



Draft Genome Sequence of *Methanothermobacter thermautotrophicus* WHS, a Thermophilic Hydrogenotrophic Methanogen from Washburn Hot Springs in Yellowstone National Park, USA

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ABSTRACT A thermophilic methanogen was enriched in coculture from Washburn Hot Springs (Yellowstone National Park, USA), grown on carbon dioxide and hydrogen, and subsequently sequenced. The reconstructed 1.65-Mb genome sequence for *Methanothermobacter thermautotrophicus* WHS contributes to our understanding of hydrogenotrophic, CO₂-reducing methanogenesis in geothermal ecosystems.

Methanogenesis is thought to be one of the earliest evolved microbial metabolisms (1, 2), and the production of methane, a potent greenhouse gas, has had a significant impact on the Earth's climate history (3). Carbon for canonical methanogenesis pathways derives from one of three primary sources: carbon dioxide, acetate, or methylated compounds (i.e., methanol, methylamines, methylsulfides) (4). The order *Methanobacteriales* is subdivided into two families, the *Methanobacteriaceae* and the *Methanothermaceae*. The genus *Methanothermobacter* falls within the family *Methanothermaceae* and is represented by the thermophilic, CO₂-reducing, hydrogenotrophic type strain *M. thermautotrophicus* ΔH, which was isolated from sewage sludge (5).

A coculture containing primarily *M. thermautotrophicus* WHS (99.6% relative abundance) was enriched from sediments obtained from Washburn Hot Springs (Yellowstone National Park [YNP], USA) and subsequently sequenced. *M. thermautotrophicus* WHS grew at an optimal temperature of 65°C in reduced medium (NaS₂) with CO₂ as the sole carbon source and H₂ as an energy source. H₂ decreased in the headspace from 95% to 0%, while CH₄ increased in the headspace from 0% to 46% over 128 h. The coculture medium steadily increased in optical density but only to a maximum of 0.04 at 80 h. Scanning electron microscopy and epifluorescence microscopy (using SYBR green DNA stain) demonstrated that the enriched methanogen formed long, thin (~3-μm-diameter) filaments similar to a methanogen previously cultivated from YNP (6).

Coculture genomic DNA was extracted using the FastDNA spin kit for soil from MP Biomedicals according to the manufacturer's procedure. Paired-end DNA sequencing (2 × 150 bp) was performed on the Illumina MiSeq platform with the v2 reagent kit. Sequence library preparation was performed with the NEBNext DNA library prep kit. Using the Illumina-utils method with default parameters (7), 317 Mb of raw reads was quality filtered and assembled using SPAdes v3.11.1 (8). Of the total quality-filtered reads (*n* = 801,518 pairs), 98.11% mapped to contigs belonging to *M. thermautotrophicus* WHS,

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and the remaining 1.89% mapped to a smaller metagenome-assembled genome (MAG) belonging to the phylum *Firmicutes* (no quality-filtered reads were unmapped). The assembled contigs were imported into *anvi'o* v4 (9) for coverage- and nucleotide frequency-based separation of the archaeal and bacterial genomes. Analysis of tetranucleotide frequencies and the mean coverage values of contigs resulted in two easily distinguishable bins, which were confirmed as members of the genera *Methanothermobacter* and *Caldanaerobacter* by *Centrifuge* v1 taxonomy identification software (10). Hidden Markov Models were used in *anvi'o* to estimate completeness and redundancy for both MAGs based on the presence of single-copy genes (162 for *Archaea* [11] and 139 for *Bacteria* [12]) and resulted in 99.38% completeness and 6.79% redundancy for *M. thermautotrophicus* WHS and 15.11% completeness and 0.72% redundancy for *Caldanaerobacter*. A full-length 16S rRNA gene sequence was recovered from the draft genome sequence of *M. thermautotrophicus* and was also recovered by cloning and sequencing from a separate DNA extraction; these two sequences were identical to one another, and phylogenetic analyses established their placement next to *M. thermautotrophicus*. We designated this strain of *M. thermautotrophicus* "WHS" for Washburn Hot Springs in Yellowstone National Park. The draft genome sequence was comprised of seven scaffolds (N_{50} , 343,349 bp) with a G+C content of 49.75% and a cumulative length of 1,654,216 bp, compared to 1,751,377 bp for the type strain, *M. thermautotrophicus* Δ H.

In *anvi'o*, open reading frames were identified using *Prodigal* v2.6.3 (13), and functional annotations were performed using the NCBI Clusters of Orthologous Groups of proteins (COGs) database (14). The potential for autotrophic CO₂ reduction was confirmed by the presence of genes essential to the methyl branch of the Wood-Ljungdahl pathway, including formate dehydrogenase, formyl-H₄F synthase, methenyl-H₄F cyclohydrase, methylene-H₄F dehydrogenase, methylene-H₄F reductase, and methyltransferase. CH₄ production was confirmed by the presence of genes for methyl coenzyme M reductase subunits, including *mcrABGCD*. Hydrogenotrophic metabolism was confirmed by the presence of multiple hydrogenase genes, including F₄₂₀-non-reducing [NiFe]-hydrogenase, heterodisulfide reductase, and F₄₂₀-reducing hydrogenase.

Nota bene, in 1980, prior to the wide availability of sequencing methods, three new strains of *M. thermautotrophicus*, YT1, YTA, and YTC, were isolated from Octopus Spring, Firehole Pool A, and Washburn Hot Springs, respectively, in Yellowstone National Park (6). Because each of these was cultivated on the same H₂/CO₂ medium and each displayed similar morphology and temperature optima, only the strain from Octopus Spring, *M. thermautotrophicus* YT1, was deposited in a culture collection (ATCC 29183). To our knowledge, however, there is no available genome sequence for this organism. Here, it is possible that we have cultivated and sequenced the genome of a population highly similar to *M. thermautotrophicus* YTC, which we are calling *M. thermautotrophicus* WHS.

Data availability. The raw sequence files (NCBI BioSample accession no. [SAMN16969497](https://www.ncbi.nlm.nih.gov/biosample/SAMN16969497)) and the draft genome sequence of *Methanothermobacter thermautotrophicus* WHS (NCBI BioSample accession no. [SAMN09381010](https://www.ncbi.nlm.nih.gov/biosample/SAMN09381010)) are available under NCBI BioProject accession no. [PRJNA475154](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA475154).

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