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GENOME SEQUENCES

Complete Genome Sequences of *Thermus thermophilus* Strains HB5002 and HB5008, Isolated from Mine Hot Spring in Japan

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ABSTRACT We isolated *Thermus thermophilus* strains HB5002 and HB5008 from Mine Hot Spring in Japan. Whole-genome sequencing revealed that they showed ~100% average nucleotide identity to each other, ≥98.53% to the *T. thermophilus* strains originating from the same spot but ≤97.64% to the *T. thermophilus* strains from geographically different places in Japan.

Thermus thermophilus was first isolated from Mine Hot Spring in Japan in 1968 (1). Since then, many *T. thermophilus* strains have been isolated from various thermal areas worldwide (2–6). The thermophile is aerobic and grows optimally at around 70°C, with a doubling time of ~1 h; basic genetic engineering techniques were developed in the mid-1980s (7). These favorable properties pushed the species as a model thermophilic organism, and researchers have intensively studied *T. thermophilus* biochemically (8, 9), structurally (10, 11), and genetically (12–14). As a 50th anniversary project, we initiated an ecological investigation of the thermophile—how *T. thermophilus* strains thrive/survive in Mine Hot Spring. In 2018, we collected a water sample from exactly the same fountain geyser at Mine Hot Spring from which the representative strains HB8 and HB27 were isolated (1). Dozens of *T. thermophilus* strains were isolated (6, 15) and preliminarily classified into several groups based on appearance—color, colony morphology, growth rate, and so on. In this study, we conducted whole-genome analyses of strains HB5002 and HB5008 by combining Oxford Nanopore Technologies (ONT) and Illumina technologies.

Cells were grown at 70°C in Lennox LB medium, and genomic DNA was purified using a blood and cell culture DNA midikit (Qiagen). For long-read sequencing, unsheared genomic DNA was pretreated with a short-read eliminator kit (Circulomics) to remove <10-kbp fragments, and a library was constructed using a ligation sequencing kit (ONT). Sequencing was performed with a GridION X5 system on a FLO-MIN106 R9.41 flow cell (ONT). Base calling was conducted using Guppy v.4.0.11 to generate 156,751 reads with an average length of 7,040 bases (total, 1.10 Gb) for HB5002 and 28,202 reads with an average length of 11,561 bases (total, 326 Mb) for HB5008. For all software, default parameters were used unless otherwise noted. The raw sequencing data were filtered (Q < 10; length, <1,000 bases) using NanoFilt v.2.3.0 (16), yielding 122,280 reads (longest read, 241,328 bases; N_{50}, 15,542 bases; total, 977 Mb) for HB5002 and 21,082 reads (longest read, 149,286 bases; N_{50}, 23,988 bases; total, 276 Mb) for HB5008. For short-read sequencing, a Nextera DNA Flex library prep kit (Illumina) was

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TABLE 1 Genome statistics and features of *Thermus thermophilus* strains HB5002 and HB5008

Strain	Chromosome or plasmid	Length (bp)	GC content (%)	No. of CDSs[a]	No. of rRNAs	No. of tRNAs	GenBank accession no.
HB5002	Chromosome	2,042,948	69.5	2,196	6	54	AP024301
	Plasmid, pHB5002b	102,370	68.4	115	0	0	AP024302
	Plasmid, pHB5002c	12,913	69.0	6	0	0	AP024303
	Plasmid, pHB5002d	7,983	69.4	15	0	0	AP024304
HB5008	Chromosome	2,041,893	69.5	2,194	6	54	AP024305
	Plasmid, pHB5008b	102,367	68.4	115	0	0	AP024306
	Plasmid, pHB5008c	7,984	69.4	15	0	0	AP024307

[a] CDSs, coding DNA sequences.

used to generate libraries with an ~700-bp insert. Sequencing was performed on a MiSeq instrument (Illumina), yielding 1,746,904 (HB5002) and 945,019 (HB5008) paired-end reads (2 × 256 bases). The raw sequencing data were filtered (Q < 30; length, <10 bases) using fastp v.0.20.1 (17), yielding 1,407,029 paired-end reads (average length, 225 bases; total, 634 Mb) for HB5002 and 724,958 paired-end reads (average length, 224 bases; total 325 Mb) for HB5008.

The trimmed long- and short-read data were assembled using Unicycler v.0.4.8 (18) and polished using Pilon v.1.23 (19), generating a single circular chromosome and three circular plasmids for HB5002 and a single circular chromosome and two circular plasmids for HB5008. Rotation and circularity were confirmed via Unicycler. Automatic annotation was conducted using DFAST v.1.2.4 (20), and the genomic features are summarized in Table 1. FastANI analysis (21) indicated that the genome sequences of HB5002 and HB5008 showed ~100% average nucleotide identity (ANI) to each other, ≥98.53% ANI to those of the strains originating from Mine Hot Spring (HB8, GenBank accession number NC_006461.1; HB27, NC_005835.1 [22]; HB5018, NZ_AP024270 [15]; HC11; NZ_AP019801 [6]) but ≤97.64% ANI to those of the strains originating in Arima Hot Spring (AA2-20, NZ_AP019792.1; AA2-29, NZ_AP019794.1) (5). The results mirrored habitat-specific genomic conservation.

Data availability. The complete genome sequences of *T. thermophilus* HB5002 and HB5008 are available from DDBJ/EMBL/GenBank under the accession numbers summarized in Table 1. The raw sequencing data were deposited in the SRA database under the accession numbers DRA011332 (HB5002) and DRA011333 (HB5008).

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