GENOME ANNOUNCEMENTS

Complete Genome Sequence of the Thermophilic Bacterium *Exiguobacterium* sp. AT1b[▽]

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Here we present the genome of strain *Exiguobacterium* sp. AT1b, a thermophilic member of the genus *Exiguobacterium* whose representatives were isolated from various environments along a thermal and physicochemical gradient. This genome was sequenced to be a comparative resource for the study of thermal adaptation with a psychroactive representative of the genus, *Exiguobacterium sibiricum* strain 255-15, that was previously sequenced by the U.S. Department of Energy's (DOE's) Joint Genome Institute (JGI) (<http://genome.ornl.gov/microbial/exig/>).

Exiguobacterium sp. AT1b (= ATCC BAA-1283) is a thermophilic, facultative anaerobic bacterium isolated from a slightly alkaline, highly carbonate hot spring water sample from Angel Terrace, which is part of Mammoth Terrace, Yellowstone National Park. The non-spore-forming, low-GC, Gram-positive, catalase-positive, nuclease-active *Exiguobacterium* AT1b strain clusters phylogenetically with other hot spring and marine isolates within *Bacillales* family XII *incertae sedis*, *Firmicutes*. Its 16S rRNA gene has 94% identity and DNA-DNA hybridization of <70% with *Exiguobacterium* 255-15 (7, 8), indicating that it is a different species from its psychroactive relative. Strain AT1b cells occur singly, in pairs, or infrequently in chains and are able to grow from 15 to 50°C; at 37°C they are ovoid rods about 2 µm in length, and they are longer at 50°C (10). Colonies appear round, shiny, irregular, elevated, and orange colored on tryptic soy agar (TSA); the pigment does not diffuse into the medium.

The genome of *Exiguobacterium* sp. AT1b was sequenced by the DOE JGI using a combination of 6.1-kb and 37.5-kb DNA libraries. General aspects of library construction and sequencing performed at the JGI can be found at <http://www.jgi.doe>

.gov/. Draft assemblies were based on 21,659 total reads. Both libraries provided 7.3× coverage of the genome. In addition to Sanger sequencing, 454 pyrosequencing was done to a depth of 20× coverage. The Phred/Phrap/Consed software package was used for sequence assembly and quality assessment (1–3) in the following finishing process. The genome was annotated at Oak Ridge National Laboratory (ORNL) using the automated annotation pipeline, which is driven by the gene prediction algorithm Prodigal (4), followed by a round of manual curation using the JGI GenePRIMP pipeline (5).

Exiguobacterium sp. AT1b contains a single replication unit consisting of 2,999,895 bp, which encodes 3,043 (96.9%) putative proteins. Genes are evenly distributed between the forward (49.9%) and reverse (50.1%) chromosome strands; the average coding sequence length is 891 bp, and 89.6% of the coding bases are in sequences encoding putative proteins. The GC content is constant across the genome with an average value of 48.5%. AT-rich fragments encode uncharacterized proteins, sulfurtransferase, and sulfur modification protein. Nine rRNA operons are present in the positive (4 copies) and negative (5 copies) strands. The average CG content of the rRNA operons is 56.1%, which is higher than the average genome value. Three transposases of the IS605 *orfB* family with 90.3 to 96.5% identity between them are predicted in the genome. DNase production is supported by the presence of restriction endonucleases, ribonucleases, and exonucleases. *Exiguobacterium* sp. AT1b contains genes for cellulose and

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hemicellulose degradation, as indicated by the presence of glycosyltransferases and glycoside hydrolases, which may be adapted to function at higher temperatures.

Having the strains and genome sequences of thermophilic *Exiguobacterium* sp. AT1b and its psychroactive relative, *Exiguobacterium sibiricum* 255-15, which was isolated from Siberian permafrost and has a growth range from -6° to 40°C (6, 9), enables investigation of the genetic basis of microbial adaptation to different temperatures and is of interest in the fields of agriculture, industrial microbiology, and astrobiology.

Nucleotide sequence accession numbers. The final annotated genome sequence was deposited in GenBank under the accession number CP001615, the IMG database under identifier (ID) 643692023, the Genomes OnLine Database (GOLD) under ID Gc01000, and the GreenGenes database under ID 284647.

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REFERENCES

1. Ewing, B., and P. Green. 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res.* **8**:186–194.
2. Ewing, B., L. Hillier, M. C. Wendl, and P. Green. 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res.* **8**:175–185.
3. Gordon, D., C. Abajian, and P. Green. 1998. Consed: a graphical tool for sequence finishing. *Genome Res.* **8**:195–202.
4. Hyatt, D., et al. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* **11**:119.
5. Pati, A., et al. 2010. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. *Nat. Methods* **7**:455–457.
6. Rodrigues, D. F., et al. 2006. Characterization of *Exiguobacterium* isolates from the Siberian permafrost. Description of *Exiguobacterium sibiricum* sp. nov. *Extremophiles* **10**:285–294.
7. Rodrigues, D. F., et al. 2008. Architecture of thermal adaptation in an *Exiguobacterium sibiricum* strain isolated from 3 million year old permafrost: a genome and transcriptome approach. *BMC Genomics* **9**:547.
8. Vishnivetskaya, T. A., S. Kathariou, and J. M. Tiedje. 2009. The *Exiguobacterium* genus: biodiversity and biogeography. *Extremophiles* **13**:541–555.
9. Vishnivetskaya, T. A., R. Siletzky, N. Jefferies, J. M. Tiedje, and S. Kathariou. 2007. Effect of low temperature and culture media on the growth and freeze-thawing tolerance of *Exiguobacterium* strains. *Cryobiology* **54**:234–240.
10. Vishnivetskaya, T. A., R. Ramaley, D. F. Rodrigues, J. M. Tiedje, and S. Kathariou. 2005. Abstracts of the Joint International Symposia for Subsurface Microbiology and Environmental Biogeochemistry, p. 78.