

# Complete Genome Sequence of the Thermophilic, Obligately Chemolithoautotrophic Hydrogen-Oxidizing Bacterium *Hydrogenobacter thermophilus* TK-6<sup>∇</sup>

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***Hydrogenobacter thermophilus* is a thermophilic, obligately chemolithoautotrophic and aerobic hydrogen-oxidizing bacterium. It is unique in its ability to fix carbon dioxide via the reductive tricarboxylic acid cycle under aerobic conditions. It utilizes molecular hydrogen, elemental sulfur, or thiosulfate as the sole energy source. Here, we report the complete genome sequence of *H. thermophilus* TK-6.**

*Hydrogenobacter thermophilus* is the first obligately chemolithoautotrophic bacterium to be found among aerobic hydrogen oxidizers (9, 10). It has a unique sulfur-containing quinone called methionaquinone and a novel aminophospholipid (7, 8, 17). It shows an unusual composition of cellular fatty acids (10). This bacterium also has a unique ability to fix carbon dioxide via the reductive tricarboxylic acid (RTCA) cycle even under aerobic conditions (14). The genus *Hydrogenobacter* belongs to the order *Aquificales*, which constitutes a distinct phylum, *Aquificae*. The *Aquificae* are known to represent the deepest branch of bacteria, based on the phylogenetic analysis of its 16S rRNA sequence (3, 12). Five complete and two draft genome sequences of *Aquificales* bacteria have been reported so far (4, 13), but no genome sequences of the genus *Hydrogenobacter* have been reported despite a large number of biochemical and enzymatic studies and despite its unique features, such as a high growth rate and high oxygen tolerance.

The complete genome sequence of *H. thermophilus* TK-6 was determined by a whole-genome shotgun strategy using the Sanger method. Genomic libraries of 3 to 4 kb and around 40 kb were constructed and sequenced. The sequences of 18,050 reads were assembled using the Paracel Genome Assembler (Paracel, Pasadena, CA) to give 6.2-fold coverage of the genome. Sequence quality was assessed and protein coding genes were predicted using Consed (6), Glimmer (5), and Critica (1). Gaps among contigs were closed by sequencing the DNA fragments amplified by PCR using appropriate primers from genomic DNA. Coding sequences were annotated based on comparison with public databases (DDBJ, COG, and KEGG). tRNAscan-SE (11) was used to find tRNA genes.

The *H. thermophilus* TK-6 genome consists of a circular chromosome of 1,743,135 bp with an average G+C content of 44.0%. No plasmids were found in the genome. The number of predicted protein coding genes was 1,864. At least 22 genes

were found to be pseudogenes. There was one copy of 16S-23S-5S rRNA and 44 tRNA genes. An intron encoding a homing endonuclease was found in the 23S rRNA gene. The genome contains two large prophage-like genomic islands of 35,329 bp and 30,893 bp.

Since *H. thermophilus* is an obligate chemolithoautotroph, it must synthesize all necessary carbon compounds by fixing carbon dioxide. The genes for the enzymes that constitute the RTCA cycle and the gluconeogenesis enzymes for the synthesis of glucose-6-phosphate through the Embden-Meyerhof-Parnas pathway were identified. The genome has four sets of membrane-bound hydrogenase genes, as reported previously (16). TK-6 can grow on thiosulfate or elemental sulfur as an energy source, as well as on molecular hydrogen (2). The *sox* gene cluster for the sulfur oxidation protein complex, the *sqr* gene for sulfide:quinone oxidoreductase, and the *sorAB* genes for sulfite:cytochrome *c* oxidoreductase were identified in the genome. Four sets of genes for terminal oxidases for aerobic respiration have been identified. TK-6 grows anaerobically by denitrification (15). The genes for all enzymes for dissimilatory reduction of nitrate to dinitrogen were identified. TK-6 also assimilates nitrate and has the genes for assimilatory nitrate reductase and nitrite reductase.

**Nucleotide sequence accession number.** The genome sequence of *Hydrogenobacter thermophilus* TK-6 reported in this paper has been deposited in the DDBJ/GenBank/EMBL database under accession number AP011112.

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