Journal of Bacteriology
March 2011, Vol. 193, No. 6, p. 1481-1482
http://dx.doi.org/10.1128/JB.01490-10

Complete Genome Sequence of the Hyperthermophilic, Piezophilic, Heterotrophic, and Carboxydotrophic Archaeon *Thermococcus barophilus* MP

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Abstract:

Thermococcus barophilus is a hyperthermophilic, anaerobic, mixed heterotrophic, and carboxydotrophic euryarchaeon isolated from the deep sea hydrothermal vent Snakepit site on the mid-Atlantic ridge at a depth of 3,550 m. *T. barophilus* is the first true piezophilic, hyperthermophilic archaeon isolated, having an optimal growth at 40 MPa. Here we report the complete genome sequence of strain MP, the type strain of *T. barophilus*. The genome data reveal a close proximity with *Thermococcus sibiricus*, another *Thermococcus* isolated from the deep biosphere and a possible connection to life in the depths.

TEXT:

Thermococcus barophilus strain MP is the first true hyperthermophilic piezophile archaeon isolated in 1993 (8) from an enrichment of chimney samples in YPS-20 rich medium at 95°C and 40 MPa. Strain MP grows from 48°C to 100°C with an optimum at 85°C and within a pressure range of 0.1 to 85 MPa with an optimum of 40 MPa (8, 10). A phylogenetic analysis using concatenated ribosomal proteins has shown that strain MP is most closely related to *Thermococcus sibiricus* strain MM379 (7). These two strains define a cluster which is almost equally distant from the *Pyrococcus* core cluster represented by *P. abyssi* GE5, *P. furiosus* DSM 3638, *P. yayanosii* CH1 and *P. horikoshii* OT3 (2,4, 6, 10) and the *Thermococcus* core cluster represented by *T. gammatolerans* EJ3, *T. kodakaraensis* KOD1 and *T. onnurineus* NA1 (3, 5, 11).

T. barophilus MP was accepted in the marine microbe sequencing project for whole genome shotgun (WGS) sequencing in 2005 (www.moore.org/microgenome) by Gordon and Betty Moore Foundation. Genomic libraries of 4 kb and 10 kb were constructed and sequenced by the Sanger method to 8X level of coverage.

Sequence reads from a total of 55488 shotgun clones were assembled and analyzed with the JCVI´s CONSED and Manatee packages, and 12 contigs were generated and connected by PCR. Preliminary ORF prediction for 2,268 genes across the genome was conducted by automated annotation with Glimmer (http://www.cbcb.umd.edu/software/glimmer) and RAST (1). The annotation was manually cured using BLAST and the nr database of the NCBI.

The *T. barophilus* MP genome consists of a circular chromosome of 2, 010, 078 bp and a circular plasmid pTBMP1 of 54,159 bp with an average G+C content, respectively, of 41.7 and 38.3 %. There are one copy of 16S-23S, two copies of 5S and 46 tRNA genes. The *T. barophilus* chromosome contains 4 CRISPR loci composed of 7 to 26 direct DNA repeats (repeat length 30 and 31 bp) interspersed with 6 to 25 nonrepetitive nucleotides of 34 to 51 nucleotides called spacers. Six CRISPR-associated genes (*cas*) that encode proteins involved in adaptation and interference were found in the vicinity of a single CRISPR locus (9), which is composed of 7 repeats (30 bp) and 6 spacers (34 to 49 bp).

Like *T. onnurineus*, and *T. gammatolerans*, *T. barophilus* possesses the carboxydotrophic pathway and bears seven different hydrogenase complexes (MBx, Mbh, Hyg 4-I, Hyg4-II, hyg4-III, SulFI and SulF II). *T. barophilus* shares 1089, 1272, 1112, and 1236 genes (> 60% identity), respectively with *T. gammatolerans*, *T. sibiricus*, *T. kodakaraensis*, and *T. onnurineus*, while 221 genes of *T. barophilus* have no homologues in the above four *Thermococcus* species. The *T. barophilus* specific gene set, which bears little homology to proteins in the database may provide clues to its adaptation to growth under the high pressure conditions which are typical of the deep-biosphere.

Nucleotide sequence accession number. The final annotated genome and plasmid of *T. barophilus* strain MP reported in this paper is now available in Genbank under accession number CP002372 and CP002373 respectively.

ACKNOWLEDGMENTS:

The authors would like to thank Daniel Prieur for helpful discussions and supports and the Gordon and Betty Moore Foundation for sequencing support at J. Craig Venter Institute. This work was in part supported by the Agence nationale de la recherche (ANR-10-BLAN- Living deep) and a partenariat Hubert Curien (PHC) Jules Verne collaboration grant by Egide to MJ and grant from the Icelandic Center for Research to VM. P.V. Was supported by a PhD fellowhip from the Ministère de l'Enseignement supérieur et de la Recherche. Thanks are also due to Justin Johnson at the JCVI.

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