

Complete Genome Sequence of the Obligate Piezophilic Hyperthermophilic Archaeon *Pyrococcus yayanosii* CH1[∇]

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***Pyrococcus yayanosii* CH1 is the first obligate piezophilic hyperthermophilic archaeon isolated from the deep-sea hydrothermal site Ashadze on the mid-Atlantic ridge at a depth of 4,100 m. This organism grows within a temperature range of 80 to 108°C and a hydrostatic pressure range of 20 to 120 MPa, with optima at 98°C and 52 MPa, respectively. Here, we report the complete genome sequence (1,716,817 bp, with a G+C content of 51.6%) of the type strain *P. yayanosii* CH1^T (= JCM 16557). This genomic information reveals a systematic view of the piezoadaptation strategy and evolution scenario of metabolic pathways in *Thermococcales*.**

Pyrococcus yayanosii CH1 is the first obligate piezophilic hyperthermophilic archaeon isolated from the deep-sea hydrothermal site named Ashadze located on the mid-Atlantic ridge at a depth of 4,100 m. *P. yayanosii* CH1 was cultivated in TRM medium (2, 7) under optimal growth conditions (98°C and 52 MPa) for 2 days. Cells were collected by centrifugation, and then the genomic DNA was prepared by the alkaline lysis method. The genome of CH1 has been sequenced by both Solexa at the Beijing Genomics Institute and 454 pyrosequencing at the Chinese National Human Genome Center in Shanghai to ensure good accuracy.

Solexa sequencing revealed 11 scaffolds ranging from 5.3 kb to 458 kb to give ~374-fold coverage of the genome. In the case of 454 pyrosequencing, 17 contigs were generated (size range of 6.5 kb to 336 kb) to give ~68.5-fold coverage of the genome. These sequencing data were combined, and gaps were closed by PCR sequencing.

The genome consists of a circular DNA molecule of 1,716,817 bp with a G+C% content of 51.6%, which is higher

than that of other related *Pyrococcus* species like *P. abyssi*, *P. horikoshii*, and *P. furiosus* (3, 5, 6).

A total of 1,926 coding sequences (CDSs) were predicted by Glimmer 3.02 and tRNAscan-SE 1.21. The average gene size is 816 bp, comprising CDSs ranging from 37 to 1,405 amino acids. There are two predicted copies of the 5S rRNA gene, one of the 16S rRNA gene, and one of the 23S rRNA gene and 46 predicted tRNAs. The genome sequence was annotated by integrated BLAST analysis against the KEGG, UniProt, and Clusters of Orthologous Groups databases.

The genome was categorized into 168 subsystems by authorized annotation using the RAST (rapid annotation using subsystem technology) server (1). The reconstructed metabolic network includes a modified Embden-Meyerhof pathway, a partial pentose phosphate (Entner-Doudoroff) pathway, and reverse tricarboxylic acid cycles.

The amino acid composition and codon usage bias of all of the predicted *P. yayanosii* CH1 open reading frames were analyzed using the Acua version 1.0 software. Observations of the asymmetries in the amino acid substitutions in 200 pairs of orthologous proteins from *P. furiosus* and *P. yayanosii* CH1 were made. Piezophily in the physicochemical properties of amino acids and in the genetic code could be ranked by PAI (pressure asymmetry index) after comparison of proteins from *P. furiosus* and *P. abyssi* as assumed by Massimo Di Giulio (4). Our data show that both alanine and arginine have higher PAI values and the ratio of appearances in the genome of *P. yayanosii* CH1 is higher than that in other *Pyrococcus* species. This result suggests that piezophilic amino acids are prone to be low in molecular weight and higher in polarity.

Taking together the results from genome annotation, phys-

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iological and genetic characterization, and high-throughput transcriptomic, proteomic, and metabolomic comparisons, a systematic view of the piezoadaptation strategy and evolution scenario of metabolic pathways in *P. yayanosii* CH1 could be reached.

Nucleotide sequence accession number. The final annotated genome of *P. yayanosii* strain CH1 reported in this paper is now accessible in GenBank under accession number CP002779.

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