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Draft Genome Sequence of the Hydrogen- and Ethanol-Producing Anaerobic Alkalithermophilic Bacterium *Caloramator celer*

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Caloramator celer **strain JW/YL-NZ35 is a Gram-positive thermophilic, alkalitolerant, and strictly anaerobic bacterium capable of producing hydrogen and ethanol under extreme conditions. The draft genome sequence presented here will provide valuable information to further explore the physiology of this species and its potential for biofuel production.**

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Caloramator celer strain JW/YL-NZ35, formerly known as *Thermobrachium celere* (equivalent to ATCC 700318 and DSM 8682) (1), is a Gram-positive thermophilic, alkalitolerant, and strictly anaerobic bacterium isolated from hot spring sediments (Ohinemutu, New Zealand), with an optimal growth temperature of 67°C, an optimal pH at 67°C of 8.2, and a doubling time reported to be as low as 10 min (2). During anaerobic fermentation, *C. celer* converts C_6 sugars to H_2, CO_2, acetate, ethanol, and formate as major metabolites. Previous studies have shown that *C. celer* is able to produce hydrogen at high yields both in a naturally occurring microbial community (3) and in pure culture (4, 5). In addition, it can produce a significant amount of ethanol depending on the growth conditions. Recently, other members of the genus *Caloramator* were investigated for their biotechnological potential (6, 7), but only the genome of one species (*Caloramator australicus* RC3T) has been revealed (8). In order to evaluate the metabolism and the potential for biofuel production of the species *C. celer* and to expand the knowledge of the genus *Caloramator*, a draft genome sequence of strain *C. celer* JW/YL-NZ35 is presented.

The genome of *C. celer* JW/YL-NZ35 was sequenced with Illumina HiSeq 2000 to get paired-end reads from short (~250 bp) and long (~3 kbp) fragment libraries, as well as with 454 sequencing to get longer single-end reads. Assembly of the genome was performed with MIRA (9) followed by contig extension and scaffolding with SSPACE (10). The genome was annotated using the RAST server (11) and, when needed, manual assessment and curation of the automatic annotation were performed by BLAST analysis (12). The improved high-quality draft (13) genome assembly has a total size of 2,644,756 bp, organized in 56 scaffolds (>1 kb) (consisting of 162 contigs with an N_{50} of 128,968 bp), the longest being 1,976,539 bp. The G+C content of genome is 31.3%. On the basis of the annotation, the genome contains 2,381 protein-coding sequences (CDSs), including 151 RNAs.

Further genome analysis provides insights into the metabolic pathways leading to H_2 and ethanol synthesis. Three operons coding for putative enzymes involved in the regeneration of NAD^+ and oxidized ferredoxin through proton reduction with consequent H_2 synthesis were identified: two putative heterotetrameric NADH-dependent [FeFe]-hydrogenases whose subunits have, respectively, from 47 to 67% and from 49 to 77% identity to genes TTE0890 to TTE0894 of *Thermoanaerobacter tengcongensis* (14), and one putative multimeric ferredoxin-dependent membrane-bound [NiFe]-hydrogenase whose subunits show from 30 to 54% identity to genes PF1423 to PF1436 of *Pyrococcus furiosus* (15). Alternatively, regeneration of NAD^+ can be carried out by the conversion of acetyl-coenzyme A (CoA) to ethanol by two putative alcohol dehydrogenases. The *C. celer* draft genome sequence will allow a more systematic investigation of the potential of this organism for bioenergy applications and will expand the knowledge of the physiology of this genus.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. CAVN000000000. The version described in this paper is the first version, accession no. CAVN010000000.

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REFERENCES

1. **Baena S, Patel BK.** 2009. Genus V. *Caloramator* Collins, Lawson, Willems, Cordoba, Fernández-Garayzábal, Garcia, Cai, Hippe and Farrow 1994, 812^{VP} emend. Chrisostomos, Patel, Dwivedi and Denman 1996, 497, p 834–838. *In* De Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (ed), Bergey's manual of systematic bacteriology, 2nd ed, vol 3. The Firmicutes. Springer-Verlag, New York, NY.
2. **Engle M, Li Y, Rainey F, DeBlois S, Mai V, Reichert A, Mayer F,**

Messner P, Wiegel J. 1996. *Thermobrachium celere* gen. nov., sp. nov., a rapidly growing thermophilic, alkalitolerant, and proteolytic obligate anaerobe. Int. J. Syst. Bacteriol. **46:**1025–1033.

3. **Koskinen PE, Lay CH, Puhakka JA, Lin PJ, Wu SY, Orlygsson J, Lin CY.** 2008. High-efficiency hydrogen production by an anaerobic, thermophilic enrichment culture from an Icelandic hot spring. Biotechnol. Bioeng. **101:**665–678.
4. **Ciranna A, Santala V, Karp M.** 2011. Biohydrogen production in alkalithermophilic conditions: *Thermobrachium celere* as a case study. Bioresour. Technol. **102:**8714–8722.
5. **Ciranna A, Santala V, Karp M.** 2012. Enhancing biohydrogen production of the alkalithermophile *Thermobrachium celere*. Int. J. Hydrogen Energ. **37:**5550–5558.
6. **Crespo CF, Badshah M, Alvarez MT, Mattiasson B.** 2012. Ethanol production by continuous fermentation of D-(+)-cellobiose, D-(+)-xylose and sugarcane bagasse hydrolysate using the thermoanaerobe *Caloramator boliviensis*. Bioresour. Technol. **103:**186–191.
7. **Fu Q, Kobayashi H, Kawaguchi H, Vilcaez J, Wakayama T, Maeda H, Sato K.** 2013. Electrochemical and phylogenetic analyses of current-generating microorganisms in a thermophilic microbial fuel cell. J. Biosci. Bioeng. **115:**268–271.
8. **Ogg CD, Patel BK.** 2011. Draft genome sequence of *Caloramator australicus* strain RC3T, a thermoanaerobe from the great artesian basin of Australia. J. Bacteriol. **193:**2664–2665.
9. **Chevreux B, Wetter T, Suhai S.** 1999. Genomic sequence assembly using trace signals and additional sequence information, p 45–56. Computer science and biology: proceedings of the German conference on bioinformatics (GCB) 99. German Conference on Bioinformatics, Hannover, Germany.
10. **Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W.** 2011. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics **27:**578–579.
11. **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. doi:10.1186/1471-2164-9-75.
12. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
13. **Chain PSG, Grafham DV, Fulton RS, FitzGerald MG, Hostetler J, Muzny D, Ali J, Birren B, Bruce DC, Buhay C, Cole JR, Ding Y, Dugan S, Field D, Garrity GM, Gibbs R, Graves T, Han S, Harrison SH, Highlander S, Hugenholtz P, Khouri HM, Kodira CD, Kolker E, Kyrpides NC, Lang D, Lapidus A, Malfatti SA, Markowitz V, Metha T, Nelson KE, Parkhill J, Pitluck S, Qin X, Read TD, Schmutz J, Sozhamannan S, Sterk P, Strausberg RL, Sutton G, Thomson NR, Tiedje JM, Weinstock G, Wollam A, Genomic Standards Consortium Human Microbiome Project Jumpstart Consortium, Detter JC.** 2009. Genome project standards in a new era of sequencing. Science **326:** 236–237.
14. **Soboh B, Linder D, Hedderich R.** 2004. A multisubunit membrane-bound [NiFe]-hydrogenase and an NADH-dependent Fe-only hydrogenase in the fermenting bacterium *Thermoanaerobacter tengcongensis*. Microbiology **150:**2451–2463.
15. **Sapra R, Verhagen MF, Adams MW.** 2000. Purification and characterization of a membrane-bound hydrogenase from the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Bacteriol. **182:**3423–3428.