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GENOME ANNOUNCEMENT

Draft Genome Sequence of *Thermus* sp. Strain RL, Isolated from a Hot Water Spring Located atop the Himalayan Ranges at Manikaran, India

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***Thermus* sp. strain RL was isolated from a hot water spring (90°C to 98°C) at Manikaran, Himachal Pradesh, India. Here we report the draft genome sequence (20,36,600 bp) of this strain. The draft genome sequence consists of 17 contigs and 1,986 protein-coding sequences and has an average G+C content of 68.77%.**

Bacteria belonging to the genus *Thermus* have been isolated from different hypothermal environments around the world. They are of special biotechnological interest due to their role in the production of thermostable enzymes and their resistance to denaturing physical and chemical factors (4). During the past few years, efforts have been directed toward sequencing complete genomes of these eubacteria to understand how biochemical processes and bacterial life are sustained under high temperature. So far, the genome sequences of *Thermus thermophilus* HB8. (GenBank accession no. AP008226), HB27 (6), and SGO-5JP17-16 (GenBank accession no. CP002777), *Thermus aquaticus* Y51MC23 (GenBank accession no. ABVK02000000), *Thermus scotoductus* SA-01 (5), and *Thermus* sp. strain CCB_US3_UF1 (10) are available.

The hot water springs located in the Himalayan ranges at Manikaran, Himachal Pradesh, India, are the hottest springs (90°C to 98°C) in India and contain low levels of helium (11). *Thermus* sp. strain RL was isolated from a hot water spring at Manikaran, India, and its genome was sequenced by using both the Roche 454 GS (FLX Titanium) system (3-kb paired-end library, 5,89,157 reads) and Sanger shotgun sequencing (40-kb library in SuperCos1 vector, 1,200 reads). Pyrosequencing and Sanger shotgun sequencing achieved ~82-fold coverage of the genome. The reads generated from both methods were assembled into 17 contigs (8 scaffolds) by using SeqMan ((DNAStar, Madison, WI) and Ray *De Novo* assembler version 0.0.3 set at a *k*-mer length of 49 (3). The final assembly was further validated by using the *in silico* tool BACCardI (2). The draft genome was annotated using RAST version 4.0 (1), NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html), and KEGG database (7). The tRNA and rRNA genes were predicted using tRNAScan-SE (9) and RNAmmer (8), respectively.

The draft sequence of *Thermus* sp. RL represents a genome size of 20,36,600 bp with an average G+C content of 68.77%. Genome annotations predicted 1,986 protein-coding genes and 710 hypothetical proteins. Strain RL has two rRNA operons (5S-16S-23S and 5S-5S-16S-23S) and 47 tRNA genes. A total of 111 tandem repeats, 2,825 CpG islands, and a single clustered regularly interspaced short palindromic repeat (CRISPR) element were found to be located on contig 3. Annotations by RAST revealed 330 subsystems and *T. thermophilus* HB27 (score, 538), HB8 (score, 526) and *T. aquaticus* (score, 412) as the closest neighbors of strain RL. Strain RL tested positive for the production of proteases on milk-casein plates and produced significantly larger zone of clearance compared to strains HB8 and HB27. Further determination of the taxonomic position of strain RL, comparative genomics of *Thermus* spp., and metagenomic analyses of the hot spring are under way. These analyses may provide significant clues in understanding the bacterial life and biochemical processes at higher temperatures and exploitation of *Thermus* sp. RL for thermostable enzymes.

Nucleotide sequence accession number. The genome sequence for *Thermus* sp. RL is available in GenBank under accession number AIJQ00000000.

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REFERENCES

1. **Aziz RK, et al.** 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics **9**:75. doi:10.1186/1471-2164-9-75.
2. **Bartels D, et al.** 2005. BACCardI—a tool for the validation of genomic assemblies, assisting genome finishing and intergenome comparison. Bioinformatics **21**:853–859.
3. **Boisvert S, Laviolette F, Corbeil J.** 2010. Ray: simultaneous assembly of reads from a mix of high-throughput sequencing technologies. J. Comput. Biol. **17**:1519–1533.
4. **Bruins ME, Janssen AEM, Boom RM.** 2001. Thermozymes and their applications. Appl. Biochem. Biotechnol. **90**:155–186.
5. **Gounder K, et al.** 2011. Sequence of the hyperplastic genome of the naturally competent *Thermus scotoductus* SA-01. BMC Genomics **12**:577. doi:10.1186/1471-2164-12-577.
6. **Henne A, et al.** 2004. The genome sequence of the extreme thermophile *Thermus thermophilus*. Nat. Biotechnol. **22**:547–553. doi:10.1038/nbt956.
7. **Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M.** 2004. The KEGG resource for deciphering the genome. Nucleic Acids Res. **32**:277–280.
8. **Lagesen K, et al.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. **35**:3100–3108.
9. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**:955–964.
10. **Teh BS, et al.** 2012. Complete genome sequence of the thermophilic bacterium *Thermus* sp. strain CCB_US3_UF1. J. Bacteriol. **194**:1240.
11. **Virk HS, Sharma AK, Sharma N.** 2002. Radon and helium monitoring in some thermal springs of North India and Bhutan. Curr. Sci. **82**:1423–1424.

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