

Draft Genome Sequence of the Gram-Positive Thermophilic Iron Reducer *Thermincola ferriacetica* Strain Z-0001^T

Bradley G. Lusk,^a Jonathan P. Badalamenti,^b Prathap Parameswaran,^a Daniel R. Bond,^b Cesar I. Torres^{a,c}

Swette Center for Environmental Biotechnology, The Biodesign Institute at Arizona State University, Tempe, Arizona, USA^a; Department of Microbiology and BioTechnology Institute, University of Minnesota-Twin Cities, Saint Paul, Minnesota, USA^b; School for Engineering of Matter, Transport and Energy, Arizona State University, Tempe, Arizona, USA^c

A 3.19-Mbp draft genome of the Gram-positive thermophilic iron-reducing *Firmicutes* isolate from the *Peptococcaceae* family, *Thermincola ferriacetica* Z-0001, was assembled at ~100× coverage from 100-bp paired-end Illumina reads. The draft genome contains 3,274 predicted genes (3,187 protein coding genes) and putative multiheme *c*-type cytochromes.

Received 10 August 2015 Accepted 13 August 2015 Published 24 September 2015

Citation Lusk BG, Badalamenti JP, Parameswaran P, Bond DR, Torres CI. 2015. Draft genome sequence of the Gram-positive thermophilic iron reducer *Thermincola ferriacetica* strain Z-0001^T. *Genome Announc* 3(5):e01072-15. doi:10.1128/genomeA.01072-15.

Copyright © 2015 Lusk et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Cesar I. Torres, cit@asu.edu, or Bradley G. Lusk, bradley.lusk@asu.edu.

Thermincola ferriacetica strain Z-0001 (DSM 14005), first isolated from a terrestrial hydrothermal spring on Kunashir Island (Kurils) (1), is a Gram-positive, thermophilic (45°C to 70°C), spore-forming bacterium that is capable of dissimilatory metal reduction and anode respiration in a microbial electrochemical cell (MXC) (2–4) and is one of only a limited number of sequenced Gram-positive thermophilic bacteria that has been documented to perform extracellular electron transfer (EET) to insoluble metal substrates (5–7). Strain Z-0001 is capable of organotrophic growth with acetate and other organic compounds while reducing extracellular electron acceptors, including amorphous Fe(III)-hydroxide, magnetite, Mn(IV), anthraquinone-2,6-disulfonate (AQDS), and anodes in MXCs (1, 2, 4). Strain Z-0001 is also capable of chemolithoautotrophic growth, using molecular hydrogen as the electron donor and Fe(III) as the electron acceptor (1). In addition, strain Z-0001 produces H₂ and CO₂ while using CO as its electron donor and acquiring its carbon from acetate (1).

Among Gram-positive bacteria, little is known regarding the mechanism for EET or how the peptidoglycan layer impacts this pathway (8–10). Direct contact-dependent electron transfer has been suggested in *Thermincola potens* JR (11) with genetic evidence for the presence of *c*-type cytochromes (12), proteins which are responsible for EET in other metal-reducing bacteria (13). In contrast to *T. potens*, *T. ferriacetica* strain Z-0001 has been suggested to transfer electrons long range via an extracellular matrix (4), suggesting it may encode additional electron transfer capabilities. *Thermincola ferriacetica* has been reported to produce current densities up to 10 A·m⁻² despite having only half the cytochrome repertoire of *Geobacter sulfurreducens* (4, 14). Further genetic comparison of these strains could help elucidate the EET mechanism(s) of strain Z-0001.

The draft assembly presented here is from an axenic culture of electrode-grown *T. ferriacetica* strain Z-0001 cells in order to eliminate contamination by iron or anthraquinone 2,6,-disulfonate (AQDS). gDNA was collected and sequenced on an Illumina

HiSeq 2000 lane, yielding >45 million 2- × 100- bp reads. Raw reads were trimmed (sliding window 3 until quality >28) and down-sampled to provide 100× coverage for assembly using the a5 pipeline (26 Mart 2013 release [15]). The 3,196,047-bp draft genome assembly yielded 53 contigs >500 bp with an *N*₅₀ of 112112 bp, an *L*₅₀ of 8, and a G+C content of 45.69%.

The draft assembly was annotated using the JGI IMG/ER pipeline, yielding 51 tRNAs, 3,274 predicted genes (3,187 predicted protein coding genes), and 35 *c*-type cytochromes with three or more heme (CXXCH)-binding motifs. BLASTN sequence analysis of its 16S rRNA gene revealed 99.9% (1,436/1,438 nt) identity with *T. potens* JR and 99.7% (1,399/1,403 nt) identity with *Thermincola carboxydophila* (5, 16). *T. ferriacetica* contains two multiheme *c*-type cytochromes and 429 genes that are not present in *T. potens*. However, based on an average nucleotide identity (ANI) of 98.3% between their genomes, these two organisms may be members of the same species (17).

Nucleotide sequence accession numbers. This whole-genome shotgun project for *T. ferriacetica* strain Z-0001 has been deposited at DDBJ/EMBL/GenBank under the accession number [LGTE00000000](https://www.ncbi.nlm.nih.gov/nuclink/LGTE00000000). The version described in this paper is version [LGTE01000000](https://www.ncbi.nlm.nih.gov/nuclink/LGTE01000000). The raw and adaptor trimmed Illumina reads were submitted to SRA under accession number [SRX1100231](https://www.ncbi.nlm.nih.gov/nuclink/SRX1100231).

ACKNOWLEDGMENTS

B.G.L., P.P., and C.I.T. were supported by Office of Naval Research grant no. N000141210344. Additional support and Illumina sequencing were provided by the Swette Center for Environmental Biotechnology at Arizona State University.

REFERENCES

- Zavarzina DG, Sokolova TG, Tourova TP, Chernyh NA, Kostrikin NA, Bonch-Osmolovskaya EA. 2007. *Thermincola ferriacetica* sp. nov., a new anaerobic, thermophilic, facultatively chemolithoautotrophic bacterium capable of dissimilatory Fe(III) reduction. *Extremophiles* 11:1–7. [http://dx.doi.org/10.1007/s00792-006-0004-7](https://doi.org/10.1007/s00792-006-0004-7).
- Marshall CW, May HD. 2009. Electrochemical evidence of direct elec-

- trode reduction by a thermophilic Gram-positive bacterium, *Thermincola ferriacetica*. Energy Environ Sci 2:699–705. <http://dx.doi.org/10.1039/b823237g>.
3. Mathis BJ, Marshall CW, Milliken CE, Makkar RS, Creager SE, May HD. 2008. Electricity generation by thermophilic microorganisms from marine sediment. Appl Microbiol Biotechnol 78:147–155. <http://dx.doi.org/10.1007/s00253-007-1266-4>.
 4. Parameswaran P, Bry T, Popat SC, Lusk BG, Rittmann BE, Torres CI. 2013. Kinetic, electrochemical, and microscopic characterization of the thermophilic, anode-respiring bacterium *Thermincola ferriacetica*. Environ Sci Technol 47:4934–4940. <http://dx.doi.org/10.1021/es400321c>.
 5. Byrne-Bailey KG, Wrighton KC, Melnyk RA, Agbo P, Hazen TC, Coates JD. 2010. Complete genome sequence of the electricity-producing “*Thermincola potens*” strain JR. J Bacteriol 192:4078–4079. <http://dx.doi.org/10.1128/JB.00044-10>.
 6. Roh Y, Liu SV, Li G, Huang H, Phelps TJ, Zhou J. 2002. Isolation and characterization of metal-reducing thermoanaerobacter strains from deep subsurface environments of the Piceance Basin, Colorado. Appl Environ Microbiol 68:6013–6020. <http://dx.doi.org/10.1128/AEM.68.12.6013-6020.2002>.
 7. Wrighton KC, Agbo P, Warnecke F, Weber KA, Brodie EL, DeSantis TZ, Hugenholtz P, Andersen GL, Coates JD. 2008. A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. ISME J 2:1146–1156. <http://dx.doi.org/10.1038/ismej.2008.48>.
 8. Beveridge TJ, Forsberg CW, Doyle RJ. 1982. Major sites of metal binding in *Bacillus licheniformis* walls. J Bacteriol 150:1438–1448.
 9. Beveridge TJ, Murray RG. 1980. Sites of metal deposition in the cell wall of *Bacillus subtilis*. J Bacteriol 141:876–887.
 10. Ehrlich HL. 2008. Are gram-positive bacteria capable of electron transfer across their cell wall without an externally available electron Shuttle? Geobiology 6:220–224. <http://dx.doi.org/10.1111/j.1472-4669.2007.00135.x>.
 11. Wrighton KC, Thrash JC, Melnyk RA, Bigi JP, Byrne-Bailey KG, Remis JP, Schichnes D, Auer M, Chang CJ, Coates JD. 2011. Evidence for direct electron transfer by a Gram-positive bacterium isolated from a microbial fuel cell. Appl Environ Microbiol 77:7633–7639. <http://dx.doi.org/10.1128/AEM.05365-11>.
 12. Carlson HK, Iavarone AT, Gorur A, Yeo BS, Tran R, Melnyk RA, Mathies RA, Auer M, Coates JD. 2012. Surface multiheme c-type cytochromes from *Thermincola potens* and implications for respiratory metal reduction by Gram-positive bacteria. Proc Natl Acad Sci USA 109:1702–1707. <http://dx.doi.org/10.1073/pnas.1112905109>.
 13. Leang C, Coppi MV, Lovley DR. 2003. OmcB, a c-Type PolyHeme cytochrome, involved in Fe(III) reduction in *Geobacter sulfurreducens*. J Bacteriol 185:2096–2103. <http://dx.doi.org/10.1128/JB.185.7.2096-2103.2003>.
 14. Methé BA, Nelson KE, Eisen JA, Paulsen IT, Nelson W, Heidelberg JF, Wu D, Wu M, Ward N, Beanan MJ, Dodson RJ, Madupu R, Brinkac LM, Daugherty SC, DeBoy RT, Durkin AS, Gwinn M, Kolonay JF, Sullivan SA, Haft DH, Selengut J, Davidsen TM, Zafar N, White O, Tran B, Romero C, Forberger HA, Weidman J, Khouri H, Feldblyum TV, Utterback TR, Van Aken SE, Lovley DR, Fraser CM. 2003. Genome of *Geobacter sulfurreducens*: metal reduction in subsurface environments. Science 302:1967–1969. <http://dx.doi.org/10.1126/science.1088727>.
 15. Tritt A, Eisen JA, Facciotti MT, Darling AE. 2012. An integrated pipeline for de novo assembly of microbial genomes. PLOS ONE 7:e42304. <http://dx.doi.org/10.1371/journal.pone.0042304>.
 16. Sokolova TG, Kostrikina NA, Chernyh NA, Kolganova TV, Tourova TP, Bonch-Osmolovskaya EA. 2005. *Thermincola carboxydiphila* gen. nov., sp. nov., a novel anaerobic, carboxydophilic, hydrogenogenic bacterium from a hot spring of the Lake Baikal area. Int J Syst Evol Microbiol 55:2069–2073. <http://dx.doi.org/10.1099/ijs.0.63299-0>.
 17. Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126–19131. <http://dx.doi.org/10.1073/pnas.0906412106>.