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GENOME ANNOUNCEMENT

Complete Genome Sequence of *Desulfurococcus fermentans*, a Hyperthermophilic Cellulolytic Crenarchaeon Isolated from a Freshwater Hot Spring in Kamchatka, Russia

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***Desulfurococcus fermentans* is the first known cellulolytic archaeon. This hyperthermophilic and strictly anaerobic crenarchaeon produces hydrogen from fermentation of various carbohydrates and peptides without inhibition by accumulating hydrogen. The complete genome sequence reported here suggested that *D. fermentans* employs membrane-bound hydrogenases and novel glycohydrolases for hydrogen production from cellulose.**

Desulfurococcus fermentans, a hyperthermophilic crenarchaeon belonging to the *Desulfurococcaceae* family, is the first reported cellulytic archaeon (6). It was isolated from a freshwater hot spring of the Uzon caldera on the Kamchatka peninsula, Russia. This obligate anaerobe grows optimally at temperatures of 80 to 82°C. It ferments cellulose and various other carbohydrates (fructose, lactose, maltose, ribose, and starch) and peptides in peptone and casein hydrolysate for growth and produces hydrogen in the process (6); hydrogen production is not impeded by hydrogen accumulation (6). In contrast, other *Desulfurococcus* species do not utilize cellulose, are inhibited by hydrogen, and require elemental sulfur for growth (1, 4, 6, 7, 9); reduction of sulfur to H_2S removes inhibition by hydrogen. *D. fermentans* neither requires nor is stimulated by elemental sulfur (6). To gain insights into the mechanisms underlying cellulose degradation and uninhibited hydrogen production abilities in *D. fermentans*, we have sequenced the genome of this crenarchaeon.

Whole-genome sequencing was performed using a combination of Illumina and 454 sequencing platforms. An Illumina GAII shotgun library, a 454 Titanium draft library, and a paired-end 454 library with an average insert size of 6.0 kb were generated. Illumina sequencing data were assembled with Velvet (8), and the consensus sequences were shredded into 1.5-kb overlapped fake reads and then assembled with the 454 data using Newbler. The Newbler assembly contained two contigs in one scaffold. The Newbler assembly was converted into a Phrap assembly by making fake reads from the consensus sequence and collecting the read pairs in the 454 paired-end library. The Phred/Phrap/Consed software package (CodonCode Corporation, Dedham, MA) was used for sequence assembly and quality assessment in the finishing process as follows. Illumina data were used to correct potential base errors and increase consensus quality using Polisher (A. Lapidus, unpublished data). Possible misassemblies were corrected with gapResolution (C. Han, unpublished data) or Dupfinisher (2) or by sequencing cloned bridging PCR fragments. Gaps between contigs were closed by editing in Consed, by PCR, and by Bubble PCR primer walks. Open reading frames were identified using Prodigal (3) as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using GenePRIMP (5). Putative protein functions were inferred by searches in the National Center for Biotechnology Information (NCBI) nonredundant, UniProt, TIGRFams, Pfam, PRIAM (PRofils pour l'Identification Automatique du Métabolisme), KEGG (Kyoto Encyclopedia of Genes and Genomes), COG (Clusters of Orthologous Groups), and InterPro databases.

The *D. fermentans* genome consists of 1,384,116 bp with a 44.8% GC content. It contains 49 tRNA genes (including three with noncanonical introns), 5 structural RNA genes (one each of 5S rRNA, 16S rRNA, 23S rRNA, archaeal type A RNase P RNA, one signal recognition particle [SRP] RNA), 1 clustered regularly interspaced short palindromic repeat (CRISPR) array, and 1,475 putative protein-coding genes of which 1,075 have predicted functions. *D. fermentans* possesses membrane-bound hydrogenases but lacks soluble hydrogenases. As expected, the *D. fermentans* genome does not carry genes encoding known sulfur-reducing enzymes. Interestingly, homologs of known cellulases are also missing in this archaeon, suggesting that it might employ new cellulose degradation systems.

Nucleotide sequence accession number. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under accession number CP003321.1.

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