

Genome Sequence of *Thermoanaerobaculum aquaticum* MP-01^T, the First Cultivated Member of *Acidobacteria* Subdivision 23, Isolated from a Hot Spring

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Thermoanaerobaculum aquaticum MP-01^T is currently the only cultivated and described member of Acidobacteria subdivision 23. Here, we report the genome sequence for this novel microorganism that was isolated from a hot spring.

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hermoanaerobaculum is the first genus to be identified within Acidobacteria subdivision 23. Environmental sequences from members of the phylum Acidobacteria are found throughout a broad diversity of ecosystems, including soils, caves, hot springs, and deep sea hydrothermal vents (1-4). Based on 16S rRNA gene sequence divergence, the phylum *Acidobacteria* is predicted to be as diverse as the much better studied *Proteobacteria* (5). Among the 26 proposed subdivisions of Acidobacteria (1), only 6 (subdivisions 1, 3, 4, 8, 10, and 23) are represented by at least one cultivated member that has been described in detail. Currently, only 17 genome sequences are publicly available. Given the scarcity of data for the diverse members of the phylum Acidobacteria, it was imperative to sequence the genome of Thermoanaerobaculum aquaticum MP-01^T, currently the only member of subdivision 23. T. aquaticum MP-01^T is a nonmotile, Gram-negative, rod-shaped bacterium. It is a strictly anaerobic chemoorganotroph isolated from Hale House Spring in Hot Springs National Park, Arkansas. The spring is covered, lacking any direct terrestrial input of carbon. The waters are oligotrophic, with the primary source of carbon input being carbonate (6). MP-01^T was found to be capable of reducing Fe(III) or Mn(IV) and was able to grow fermentatively on proteinaceous compounds (7).

Genomic DNA was isolated from MP-01^T cells using the phenol-chloroform method, as described previously (8). A draft genome sequence was determined from a 350-bp insert library prepared with the Illumina TruSeq LT kit and sequenced using the Illumina MiSeq instrument for 2 \times 150 bp paired-end sequencing. Raw reads were trimmed within CLC Genomics Workbench 7.0 (CLC bio, Cambridge, MA) to remove adapters, bases below Q30 (Phred 33), and 10 bp from each end. The trimmed sequences (>100 bp) were assembled and scaffolded *de novo* within CLC, retaining scaffolds of >800 bp. The assembly produced 68 scaffolds, for a final assembly size of 2.66 Mbp. The N_{50} of the genome is 115.9 kbp, and the largest scaffold is 280.1 kbp.

The NCBI Prokaryotic Genome Annotation Pipeline was used for gene annotation (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The genome contains 2,253 coding regions and 49 RNAs. Interestingly, while no autotrophic growth was ob-

served in isolated cultures of MP-01^T, genes required to fix carbon through reductive carboxylation are present, indicating that the potential may exist. During fermentation, MP-01^T was observed to produce hydrogen, butyrate, and lactate (7), which was confirmed by the presence of genes responsible for the metabolism of glucose, fructose, and pyruvate. No evidence for the ability to reduce sulfate or nitrate was found. Given the low relative abundance of MP-01^T observed in previous studies at Hale Springs (9), it is possible that MP-01^T subsists off the metabolic end products of other more dominant members of the community.

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited in GenBank under the accession no. JMFG00000000.

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REFERENCES

- 1. Barns SM, Cain EC, Sommerville L, Kuske CR. 2007. *Acidobacteria* phylum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum. Appl. Environ. Microbiol. 73:3113–3116. http://dx.doi.org/10.1128/AEM.02012-06.
- Barns SM, Takala SL, Kuske CR. 1999. Wide distribution and diversity of members of the bacterial kingdom *Acidobacterium* in the environment. Appl. Environ. Microbiol. 65:1731–1737.
- 3. Zimmermann J, Gonzalez JM, Saiz-Jimenez C, Ludwig W. 2005. Detection and phylogenetic relationships of highly diverse uncultured acidobacterial communities in Altamira cave using 23S rRNA sequence analyses. Geomicrobiol. J. 22:379–388. http://dx.doi.org/10.1080/01490450500248986.
- 4. Izumi H, Nunoura T, Miyazaki M, Mino S, Toki T, Takai K, Sako Y, Sawabe T, Nakagawa S. 2012. Thermotomaculum hydrothermale gen. nov., sp. nov., a novel heterotrophic thermophile within the phylum Acidobacteria from a deep-sea hydrothermal vent chimney in the southern Okinawa trough. Extremophiles 16:245–253. http://dx.doi.org/10.1007/s00792-011-0425-9.
- Janssen PH. 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Appl. Environ. Microbiol. 72: 1719–1728. http://dx.doi.org/10.1128/AEM.72.3.1719-1728.2006.
- Bell RW, Hays PD. 2007. Influence of locally derived recharge on the water quality and temperature of springs in Hot Springs National Park, Arkansas.

- Scientific investigations report 2007-5004. U.S. Geological Survey, Reston, VA. http://pubs.usgs.gov/sir/2007/5004/pdf/SIR2007-5004.pdf.
- 7. Losey NA, Stevenson BS, Busse HJ, Sinninghe Damsté JS, Rijpstra WI, Rudd S, Lawson PA. 2013. *Thermoanaerobaculum aquaticum* gen. nov., sp. nov., the first cultivated member of *Acidobacteria* subdivision 23, isolated from a hot spring. Int. J. Syst. Evol. Microbiol. 63:4149–4157. http://dx.doi.org/10.1099/ijs.0.051425-0.
- 8. Lawson PA, Gharbia SE, Shah HN, Clark DR. 1989. Recognition of *Fusobacterium nucleatum* subgroups Fn-1, Fn-2 and Fn-3 by ribosomal RNA gene restriction patterns. FEMS Microbiol. Lett. 53:41–45.
- Marks CR, Stevenson BS, Rudd S, Lawson PA. 2012. Nitrospiradominated biofilm within a thermal artesian spring: a case for nitrificationdriven primary production in a geothermal setting. Geobiology 10: 457–466. http://dx.doi.org/10.1111/j.1472-4669.2012.00335.x.