



Draft Genome Sequence of *Anoxybacillus ayderensis* Strain MT-Cab (*Firmicutes*)

Vera Thiel,^{a,b} Marcus Tank,^{a,b} Lynn P. Tomsho,^a Richard Burhans,^a Scott E. Gay^a
Trinity L. Hamilton,^{c,d} Stephan C. Schuster,^{a,e}  Donald A. Bryant^{a,e,f}

Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania, USA^a; Department of Biological Sciences, Tokyo Metropolitan University, Hachioji, Tokyo, Japan^b; Department of Geosciences, Penn State Astrobiology Research Center (PSARC), The Pennsylvania State University, University Park, Pennsylvania, USA^c; Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, USA^d; Singapore Center for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore^e; Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana, USA^f

ABSTRACT The draft genome of the Gram-positive spore-forming *Anoxybacillus ayderensis* strain MT-Cab (*Firmicutes*), isolated from an enrichment culture of *Chloracidobacterium thermophilum*, was sequenced and comprises 2,577,015 bp in 92 contigs. The draft genome is predicted to consist of 2,699 protein-coding genes, 73 tRNA-coding genes, and an estimated 8 rRNA operons.

Anoxybacillus ayderensis strain MT-Cab is a thermophilic heterotrophic spore-forming member of the phylum *Firmicutes*. The strain was obtained from the original enrichment culture of *Chloracidobacterium thermophilum* isolated from a phototrophic microbial mat in an effluent channel of Octopus Spring, an alkaline siliceous hot spring in the Lower Geyser Basin of Yellowstone National Park, WY (1–3).

Strain MT-Cab was isolated in axenic culture, and its genome was sequenced to understand possible interactions among *C. thermophilum* and its chemoheterotrophic partners. *A. ayderensis* strain MT-Cab was grown in liquid LB medium; genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) 2012 protocol of the DOE Joint Genome Institute (<http://jgi.doe.gov/user-program-info/pmo-overview/protocols-sample-preparation-information/>) and sequenced on an Illumina MiSeq instrument. The draft genome was assembled with Newbler (version 2.9; Roche) from 2,347,496 reads that had an average length of 301 bp. Following assembly, contigs with $\geq 150\times$ coverage were maintained for genome analysis. The resulting 92 contigs comprised 2,577,015 bp, with an average G+C content of 42%.

The genome sequences of strain MT-Cab are 97.6% identical by average nucleotide identity (ANI) to the type genome of *Anoxybacillus ayderensis* (4), with 89.5% coverage of the genome. Annotation using RAST (5) predicted 2,699 protein-coding genes and 73 tRNA genes. Assembly of ribosomal rRNA genes was incomplete because of the presence of multiple copies. Based on coverage, 8 rRNA operons are predicted, which corresponds to the same number found in the *Anoxybacillus flavithermus* MK1 genome (6). Phyla-AMPHORA (7) identified all 168 *Firmicutes*-specific phylogenetic marker genes. Based on gene content, strain MT-Cab is predicted to be a spore-forming facultatively anaerobic chemoorganoheterotroph. Genes encoding enzymes for glycolysis, the tri-carboxylic acid cycle, and the oxidative pentose phosphate pathway are complete. The genome lacks genes for nitrate and nitrogen reduction but has transporters for ammonia and several amino acids. Genes encoding branched-chain amino acid biosynthesis, as well as assimilatory sulfate reduction, are present in strain MT-Cab but missing in the *C. thermophilum* genome (8), indicating a possible cross-feeding in the mixed enrichment culture.

Received 27 April 2017 Accepted 9 May 2017 Published 29 June 2017

Citation Thiel V, Tank M, Tomsho LP, Burhans R, Gay SE, Hamilton TL, Schuster SC, Bryant DA. 2017. Draft genome sequence of *Anoxybacillus ayderensis* strain MT-Cab (*Firmicutes*). *Genome Announc* 5:e00547-17. <https://doi.org/10.1128/genomeA.00547-17>.

Copyright © 2017 Thiel et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Donald A. Bryant, dab14@psu.edu.

A complete respiratory electron transport chain is encoded in the genome, including an NADH dehydrogenase containing 11 subunits, succinate dehydrogenase, a menaquinol-cytochrome *c* reductase, 3 quinol oxidases (cytochrome *bd* and 2 *aa*₃ type), and 2 cytochrome *c* oxidases [cytochrome *bb*₃ quinol and *b(o/a)*₃ types]. Genes encoding enzymes involved in mixed acid as well as lactate and butyrate, fermentation could enable anaerobic growth. The genome encodes a complete pathway for menaquinone synthesis. The presence of the sporulation-specific master regulator gene *spo0A* and RNA polymerase sigma factors *sigE*, *sigF*, *sigG*, and *sigK* indicates the potential for sporulation, which has been observed in culture. Similar to other *Anoxybacillus* and *Bacillus* species, strain MT-Cab is pale yellow in color due to the presence of carotenoids, putatively glycosyl-apo-8-lycopene, as identified in previous studies (9) and suggested by high-performance liquid chromatography (HPLC) analysis. The *crtBEIMN* and *Nb* genes for carotenoid biosynthesis, as well as a gene encoding a glycosyltransferase, were identified in the genome. Precursors for the synthesis of carotenoids and other isoprenoid compounds are produced by the 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DOXP) pathway.

Accession number(s). The draft genome sequence of *Anoxybacillus ayderensis* strain MT-Cab has been deposited at DDBJ/EMBL/GenBank as a whole-genome shotgun project under the accession number [NDEZ00000000](https://ncbi.nlm.nih.gov/nucl/NDEZ00000000).

ACKNOWLEDGMENTS

This sample was collected under Yellowstone National Park collection permit no. YELL-SCI-0129.

The collection of mat material was funded by the National Science Foundation (grant EF-0805385). This research was supported by the Genomic Science Program (GSP), Office of Biological and Environmental Research (OBER), U.S. Department of Energy (DOE), and is a contribution of the Pacific Northwest National Laboratory (PNNL) Foundational Scientific Focus Area. This work was also funded by grant DE-FG02-94ER20137 (to D.A.B.) from the Photosynthetic Systems Program, Division of Chemical Sciences, Geosciences, and Biosciences (CSGB), Office of Basic Energy Sciences of the U.S. Department of Energy.

REFERENCES

- Bryant DA, Costas AMG, Maresca JA, Chew AGM, Klatt CG, Bateson MM, Tallon LJ, Hostetler J, Nelson WC, Heidelberg JF, Ward DM. 2007. *Candidatus Chloracidobacterium thermophilum*: an aerobic phototrophic *Acidobacterium*. *Science* 317:523–526. <https://doi.org/10.1126/science.1143236>.
- Tank M, Bryant DA. 2015. *Chloracidobacterium thermophilum* gen. nov., sp. nov.: an anoxygenic microaerophilic chlorophotoheterotrophic acidobacterium. *Int J Syst Evol Microbiol* 65:1426–1430. <https://doi.org/10.1099/ijs.0.000113>.
- Tank M, Bryant DA. 2015. Nutrient requirements and growth physiology of the photoheterotrophic acidobacterium, *Chloracidobacterium thermophilum*. *Front Microbiol* 6:226. <https://doi.org/10.3389/fmicb.2015.00226>.
- Belduz AO, Canakci S, Chan KG, Kahar UM, Chan CS, Yaakop AS, Goh KM. 2015. Genome sequence of *Anoxybacillus ayderensis* AB04^T isolated from the Ayder hot spring in Turkey. *Stand Genomic Sci* 10:70. <https://doi.org/10.1186/s40793-015-0065-2>.
- Overbeek RA, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
- Saw JH, Mountain BW, Feng L, Omelchenko MV, Hou S, Saito JA, Stott MB, Li D, Zhao G, Wu J, Galperin MY, Koonin EV, Makarova KS, Wolf YI, Rigden DJ, Dunfield PF, Wang L, Alam M. 2008. Encapsulated *in silico*: genome, proteome and physiology of the thermophilic bacterium *Anoxybacillus flavithermus* WK1. *Genome Biol* 9:R161. <https://doi.org/10.1186/gb-2008-9-11-r161>.
- Wang Z, Wu M. 2013. A phylum-level bacterial phylogenetic marker database. *Mol Biol Evol* 30:1258–1262. <https://doi.org/10.1093/molbev/mst059>.
- Garcia Costas AM, Liu Z, Tomsho LP, Schuster SC, Ward DM, Bryant DA. 2012. Complete genome of “*Candidatus Chloracidobacterium thermophilum*,” a chlorophyll-based photoheterotroph belonging to the phylum *Acidobacteria*. *Environ Microbiol* 14:177–190. <https://doi.org/10.1111/j.1462-2920.2011.02592.x>.
- Perez-Fons L, Steiger S, Khaneja R, Bramley PM, Cutting SM, Sandmann G, Fraser PD. 2011. Identification and the developmental formation of carotenoid pigments in the yellow/orange *Bacillus* spore-formers. *Biochim Biophys Acta* 1811:177–185. <https://doi.org/10.1016/j.bbali.2010.12.009>.