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GENOME ANNOUNCEMENT

Complete Genome Sequence of the Hyperthermophilic Cellulolytic Crenarchaeon "*Thermogladius cellulolyticus*" 1633

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Strain 1633, a novel member of the genus *Thermogladius*, isolated from a freshwater hot spring, is an anaerobic hyperthermophilic crenarchaeon capable of fermenting proteinaceous and cellulose substrates. The complete genome sequence reveals genes for protein and carbohydrate-active enzymes, the Embden-Meyerhof pathway for glucose metabolism, cytoplasmic NADP-dependent hydrogenase, and several energy-coupling membrane-bound oxidoreductases.

The genus *Thermogladius* (4) belongs to the order *Desulfurococcales*, comprising coccoidal thermophilic crenarchaeotes (2). The only previously described *Thermogladius* species, *T. shockii* WB1, isolated from a thermal pool in Yellowstone National Park, USA, is an anaerobic hyperthermophile capable of fermenting proteinaceous substrates (4). Strain 1633 was isolated from the hot spring in the Uzon Caldera in Kamchatka (Russia) in an enrichment culture on carboxymethyl cellulose. Strain 1633 is an obligate anaerobe growing optimally at a temperature of 84°C and pH 7.1. Unlike *T. shockii*, strain 1633 is able to grow by fermentation not only on proteinaceous substrates but also on cellulose (filter paper, microcrystalline and carboxymethyl cellulose). Elemental sulfur is not obligately required but stimulates growth and is reduced to H_2S. To understand the metabolic properties of strain 1633, its complete genome sequence was determined.

The genome was sequenced using the Roche 454 GS FLX pyrosequencing platform. We obtained a library of 227,415 single-strand reads; the reads were assembled into 2 contigs by the Newbler Assembler 1.1 (454 Life Sciences, Branford, CT). The genome was finished by filling gaps with sequencing and primer walking of PCR products with an ABI 3730 capillary sequencer (Applied Biosystems, CA).

The complete genome of strain 1633 consists of 1,356,318 bp in a single circular chromosome with an average G+C content of 55.64%. The 16S rRNA sequence is 98.1% identical to that of *T. shockii*, suggesting that strain 1633 belong to the *Thermogladius* genus. In contrast with *T. shockii*, the 16S rRNA gene of strain 1633 contains no introns, further suggesting that this strain may be classified as a novel species, "*Thermogladius cellulolyticus*." A total of 1,414 protein coding genes was predicted by Glimmer (1), covering 91% of the chromosome. A whole-genome annotation and analysis were performed with the AutoFACT tool (3), followed by a round of manual curation.

Metabolic pathway analysis revealed that utilization of polysaccharides involves the function of several encoded glycoside hydrolases and more than 20 glycosyl transferases. Most of these enzymes are encoded at two genomic islands. However, glycoside hydrolases belong to family 57, comprising alpha-amylases and some other enzymes for hydrolysis of alpha-linked polysaccharides, while known cellulases (endo-beta-1,4-glucanases, etc.) were not found, suggesting the existence of peculiar mechanisms of cellulose hydrolysis in *T. cellulolyticus*. Similar to the situation with *Desulfurococcus kamchatkensis* (5), glucose oxidation proceeded in the modified Embden-Meyerhof pathway (8) while the tricarboxylic acid cycle was not encoded. ATP could be gained either by substrate-level phosphorylation or by a membrane-bound ATP synthase utilizing a chemiosmotic gradient generated by several membrane-bound oxidoreductases, including MBH-related ferredoxin-oxidizing hydrogenases and MBX-related ferredoxin-NADPH oxidoreductase (6) similar to ones found in *D. kamchatkensis* (5) and *Thermosphaera aggregans* (7). The *T. cellulolyticus* genome also encodes group 3b cytoplasmic NADP-dependent hydrogenase (9), previously not described in *Crenarchaeota*. Making the genome sequence of *T. cellulolyticus* available will allow comprehensive comparisons with other *Desulfurococcales* and enable further investigation into the mechanisms of cellulose utilization in archaea.

Nucleotide sequence accession number. The complete genome sequence of *T. cellulolyticus* 1633 was deposited in GenBank under accession number CP003531.

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REFERENCES

1. **Delcher AL, Harmon D, Kasif S, White O, Salzberg SL.** 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res. **27**:4636–4641.
2. **Kletzin A.** 2007. General characteristics and important model organisms, p 14–92. *In* Cavicchioli R (ed), Archaea: molecular and cellular biology. ASM Press, Washington, DC.
3. **Koski LB, Gray MW, Langi BF, Burger G.** 2005. AutoFACT: an automatic functional annotation and classification tool. BMC Bioinformatics **6**:151.
4. **Osburn MR, Amend JP.** 2011. Thermogladius shockii gen. nov., sp. nov., a hyperthermophilic crenarchaeote from Yellowstone National Park, U. S. A. Arch. Microbiol. **193**:45–52.

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5. **Ravin NV, et al.** 2009. Complete genome sequence of the anaerobic, protein-degrading hyperthermophilic crenarchaeon *Desulfurococcus kamchatkensis*. J. Bacteriol. **191**:2371–2379.
6. **Sapra R, Bagramyan K, Adams MWW.** 2003. A simple energy-conserving system: proton reduction coupled to proton translocation. Proc. Natl. Acad. Sci. U. S. A. **100**:7545–7550.
7. **Spring S, et al.** 2010. Complete genome sequence of *Thermosphaera aggregans* type strain (M11TL^T). Stand. Genomic Sci. **2**:245–259.
8. **Verhees CH, et al.** 2003. The unique features of glycolytic pathways in *Archaea*. Biochem. J. **375**:231–246.
9. **Vignais PM, Billoud B.** 2007. Occurrence, classification, and biological function of hydrogenases: an overview. Chem. Rev. **107**:4206–4272.