

GENOME ANNOUNCEMENTS

Complete Genome Sequence of the Hyperthermophilic, Piezophilic, Heterotrophic, and Carboxydrotrophic Archaeon *Thermococcus barophilus* MP[∇]

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***Thermococcus barophilus* is a hyperthermophilic, anaerobic, mixed heterotrophic, and carboxydrotrophic 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.**

Thermococcus barophilus strain MP is the first true hyperthermophilic, piezophilic archaeon and was 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 (1a, 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 (www.moore.org/microgenome) for whole-genome shotgun (WGS) sequencing in 2005 by the Gordon and Betty Moore Foundation. Genomic libraries of 4 kb and 10 kb were constructed and sequenced by the Sanger method to an 8-fold level of coverage.

Sequence reads from a total of 55,488 shotgun clones were assembled and analyzed with the JCVI CONSED and Manatee packages, and 12 contigs were generated and connected by

PCR. A preliminary open reading frame 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 average G+C contents of 41.7 and 38.3%, respectively. There are one copy of 16S-23S and two copies of 5S and 46 tRNA genes. The *T. barophilus* chromosome contains four CRISPR loci composed of 7 to 26 direct DNA repeats (repeat length, 30 and 31 bp) interspersed with 6 to 25 nonrepetitive sections 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 seven repeats (30 bp) and six spacers (34 to 49 bp).

Like *T. onnurineus* and *T. gammatolerans*, *T. barophilus* possesses the carboxydrotrophic pathway and bears seven different hydrogenase complexes (MBx, Mbh, Hyg 4-I, Hyg4-II, hyg4-III, SulFI, and SulF II). *T. barophilus* shares 1,089, 1,272, 1,112, and 1,236 genes (>60% identity) with *T. gammatolerans*, *T. sibiricus*, *T. kodakaraensis*, and *T. onnurineus*, respectively, 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.

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Nucleotide sequence accession numbers. The final annotated genome and plasmid of *T. barophilus* strain MP reported in this paper are now available in GenBank under accession numbers CP002372 and CP002373, respectively.

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REFERENCES

1. **Aziz, R. K., et al.** 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* **9**:75.
- 1a. **Birrien, J. L., et al.** 14 January 2011, posting date. *Pyrococcus yayanosii* sp. nov., the first obligate piezophilic hyperthermophilic archaeon isolated from deep-sea hydrothermal vent. *Int. J. Syst. Evol. Microbiol.*, in press. doi: 10.1099/ijs.0.024653-0. [E-pub ahead of print.]
2. **Cohen, G. N., et al.** 2003. An integrated analysis of the genome of the hyperthermophilic archaeon *Pyrococcus abyssi*. *Mol. Microbiol.* **47**:1495–1512.
3. **Fukui, T., et al.** 2005. Complete genome sequence of the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1 and comparison with *Pyrococcus* genomes. *Genome Res.* **15**:352–363.
4. **Kawarabayasi, Y., et al.** 1998. Complete sequence and gene organization of the genome of a hyper-thermophilic archaeobacterium, *Pyrococcus horikoshii* OT3. *DNA Res.* **30**:55–76.
5. **Lee, H. S., et al.** 2008. The complete genome sequence of *Thermococcus onnurineus* NA1 reveals a mixed heterotrophic and carboxydrotrophic metabolism. *J. Bacteriol.* **190**:7491–7499.
6. **Maeder, D. L., et al.** 1999. Divergence of the hyperthermophilic archaea *Pyrococcus furiosus* and *Pyrococcus horikoshii* inferred from complete genomic sequences. *Genetics* **152**:1299–1305.
7. **Mardanov, A. V., et al.** 2009. Metabolic versatility and indigenous origin of the archaeon *Thermococcus sibiricus*, isolated from a Siberian oil reservoir, as revealed by genome analysis. *Appl. Environ. Microbiol.* **75**:4580–4588.
8. **Marteinsson, V. T., et al.** 1999. *Thermococcus barophilus* sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. *Int. J. Syst. Bacteriol.* **49**:351–359.
9. **Rousseau, C., M. Gonnet, M. Le Romancer, and J. Nicolas.** 2009. CRISPI: a CRISPR interactive database. *Bioinformatics* **25**:3317–3318.
10. **Zeng, X., et al.** 2009. *Pyrococcus* CH1, an obligate piezophilic hyperthermophile: extending the upper pressure-temperature limits for life. *ISME J.* **3**:873–876.
11. **Zivanovic, Y., et al.** 2009. Genome analysis and genome-wide proteomics of *Thermococcus gammatolerans*, the most radioresistant organism known amongst the Archaea. *Genome Biol.* **10**:R70.