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, methysulfides) (4). The order Methanobacterales 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, 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 x 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 anv’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 anv’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 (N50 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 ΔH.

In anv’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-nonreducing [NiFe]-hydrogenase, heterodisulfide reductase, and F430-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) and the draft genome sequence of Methanothermobacter thermautotrophicus WHS (NCBI BioSample accession no. SAMN09381010) are available under NCBI BioProject accession no. PRJNA475154.

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