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GENOME SEQUENCES

Complete Genome Sequencing of *Thermus thermophilus* Strain HC11, Isolated from Mine Geyser in Japan

Kentaro Miyazaki[a,b]

[a]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan
[b]Department of Computational Biology and Medical Sciences, The University of Tokyo, Kashiwa, Chiba, Japan

ABSTRACT *Thermus thermophilus* strain HC11 was isolated from Mine Geyser in Japan, where type strain HB8 was isolated 50 years ago. In this article, the complete genome sequence of HC11 is presented. HC11 shares the highest average nucleotide identity with HB8 among known *T. thermophilus* genomes (93.1%) with no genetic rearrangements.

Thermus thermophilus is an extremely thermophilic bacterium that grows optimally between 70 and 75°C. It was first isolated by Tairo Oshima about a half-century ago from Mine Geyser in Japan (1). Since then, many *Thermus* strains have been isolated from high-temperature environments (2, 3). The strains HB8 and HB27, both isolated from Mine Geyser, have been extensively studied due to their high thermostability (4–7). Several different techniques have been used, including genetic engineering (8), protein engineering (6, 9), structural genomics (10–12), and functional genomics (13).

I collected boiling water samples at Mine Geyser on the Izu Peninsula. The sample was spread over LB (1% [wt/vol] tryptone, 0.5% [wt/vol] yeast extract, 0.5% [wt/vol] NaCl) agar (1.5% [wt/vol]) plates and incubated at 65°C overnight. Several single colonies were isolated, and DNA sequencing analysis of 16S rRNA genes suggested that all the isolates belonged to *T. thermophilus* (>99% identity to the *T. thermophilus* HB8 gene). The whole-genome sequence was analyzed in one of the strains, HC11. Cells were grown to saturation in LB broth, and genomic DNA was purified using the MagAttract high-molecular-weight (HMW) DNA kit (Qiagen). Long- and short-read sequencing was performed using GridION (Oxford Nanopore Technologies [ONT]) and MiSeq (Illumina), respectively. Software analyses throughout this study were conducted using default parameter settings.

For long-read sequencing, genomic DNA was treated with Short Read Eliminator (Circulomics). A library was constructed with 1.0 μg of the resulting DNA using a ligation sequencing kit (ONT). The library was then analyzed on a FLO-MIN 106 R9.41 flow cell (ONT) for 12 h. Base-calling was conducted using Guppy v.3.0.3 to generate 68,638 reads (956 Mb) with an average length of 13,929 bases. The raw reads were filtered (average Phred quality values of >8.0) using NanoFilt v.2.3.0 (14). The longest read was 192,749 bases.

For short-read sequencing, a Nextera DNA Flex library prep kit (Illumina) was used to generate paired-end libraries with insertions that were approximately 350 bp long. Sequencing was performed using a MiSeq reagent kit v.2 (300 cycles) with reads that were 156 bp long. Adapter sequences and low-quality data were trimmed using fastp v.0.14.1 (15), yielding 2.59 million paired-end reads, spanning 398 Mb with an average length of 153.6 bp.

The long-read and short-read data were assembled *de novo* using Unicycler v.0.4.7 (16), followed by assembly polishing with Pilon v.1.23 (17). This yielded a single circular chromosome (1,910,731 bp, G+C content of 69.4%) and a plasmid (258,759 bp, G+C

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Address correspondence to miyazaki-kentaro@aist.go.jp.

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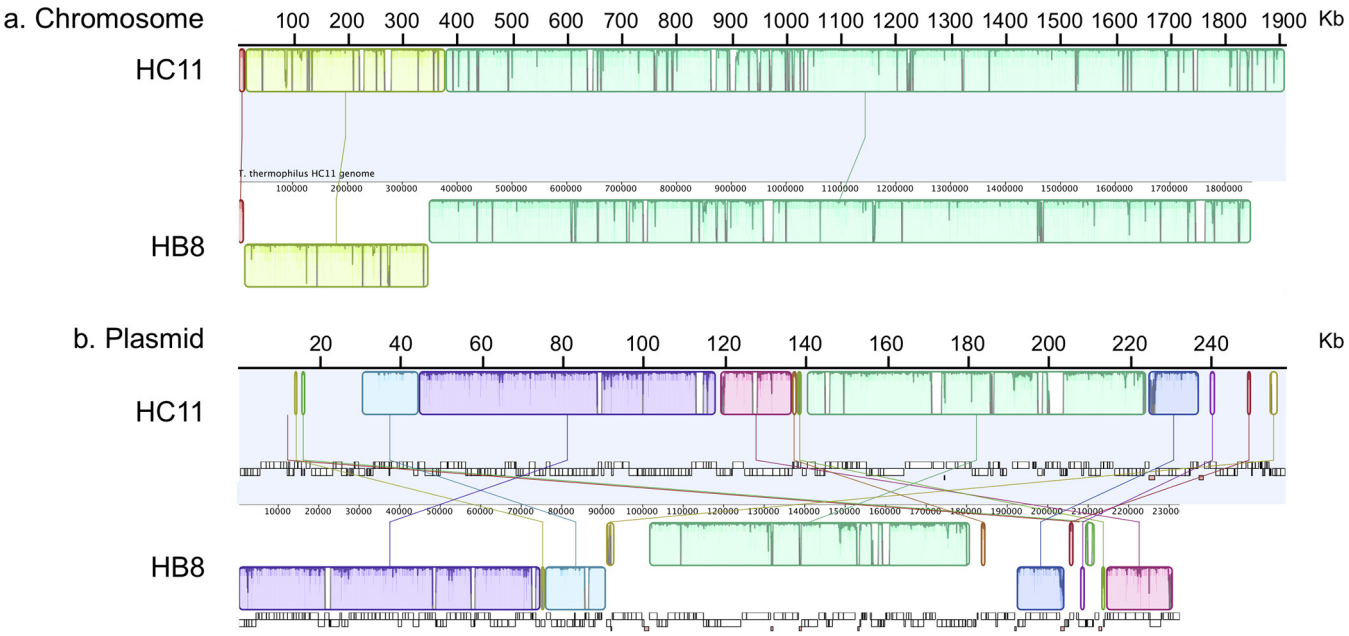

FIG 1 Genomic sequence alignment of *T. thermophilus* strains HC11 and HB8. (a) Chromosomes; (b) plasmids. The alignment was generated using Mauve software (http://darlinglab.org/mauve/mauve.html) with default settings.

content of 69.1%). The obtained sequence data were submitted to a Web-based annotation pipeline, DFAST v.1.1.0 (18), for automated annotation. The chromosome sequence of HC11 was most similar to HB8 (GenBank accession number NC_006461) among known *T. thermophilus* genomes, sharing an average nucleotide identity of 93.1% with HB8. Despite the known dynamic evolution patterns in *Thermus* species genomes (19, 20), no genetic rearrangements were observed (Fig. 1a). However, even though the nucleotide identity between the HC11 plasmid (pHC11) and the HB8 plasmid (pTT27) was high (97.8%), numerous genetic rearrangements were observed (Fig. 1b).

T. thermophilus strains are polyploids with four to five sets of chromosomes (21). A previous study of chromosome and plasmid copy numbers showed that they are detected in equal numbers. In the present study, the relative chromosome and plasmid copy numbers were estimated from the coverage of short reads to the complete chromosome/plasmid sequences. Sequence coverage was 180.4× ± 27.5× for the chromosome and 146.3× ± 79.8× for the plasmid. This suggested that the copy numbers were similar but a bit higher for the chromosome.

Data availability. The complete genome sequence of *T. thermophilus* HC11 is available from DDBJ/EMBL/GenBank under the accession numbers AP019801 for the chromosome and AP019802 for the plasmid. Raw sequencing data were deposited in the SRA database under the accession numbers DRR184352 (Illumina) and DRR184353 (Nanopore). The BioProject number is PRJDB8536, and the BioSample number is SAMD00178645.

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