

Complete Genome Sequence of the Hyperthermophilic Archaeon *Thermococcus* sp. Strain AM4, Capable of Organotrophic Growth and Growth at the Expense of Hydrogenogenic or Sulfidogenic Oxidation of Carbon Monoxide

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Analysis of the complete genome of *Thermococcus* sp. strain AM4, which was the first lithotrophic *Thermococcales* isolate described and the first archaeal isolate to exhibit a capacity for hydrogenogenic carboxyd-otrophy, reveals a proximity with *Thermococcus gammatolerans*, corresponding to close but distinct species that differ significantly in their lithotrophic capacities.

Thermococcus sp. strain AM4 was isolated from 1 of 13 enrichments of coccoid cells obtained from hydrothermal venting structures (East Pacific Rise; 13°N, 2,600-m depth) anaerobically on CO at 80°C and growing with H₂ and CO₂ production (9). Carboxyd-otrophic growth of strain AM4 required the presence of 50 mg/liter yeast extract. In the absence of CO, AM4 could grow on peptone or yeast extract with elemental sulfur as the electron acceptor.

Within the marine microbe sequencing project (www.moore.org/microgenome), genomic libraries for *Thermococcus* sp. AM4 were constructed and sequenced by the Sanger method to an 8-fold level of coverage. Sequence reads were assembled with the J. Craig Venter Institute (JCVI) Consed and Manatee packages to 17 contigs.

The contigs were connected by PCR. Open reading frames were predicted with Glimmer (<http://www.cbc.umd.edu/software/glimmer>) and RAST (1). The annotation was manually cured using the BLAST and the NCBI nr databases.

The *Thermococcus* sp. AM4 genome consists of a circular chromosome of 2,086,428 bp without extrachromosomal elements. It contains 2,235 protein-coding genes, 46 tRNA genes, and two copies of 5S and one copy of 16S-23S rRNA genes.

Strain AM4 is most closely related to *Thermococcus gammatolerans* EJ3 (3, 13). The *in silico* hybridization yielded an average nucleotide identity (ANI) of shared protein-coding genes of 87% (about 80% of genes shared). This ANI value is

lower than the 95 to 96% value shown to correspond to the 70% DNA-DNA hybridization level accepted to delimit microbial species (2, 7, 11). Thus, AM4 and *T. gammatolerans* represent phylogenetically close but distinct species.

Genomic comparisons have revealed an organotrophic potential comparable in the two thermococci (13). Both genomes encode a CO dehydrogenase (CODH) which allows litho-er-otrophic growth on CO plus sulfur with H₂S formation (D. A. Kozhevnikova and T. G. Sokolova, unpublished data). However, AM4 possesses an extra CODH clustered in the genome with an energy-converting hydrogenase. This gene cluster, also conserved in *Thermococcus onnurineus* and *Thermococcus barophilus* (5, 6, 10, 12), enables these species to grow on CO following the reaction CO + H₂O → H₂ + CO₂ (5, 10), while *T. gammatolerans* is unable to produce hydrogen from CO and H₂O (T. G. Sokolova, unpublished data). Noticeably, *T. gammatolerans* and AM4 possess a formate hydro-ge-n-lyase gene cluster, but only *T. gammatolerans* possesses the formate transporter gene that allows this species to grow via hydrogenogenic oxidation of exogenous formate (4).

Like all sequenced genomes of the *Thermococcales*, the AM4 genome encodes a type III RubisCO but lacks a phosphoribulokinase to provide RubisCO its substrate in the Calvin cycle. Given the extensive requirements of carboxyd-otrophic thermococci for amino acids (5, 13), possession of an autotrophic CO₂ fixation pathway would be paradoxical. Furthermore, the hypothetical CO₂ fixation cycle that involves RubisCO and can proceed without phosphoribulokinase (5, 8) cannot be closed in thermococci, including carboxyd-otrophic ones, due to the lack of transaldolase genes. Nor is acetyl coenzyme A synthase, a key enzyme of the Wood-Ljungdahl pathway, encoded by any of the *Thermococcales* genomes. Thus, carboxyd-otrophy, while providing energy to diverse thermococci, cannot be considered to contribute to the primary production of hydrothermal vent communities.

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Nucleotide sequence accession number. The final annotated genome of *Thermococcus* sp. AM4 is available in GenBank under accession number CP002952.

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