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GENOME ANNOUNCEMENT

Complete Genome Sequence of the Hyperthermophilic Archaeon *Pyrococcus* sp. Strain ST04, Isolated from a Deep-Sea Hydrothermal Sulfide Chimney on the Juan de Fuca Ridge

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***Pyrococcus* sp. strain ST04 is a hyperthermophilic, anaerobic, and heterotrophic archaeon isolated from a deep-sea hydrothermal sulfide chimney on the Endeavour Segment of the Juan de Fuca Ridge in the northeastern Pacific Ocean. To further understand the distinct characteristics of this archaeon at the genome level (polysaccharide utilization at high temperature and ATP generation by a Na^+ gradient), the genome of strain ST04 was completely sequenced and analyzed. Here, we present the complete genome sequence analysis results of *Pyrococcus* sp. ST04 and report the major findings from the genome annotation, with a focus on its saccharolytic and metabolite production potential.**

Pyrococcus sp. strain ST04 was isolated in May 2004 from a piece of a hydrothermal sulfide chimney that was emitting hydrothermal fluid at temperatures up to 302°C at a depth of 2,290 m (10). The sample was collected from the Stonehenge mound in the Mothra vent field along the Endeavor Segment of the Juan de Fuca Ridge using the deep-sea research submarine *Alvin*. Unlike other *Pyrococcus* strains isolated from this area, strain ST04 grows well at 95°C on maltose, cellobiose, or peptides with concomitant production of H_2S from elemental sulfur and H_2 in relatively equal proportions (7). Further understanding of its metabolism at the genome level would explain its respiration mechanisms, as well as how it utilizes different carbohydrates.

Genomic DNA from *Pyrococcus* sp. strain ST04 was isolated as described previously (5) and sequenced completely using a GS-FLX Titanium genome sequencer (Macrogen, Seoul, South Korea) with approximately 73-fold coverage. The open reading frames (ORFs) were predicted using GeneMarkS (2), Glimmer 3.02 (3), and FgenesB (Softberry, Inc., Mount Kisco, NY), and functional analyses of the predicted ORFs were conducted using BLASTP (1) and InterProScan (12). Transfer RNA (tRNA) was predicted using tRNAscan-SE, and the rRNA operon was predicted using RNAmmer (4, 6).

The complete genome of *Pyrococcus* sp. strain ST04 consists of a circular chromosome of 1,736,885 bp containing 1,748 ORFs and 41 pseudogenes with a GC content of 42.3%. This genome has 46 tRNAs and one rRNA operon consisting of two 5S rRNA genes, one 16S rRNA gene, and one 23S rRNA gene. It has two CRISPR loci consisting of CRISPR-associated *cas* genes, as well as 9 or 15 repeats, respectively, probably explaining why it has no prophage in the genome. Pathway analysis of the *Pyrococcus* sp. strain ST04 genome showed that it encodes all of the enzymes of the archaeal Embden-Meyerhof glycolytic pathway (11). Polysaccharides such as starch and, potentially, cellulose are thought to be degraded extracellularly by amylopullulanase (Py04_0209) and β-1,4-endoglucanase (Py04_1787) and then hydrolyzed intracellularly by α-amylase (Py04_0423), β-endoglucanase (Py04_1747), and α- and β-glucosidases (Py04_1214 and Py04_0550) (7). While this strain has a complete glycolytic pathway, it has an incomplete citric acid cycle due to the absence of genes for succinyl-coenzyme A ligase, succinate dehydrogenase, and malate dehydrogenase. It is noteworthy that the glycolytic pathway produces reduced ferredoxin instead of NADH as an electron carrier. During electron transfer from ferredoxin to protons, a membrane-bound hydrogenase (Py04_1312-1325) appears to also translocate either H^+ or Na^+ across the membrane, generating an ion gradient (9). A membrane-bound, Na^+-translocating ATP synthase (Py04_0327-0335) then produces ATP from this gradient (8).

This complete genome analysis of *Pyrococcus* sp. ST04 provides novel insights into H_2 and ATP generation by a Na^+ gradient, as well as efficient polysaccharide catabolism at high temperature, suggesting the potential application of thermostable polysaccharide degradation enzymes in food and bioindustries.

Nucleotide sequence accession number. The complete genome sequence of *Pyrococcus* sp. strain ST04 is now available in GenBank database under accession number CP003534.

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