥99% amino acid identity with those of DSM 2542<sup>T</sup> (4), which would enable carbon monoxide-dependent hydrogen production.

**Data availability.** The draft genome sequences were deposited in DDBJ/ENA/ GenBank under the accession numbers BHZK01000001 to BHZK01000011 (for the scaffolds) and AP019364 (for the circular plasmid). The raw reads were deposited in SRA/DRA/ERA under the accession number DRA007789.

## **ACKNOWLEDGMENTS**

We thank Yuto Fukuyama and Tatsuki Oguro from Kyoto University and the crew of the *Nansei-Maru* vessel at Kagoshima University for providing technical assistance with the sampling of marine sediments. We also thank Shigeko Kimura and Kimiho Omae from Kyoto University for their technical assistance with DNA sequencing. Part of the computational analysis was performed at the Super Computer System, Institute for Chemical Research, Kyoto University.

This work was supported by Grant-in-Aid for Scientific Research 16H06381 (to Y.S.) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT).

M.I., T.Y., and Y.S. designed the work and drafted the paper. S.O. and H.M. conducted sampling of marine sediments. A.T. and Y.O. isolated the strain. M.I., Y.O., and T.H. performed genome sequencing and data analysis. All authors edited and approved the paper.

We declare no conflicts of interest with regard to the contents of this article.

## REFERENCES

- Zhou J, Wu K, Rao CV. 2016. Evolutionary engineering of *Geobacillus thermoglucosidasius* for improved ethanol production. Biotechnol Bioeng 113:2156–2167. https://doi.org/10.1002/bit.25983.
- Cripps RE, Eley K, Leak DJ, Rudd B, Taylor M, Todd M, Boakes S, Martin S, Atkinson T. 2009. Metabolic engineering of *Geobacillus thermoglucosi-dasius* for high yield ethanol production. Metab Eng 11:398–408. https://doi.org/10.1016/j.ymben.2009.08.005.
- Suzuki Y, Kishigami T, Inoue K, Mizoguchi Y, Eto N, Takagi M, Abe S. 1983. Bacillus thermoglucosidasius sp. nov., a new species of obligately thermophilic bacilli. Syst Appl Microbiol 4:487–495. https://doi.org/10.1016/S0723-2020(83)80006-X.
- Mohr T, Aliyu H, Küchlin R, Polliack S, Zwick M, Neumann A, Cowan D, de Maayer P. 2018. CO-dependent hydrogen production by the facultative anaerobe *Parageobacillus thermoglucosidasius*. Microb Cell Fact 17:108. https://doi.org/10.1186/s12934-018-0954-3.
- Sheng L, Zhang Y, Minton NP. 2016. Complete genome sequence of Geobacillus thermoglucosidasius NCIMB 11955, the progenitor of a bioethanol production strain. Genome Announc 4:e01065-16. https://doi .org/10.1128/genomeA.01065-16.
- Chen J, Zhang Z, Zhang C, Yu B. 2015. Genome sequence of *Geobacillus thermoglucosidasius* DSM2542, a platform hosts for biotechnological applications with industrial potential. J Biotechnol 216:98–99. https://doi.org/10.1016/j.ibiotec.2015.10.002.
- Zhao Y, Caspers MP, Abee T, Siezen RJ, Kort R. 2012. Complete genome sequence of *Geobacillus thermoglucosidans* TNO-09.020, a thermophilic sporeformer associated with a dairy-processing environment. J Bacteriol 194:4118. https://doi.org/10.1128/JB.00318-12.
- Brumm PJ, Land ML, Mead DA. 2015. Complete genome sequence of Geobacillus thermoglucosidasius C56-YS93, a novel biomass degrader isolated from obsidian hot spring in Yellowstone National Park. Stand Genomic Sci 10:73. https://doi.org/10.1186/s40793-015-0031-z.
- Brumm P, Land ML, Hauser LJ, Jeffries CD, Chang Y-J, Mead DA. 2015.
  Complete genome sequence of Geobacillus strain Y4. 1MC1, a novel

- CO-utilizing *Geobacillus thermoglucosidasius* strain isolated from bath hot spring in Yellowstone National Park. Bioenerg Res 8:1039–1045. https://doi.org/10.1007/s12155-015-9585-2.
- Yoneda Y, Yoshida T, Kawaichi S, Daifuku T, Takabe K, Sako Y. 2012. Carboxydothermus pertinax sp. nov., a thermophilic, hydrogenogenic, Fe(III)-reducing, sulfur-reducing carboxydotrophic bacterium from an acidic hot spring. Int J Syst Evol Microbiol 62:1692–1697. https://doi.org/ 10.1099/ijs.0.031583-0.
- 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.
- O'Connell J, Schulz-Trieglaff O, Carlson E, Hims MM, Gormley NA, Cox AJ. 2015. NxTrim: optimized trimming of Illumina mate pair reads. Bioinformatics 31:2035–2037. https://doi.org/10.1093/bioinformatics/ btv057.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski 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.
- Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 1303:3997 [q-bio.GN]. https://arxiv.org/ abs/1303.3997.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/ bioinformatics/btp352.
- Murphy RR, O'Connell J, Cox AJ, Schulz-Trieglaff O. 2015. NxRepair: error correction in *de novo* sequence assembly using Nextera mate pairs. PeerJ 3:e996. https://doi.org/10.7717/peerj.996.
- 17. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic

Volume 8 Issue 5 e01666-18 mra.asm.org 2



- genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. https://doi.org/10.1093/bioinformatics/btx713.
- Yoon SH, Ha SM, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286. https://doi.org/10.1007/s10482-017 -0844-4.
- 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.
- 20. Kananavičiūtė R, Butaitė E, Citavičius D. 2014. Characterization of two novel plasmids from *Geobacillus* sp. 610 and 1121 strains. Plasmid 71:23–31. https://doi.org/10.1016/j.plasmid.2013.10.002.

Volume 8 Issue 5 e01666-18 mra.asm.org **3**