

Draft Genome Sequence of *Thermoanaerobacterium saccharolyticum* Strain NTOU1, a Thermophilic Bacterium Isolated from Marine Shallow Hydrothermal Vents

Engkong Tan,^a Yusan Chen,^b Jung Kuan,^b Chiajui Lin,^b S. S. S. De S. Jagoda,^a Fupang Lin,^b Wenshyong Tzou,^b Shigeharu Kinoshita,^a Shugo Watabe,^c Shuichi Asakawa,^a Shiumei Liu^b

Laboratory of Aquatic Molecular Biology and Biotechnology, Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, the University of Tokyo, Tokyo, Japan^a; Institute of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan^b; School of Marine Biosciences, Kitasato University, Kanagawa, Japan^c

E.T., Y.C., J.K., and C.L. contributed equally to this work, and S.A. and S.L. contributed equally to this work.

Thermoanaerobacterium saccharolyticum strain NTOU1 has the ability to utilize several kinds of sugars in lignocellulosic biomass to produce ethanol more efficiently than other bacteria. Here, we report the draft genome sequence and annotation of this strain, which may provide insights into the possible genes and metabolic pathways related to ethanol production.

Received 2 September 2014 Accepted 8 September 2014 Published 9 October 2014

Citation Tan E, Chen Y, Kuan J, Lin C, Jagoda SSSDS, Lin F, Tzou W, Kinoshita S, Watabe S, Asakawa S, Liu S. 2014. Draft genome sequence of *Thermoanaerobacterium* saccharolyticum strain NTOU1, a thermophilic bacterium isolated from marine shallow hydrothermal vents. Genome Announc. 2(5):e01019-14. doi:10.1128/genomeA.01019-14. Copyright © 2014 Tan et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

 $Address\ correspondence\ to\ Shuichi\ Asakawa, asakawa@mail.ecc.u-tokyo.ac.jp,\ or\ Shiumei\ Liu,\ smliu@mail.ntou.edu.tw.$

s an alternative to fossil fuels, the biofuel-like ethanol pro-Aduced from bacteria has received great interest (1). Converting biomass to sugars is required but is an expensive step in biofuel production (2). One way to solve this problem is to use thermophilic bacteria to produce ethanol (3), as it works with a shorter fermentation time at higher temperatures. Bacteria belonging to the genus Thermoanaerobacterium have the ability to ferment both pentose and hexose components in hemicelluloses, and ethanol is generated as a by-product during fermentation at 70°C (4). Thermoanaerobacterium saccharolyticum strain NTOU1, a Gramnegative thermophilic bacterium, was isolated from shallow marine hydrothermal vents off Gueishandao Island in Taiwan. Strain NTOU1 is of particular interest, because it has been reported that among wild-type anaerobic thermophilic ethanogens, this strain had the highest ethanol yield rate (5). Here, we attempted to sequence, assemble, and annotate the draft genome sequence of NTOU1.

We performed whole-genome shotgun sequencing using the Ion Torrent PGM sequencer (Life Technologies). To achieve the best sequencing result, a 400-bp sequencing kit and 318 Chip (Life Technologies, Japan) were selected. *De novo* genome assembly was done by MIRA software version 3.9.18 (http://sourceforge.net /projects/mira-assembler/), and the assembled genome sequence was then annotated with Rapid Annotations using Subsystems Technology (RAST) 4.0 (6). Furthermore, rRNAs and tRNAs were predicted by RNAmmer 1.2 (7) and tRNAscan-SE 1.21 (8), respectively.

A total of 826,957,387 bp (2,694,361 reads) were sequenced and applied to MIRA. One hundred one contigs of >500 bp were assembled, and the total length is 2,833,212 bp, with an N_{50} of 59,076 bp. The average G+C content is 34.84%. The NTOU1 draft genome sequence showed approximately similar total lengths and G+C contents as the available genomes of the same species (*T. saccharolyticum* JW/SL-YS485, GenBank accession no. NC_017992; genome size, 2.88 Mb, G+C content, 35.1%). Annotation revealed that NTOU1 contains 3,101 protein-coding genes, 7 rRNAs, and 54 tRNAs. Furthermore, 21 protein-coding sequences related to xylose utilization, including xylose isomerase, xylulose kinase, and beta-xylosidase, 20 coding sequences related to L-arabinose utilization were also annotated by RAST. This draft genome may provide insight into the possible genes and metabolic pathways related to ethanol production. Also, a Pacific Biosciences RSII sequencing and assembly of this strain is ongoing, with the hope of achieving the complete genomic sequence of this strain.

Nucleotide sequence accession numbers. The accession numbers of this draft genome sequence in DDBJ/EMBL/GenBank are BBKT01000001 to BBKT01000101.

ACKNOWLEDGMENT

This work was partly supported by JST, CREST.

REFERENCES

- Farrell AE, Plevin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM. 2006. Ethanol can contribute to energy and environmental goals. Science 311:506–508. http://dx.doi.org/10.1126/science.1121416.
- Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD. 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315:804–807. http://dx.doi.org/10.1126/ science.1137016.
- Lynd LR, van Zyl WH, McBride JE, Laser M. 2005. Consolidated bioprocessing of cellulosic biomass: an update. Curr. Opin. Biotechnol. 16: 577–583. http://dx.doi.org/10.1016/j.copbio.2005.08.009.
- Shaw AJ, Podkaminer KK, Desai SG, Bardsley JS, Rogers SR, Thorne PG, Hogsett DA, Lynd LR. 2008. Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. Proc. Natl. Acad. Sci. U. S. A. 105:13769–13774. http://dx.doi.org/10.1073/pnas.0801266105.

- Tsai TL, Liu SM, Lee SC, Chen WJ, Chou SH, Hsu TC, Guo GL, Hwang WS, Wiegel J. 2011. Ethanol production efficiency of an anaerobic hemicellulolytic thermophilic bacterium, strain NTOU1, isolated from a marine shallow hydrothermal vent in Taiwan. Microbes Environ. 26:317–324. http://dx.doi.org/10.1264/jsme2.ME10202.
- 6. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O.

2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/1471-2164-9-75.

- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25: 955–964. http://dx.doi.org/10.1093/nar/25.5.0955.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100–3108. http://dx.doi.org/10.1093/nar/ gkm160.