**Methylothermus subterraneus** sp. nov., a moderately thermophilic methanotroph isolated from a terrestrial subsurface hot aquifer

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A novel methane-oxidizing bacterium, strain HTM55T, was isolated from subsurface hot aquifer water from a Japanese gold mine. Strain HTM55T was a Gram-negative, aerobic, motile, coccoid bacterium with a single polar flagellum and the distinctive intracytoplasmic membrane arrangement of a type I methanotroph. Strain HTM55T was a moderately thermophilic, obligate methanotroph that grew on methane and methanol at 37–65 °C (optimum 55–60 °C). The isolate grew at pH 5.2–7.5 (optimum 5.8–6.3) and with 0–1 % NaCl (optimum 0–0.3 %). The ribulose monophosphate pathway was operative for carbon assimilation. The DNA G+C content was 54.4 mol% and the major fatty acids were C16:0 (52.0 %) and C18:1ω7c (34.8 %). Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain HTM55T was closely related to *Methylothermus thermalis* MYHTT (99.2 % 16S rRNA gene sequence similarity), which is within the class Gammaproteobacteria. However, DNA–DNA relatedness between strain HTM55T and *Methylothermus thermalis* MYHTT was < 39 %. On the basis of distinct phylogenetic, chemotaxonomic and physiological characteristics, strain HTM55T represents a novel species of the genus *Methylothermus*, for which the name *Methylothermus subterraneus* sp. nov. is proposed. The type strain is HTM55T (= JCM 13664T = DSM 19750T).

The existence of aerobic methanotrophs inhabiting extreme environments has been verified so far by cultivation experiments and direct detection of the methane monooxygenase genes specific to aerobic methanotrophs. Thermophilic methanotrophs have been isolated from such extreme environments and consist of the gammaproteobacterial (type I) genera *Methylothermus*, *Methylocaldum* and *Methylococcus* and the verrucomicrobial genus *Methylacidiphilum* (Bodrossy et al., 1997; Bowman et al., 1993; Eshinimaev et al., 2004; Op den Camp et al., 2009; Tsubota et al., 2005). The genus *Methylothermus* represents the most thermophilic methanotrophs isolated so far. It was reported that *Methylothermus* sp. strain HB, isolated from Hungarian hot springs, grew at temperatures up to 72 °C (Bodrossy et al., 1999). Unfortunately, strain HB was lost before it received a validly published name within the genus *Methylothermus*. The genus name *Methylothermus* was validly published by Tsubota et al. (2005), with strain MYHTT as the type strain of the type species *Methylothermus thermalis* gen. nov., sp. nov. Although strains HB and MYHTT seemed to belong to different species within the genus *Methylothermus*, on the basis of their low 16S rRNA gene sequence similarity (92.6 %), *Methylothermus thermalis* is currently the only species and strain MYHTT is the only currently extant strain of the genus *Methylothermus*.

In a previous study by our laboratory, a 16S rRNA gene sequence that was closely related to the genus *Methylothermus*...
was identified in subsurface geothermal aquifer water (71.5 °C) in the Hishikari gold mine, Japan (Inagaki et al., 2003). Further culture-independent analyses of microbial mats found on the tunnel floor in the gold mine, where the geothermal water (69 °C) was naturally discharging through the cracks of the basement rocks, demonstrated the predominance of methanotrophs of the genus *Methylothermus* in the mat community (Hirayama et al., 2005b). On the basis of these results, cultivation experiments were attempted by targeting a variety of metabolic pathways and several novel thermophilic chemolithoautotrophs have been isolated and characterized (Hirayama et al., 2005a; Takai et al., 2002, 2003). In this report, we describe a novel thermophilic methanotroph, strain HTM55T, from the gold mine microbial communities. Based on the taxonomic characterization, this report proposes that strain HTM55T represents a novel species of the genus *Methylothermus*.

Sample mixtures of hot water and microbial mats were taken from the discharge point (69 °C) of the subsurface geothermal aquifer water in the Hishikari gold mine, Kagoshima, Japan. The site description has previously been reported in detail (Hirayama et al., 2005b). For cultivation, Mmj medium was prepared by adding 15 μM CuSO4 to mj medium [containing Γ−1: 0.5 g NaHCO3, 0.25 g NH4Cl, 0.25 g NaNO3, 3.0 g NaCl, 14 mg K2HPO4, 80 mg CaCl2, 0.34 g MgSO4·7H2O, 0.42 g MgCl2·6H2O, 33 mg KCl, 0.05 mg NiCl2·6H2O, 0.05 mg Na2SeO3·5H2O, 0.01 mg Na2WO4, 2 mg Fe(NH4)2(SO4)2·6H2O, 1 ml trace mineral solution (Balch et al., 1979) and 1 ml vitamin mixture (Balch et al., 1979)]. Enrichments were started by inoculating 3 ml Mmj medium with 1 ml sample in 15 ml tubes. The headspace gas was composed of CH4/N2/CO2/O2 (40:40:15:5; 200 kPa). The final pH of Mmj medium was prepared by adding 15 μM CuSO4 to mj medium [containing Γ−1: 0.5 g NaHCO3, 0.25 g NH4Cl, 0.25 g NaNO3, 3.0 g NaCl, 14 mg K2HPO4, 80 mg CaCl2, 0.34 g MgSO4·7H2O, 0.42 g MgCl2·6H2O, 33 mg KCl, 0.05 mg NiCl2·6H2O, 0.05 mg Na2SeO3·5H2O, 0.01 mg Na2WO4, 2 mg Fe(NH4)2(SO4)2·6H2O, 1 ml trace mineral solution (Balch et al., 1979) and 1 ml vitamin mixture (Balch et al., 1979)]. Enrichments were started by inoculating 3 ml Mmj medium with 1 ml sample in 15 ml tubes. The headspace gas was composed of CH4/N2/CO2/O2 (40:40:15:5; 200 kPa). The final pH of Mmj medium was approximately pH 6.2. The tubes were incubated for 2 weeks at 55, 60 or 70 °C.

Methane-oxidizing coccolid cells grew only in the tube incubated at 55 °C. The enriched culture was purified by a serial dilution-to-extinction technique, which was repeated at least four times before the culture was identified as being pure. The following is a brief description of the technique. An active culture was diluted 1:10 in Mmj medium nine times consecutively. Each tube was incubated under the same conditions as for the enrichment. The most dilute tube indicating cell growth was further diluted and incubated in the same manner. Finally, a 1:2 dilution was conducted to obtain a pure culture that theoretically originated from a single cell. The purity of the culture was verified by successful direct sequencing of the partial 16S rRNA gene at least three times using independently grown cultures. Growth of the isolate on multicarbon compounds was examined repeatedly but was not observed, indicating that there were no heterotrophic contaminants in the culture. The isolate was designated strain HTM55T. Strain HTM55T was maintained and examined afterwards in Mmj medium supplemented with a reduced CuSO4 concentration (1 μM) because of an obvious inhibitory effect of CuSO4 at concentrations above 4 μM. All cultivations were in liquid Mmj medium with the same headspace gas composition as the enrichment, unless otherwise mentioned. Colony formation was examined twice on Mmj agar with incubation in a gas-tight chamber containing CH4/N2/CO2/O2 (40:45:5:10; 100 kPa); however, colony formation was not observed after 3 weeks. Therefore, the ability of strain HTM55T to form colonies has not yet been demonstrated.

Cell morphology was determined by phase-contrast microscopy with an Olympus BX51 microscope and by electron microscopy with a JEOL JEM-1210 electron microscope at 120 kV. Cells of strain HTM55T in exponential and early stationary phase were coccolid, 0.8–1.4 μm in diameter and motile with a single polar flagellum (Fig. 1a, b). Electron microscopy of negatively stained cells revealed a lot of flagella removed from the cells, with only a few of the cells connected to the flagellum. It is unclear whether the flagella fell out actively or whether they were removed passively by physical impact during cultivation or sample preparation. No cyst-like cells were evident in the culture after 1 month of storage at growth temperatures. A bundle of type I intracytoplasmic membranes was observed in the ultrathin sections of the cells (Fig. 1c, d). Most of the cells contained intracellular inclusions, seen as low-electron density granules, probably comprising poly-β-hydroxybutyrate (Fig. 1b, c).

Utilization of various energy and carbon sources other than methane was examined by adding one of the following substrates to Mmj medium: 0.05% (w/v) formate, acetate, citrate, succinate, glucose, fructose, ribose, mannitol, methylamine, dimethylamine, methanol, ethanol and 2-propanol or 0.1% (w/v) yeast extract and Casamino acids. Methane in the headspace of the tube was replaced with N2 in these experiments. Strain HTM55T was found to grow only on methane and methanol as the carbon and energy source. Growth on methanol was further tested at different concentrations (0.025–3 %, v/v). Strain HTM55T grew at all methanol concentrations tested and the highest cell density was achieved at 0.5% methanol. None of the other substrates supported growth. Nitrogen sources were examined by adding one of the following substrates (0.05%, w/v) to Mmj medium instead of both NH4Cl and NaNO3: NH4Cl, NaNO3, Tris, urea, formamide, methylamine, dimethylamine, Casamino acids, glycine, L-tryptophan, L-lysine, L-glutamic acid or L-aspartic acid. Strain HTM55T grew well with NH4Cl, NaNO3, Tris, Casamino acids and L-aspartic acid as the nitrogen source. Weak growth was observed with Tris, urea, L-lysine and L-glutamic acid. No growth was observed with any of the other nitrogen sources tested (formamide, methylamine, dimethylamine, glycine and L-tryptophan). In addition, no growth was found with N2 in the headspace gas in the absence of any other nitrogen compounds. PCR experiments using two pairs of reported primers to amplify the *nifH* gene, which encodes the nitrogenase iron protein involved in nitrogen fixation, also did not generate plausible amplification products from strain HTM55T DNA (Mehta et al., 2003; Zehr & McReynolds, 1989). Three strains, *Methylococcus*...
capsulatus ATCC 19069T, Methylosinus sporium DSM 17706T and Methylocella silvestris DSM 15510T, were used as positive controls in the nifH amplification.

Growth conditions for temperature, pH, NaCl concentration and Cu²⁺ concentration on methane were examined in Mmj medium and are summarized in Table 1. Strain HTM55T grew at 37–65°C (optimum 55–60°C); no growth was observed below 33°C or above 68°C. The effects of pH and NaCl concentration on growth were tested at 55°C. The pH of Mmj medium was adjusted with HCl or NaOH after the tube was sealed with headspace gas. Strain HTM55T grew at pH 5.2–7.5 (optimum pH 5.8–6.3); no growth was observed at pH 5.0 or 8.0. Strain HTM55T was moderately acidophilic compared to other thermophilic type I methanotrophs (Table 1). Strain HTM55T grew with 0–1 % (w/v) NaCl (optimum 0–0.3 % NaCl); no growth was observed with 1.5 % NaCl. The effect of Cu²⁺ concentration on growth was tested under optimum growth conditions (55°C, pH 6.3 and 0.3 % NaCl). Strain HTM55T grew with 0–3 μM CuSO₄ and optimum growth was observed with 0.5 μM CuSO₄ (specific growth rate: 0.30 h⁻¹; doubling time: 2.3 h). The final cell yield was 2–7×10⁷ ml⁻¹ and the OD₆₆₀ was 0.07–0.25. CuSO₄ concentrations above 4 μM had an inhibitory effect on growth and the presence of 20 μM CuSO₄ completely inhibited the growth of strain HTM55T. The growth rate and cell yield on methanol (0.5 %, v/v) were equal to those on methane.

The presence of soluble methane monooxygenase (sMMO) in strain HTM55T was tested both by an enzyme activity assay and PCR amplification. To measure sMMO activity, the naphthalene oxidation assay for a liquid culture (Csáki et al., 2003) was performed with whole cells of strain HTM55T grown without a Cu²⁺ supplement. The genes mmoX, mmoY, mmoZ, mmoB and mmoC, which encode sMMO, were examined by PCR amplification as described elsewhere (McDonald et al., 1995; Miguez et al., 1997). The presence of sMMO in strain HTM55T was not proven by any of these methods. Meanwhile, the presence of particulate monooxygenase (pMMO) was confirmed by the identification of pmoA by PCR and sequencing experiments (see below).

To determine carbon fixation capabilities, a cell-free extract was prepared from a cell pellet (0.3–0.5 g, wet weight) harvested during the exponential growth phase. The cells were resuspended in 3 ml 0.1 M potassium phosphate buffer (pH 7.0) to assay for hexulose phosphate synthase and hydroxypropionate reductase or 3 ml 0.1 M HEPES/NaOH (pH 8.3), 10 mM MgCl₂ buffer to assay for ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). The cell suspension was sonicated for four periods of 30 s (150 W, 20 kHz) with ice-cooling and then centrifuged at 15 000 g for 10 min. The supernatant was used for the enzyme activity assays. For hexulose phosphate synthase in the ribulose monophosphate (RuMP) pathway, the colorimetric assay was carried out as described by Bodrossy et al. (1997), except for a minor change in the formaldehyde concentration in this study. The formaldehyde concentration in the enzyme reaction solution was reduced to one-tenth of the original method because the original concentration (67 mM) failed.
to lead to reasonable colour development in the following colorimetric assay developed by Nash (1953). Two negative controls were employed in the colorimetric assay: (1) a reaction without ribulose-5-phosphate; and (2) a reaction with the inactivated enzyme by heating at 96°C for 15 min.

Hydroxypyruvate reductase in the serine pathway was assayed as described by Large & Quayle (1963) and Rubisco in the Calvin–Benson cycle was assayed as described by Maeda et al. (2002).

Hexulose phosphate synthase activity was detected, indicating that strain HTM55T assimilated carbon by the RuMP pathway. The presence of the RuMP pathway was further suggested by the presence of the hps and phi genes encoding 3-hexulose-6-phosphate synthase and 6-phospho-3-hexulose isomerase, respectively. The complete sequences of hps and phi from strain HTM55T were determined on the basis of the results of the metagenomic analyses used for this gold mine environment (H. Takami, S. Nishi & A. Toyoda, unpublished data).

Neither activity of hydroxypyruvate reductase in the serine pathway nor activity of Rubisco in the Calvin–Benson cycle was detected. PCR amplification of cbbL encoding the large subunit of Rubisco was also attempted using three pairs of primers as reported elsewhere (Alfreider et al., 2003; Elsaied & Naganuma, 2001; Nanba et al., 2004), but no plausible amplification products were generated.

In each experiment for metabolic enzymes and genes, Methylothermus subterraneus sp. nov. was used as positive or negative controls. Representative metabolic characteristics of strain HTM55T and those of physiologically and phylogenetically related gammaproteobacterial methanotrophs are summarized in Table 1.

For analysis of the whole-cell fatty acids of strain HTM55T, fatty acid methyl esters were prepared as described previously (Hirayama et al., 2005a) using cells grown exponentially at 55°C. Fatty acid methyl esters were extracted with hexane and analysed using a Finnigan TRACE DSQ GC-MS system (Thermo Fisher Scientific) equipped with a polar capillary column SP-2560 (Supelco, 100 m × 0.25 mm × 0.2 μm) under a helium flow of 1.8 ml min⁻¹ and an oven temperature program increasing from 155°C (1 min) to 175°C at 1°C min⁻¹. The major fatty acids of strain HTM55T and related methanotrophs are summarized in Table 1 and the

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*The value is a mean of three determinations.

Table 1. Characteristics of strain HTM55T and physiologically and phylogenetically related gammaproteobacterial methanotrophs

Strains: 1, HTM55T (this study); 2, Methylothermus thermalis MYHTT (Tsubota et al., 2005); 3, Methylocaldum (Methylocaldum szegediense OR2T, strain O-12 and/or strain H-11; Bodrossy et al., 1997; Eshinimaev et al., 2004); 4, Methylococcus thermophilus (6 strains; Bowman et al., 1993); 5, Methylohalobius crimeensis 10KiT and 4Kr (Heyer et al., 2005). +, Positive; +/−, strain dependent; −, negative; ND, no data available.
detailed compositions are given in Supplementary Table S1 (available in IJSEM Online). The major fatty acids of strain HTM55T were C16:0 (52.0 %) and C18:1ω7c (11-ct-octadecenoic acid; 34.8 %). C16:0 is a major component in thermophilic and thermotolerant species of proteobacterial type I methanotrophs. Mesophilic strains of the genus *Methylohalobius*, the closest genus to *Methylothermus*, also indicate relatively high C16:0 ratios (Heyer et al., 2005). Other type I methanotrophs belonging to the genera *Methylomonas*, *Methylbacterium*, *Methylococccus*, * METHYLOSPAHERA* and *Methylosoma* contain less than 20 % C16:0 fatty acids (Bowman et al., 1993, 1995, 1997; Kalyuzhnaya et al., 2005; Rahalkar et al., 2007). It was revealed that both strain HTM55T and *Methylothermus thermalis* MYHTT possess C18:1ω7c fatty acids as major components. Other studies showed that C18:1ω7c fatty acids are, if contained, only minor components in most type I methanotrophs, including the thermophilic strains of the genera *Methylocaulidum* and *Methylococcus* (Bowman et al., 1993, 1995, 1997; Eshinimaev et al., 2004; Kalyuzhnaya et al., 2005; Rahalkar et al., 2007). An exception is the mesophilic strains of *Methylohalobius*, which are reported to contain more than 50 % C18:1ω7c (Heyer et al., 2005). The thermotolerant *Methylohalobius* sp. IT-9 (Hirayama et al., 2007) also contains 49.6 % C18:1ω7c (H. Hirayama, T. Nunoura & K. Takai, unpublished data). The predominance of fatty acid C18:1ω7c may be one of the characteristics commonly found in the phylogenetically related genera *Methylothermus* and *Methylohalobius*. Meanwhile, C18:1ω7c fatty acids are generally abundant in the type II methanotrophs of the class *Alphaproteobacteria* (Bowman et al., 1993; Dedys et al., 2007).

The G + C content of the genomic DNA was determined as described by Tamaoka & Komagata (1984). The G + C content of strain HTM55T was 54.4 mol%, which is similar to the values of thermophilic members of the genus *Methylocalidum* (56.5–58.5 mol%) (Bodrossy et al., 1997; Eshinimaev et al., 2004). However, the G + C content of strain HTM55T was far lower than that of *Methylothermus thermalis* MYHTT (62.5 mol%; Table 1).

The nearly complete sequence (1444 bp) of the 16S rRNA gene from strain HTM55T was amplified by PCR as described previously (Hirayama et al., 2005b) and directly sequenced from both strands with a DNA sequencer (model 3130; Applied Biosystems). The 16S rRNA gene sequence was subjected to similarity analysis using the FASTA algorithm (http://fasta.ddbj.nig.ac.jp/top-j.html) and GENETYX-MAC version 14.0.5 software (Genetyx). The closest relatives of strain HTM55T were the environmental clone HAU-DUB26, which had been obtained previously from the same gold mine environment (99.6 % 16S rRNA gene sequence similarity; Hirayama et al., 2005b) and *Methylothermus thermalis* MYHTT (99.2 %; Tsubota et al., 2005). The results strongly indicated that strain HTM55T belonged to the genus *Methylothermus*. Strain HTM55T was moderately related to *Methylothermus* sp. HB (92.6 %; Bodrossy et al., 1999) and the mesophilic and halophilic strain *Methylohalobius crimeensis* 10KII (90.5 %; Heyer et al., 2005). Members of other methanotrophic genera were only distantly related to strain HTM55T (≤88.1 %).

For phylogenetic analysis, 16S rRNA gene sequences from type I and type X methanotrophic bacteria of the class *Gammaproteobacteria* were aligned using ARB software and corrected manually where necessary. Only unambiguously aligned nucleotide positions were used for the analysis. A phylogenetic tree was constructed using 835 sequence positions using the neighbour-joining algorithm in the SeaView version 4.0 package and evolutionary distances estimated with Kimura’s correction. The phylogenetic tree indicated that strain HTM55T belonged to a deeply branching and distinct lineage comprising the genera *Methylothermus* and *Methylohalobius* within the type I and type X methanotrophs of the class *Gammaproteobacteria* (Fig. 2).

To amplify the full sequence of pmoA, the following primers were developed on the basis of full sequences of the pmoCAB operons (encoding pMMO) from the GenBank database: pmoC-F2 5’-GGTCCGTTCTAGTGYTGCC-3’ and pmoB-R1 5’-TACGTCRWACAGTGRAT-3’, corresponding to positions 646–665 of pmoC and 139–157 of pmoB, respectively, of *Methylococcus capsulatus* Bath (GenBank accession no. U94337). Amplification was performed with Ex Taq polymerase (TaKaRa Bio) with the following 30-cycle programme: 96 °C for 25 s, 45 °C for 45 s and 72 °C for 90 s. The PCR fragment (approx. 1.3 kb) was directly sequenced from both strands and subjected to similarity analysis as described above. As summarized in Supplementary Table S2, the pmoA sequence of strain HTM55T was very similar to the environmental pmoA clone pmoU-1, which was isolated from the same gold mine environment (99.0 % gene sequence similarity, 100 % amino acid sequence similarity; Hirayama et al., 2005b), *Methylothermus thermalis* MYHTT (98.7 % gene sequence similarity, 100 % amino acid sequence similarity) and *Methylothermus* sp. HB (90.6 % gene sequence similarity, 97.7 % amino acid sequence similarity). Strain HTM55T was also related to *Methylohalobius crimeensis* 10KII (83.0 % gene sequence similarity, 92.1 % amino acid sequence similarity). The alignment of the deduced partial pmoA sequences from various methanotrophs (nine genera of type I and one genus of type II) presented several distinctive residues that were shared with the genera *Methylothermus* and *Methylohalobius* (Supplementary Fig. S1).

The similarities between members of the genera *Methylothermus* and *Methylohalobius* in the 16S rRNA gene and pmoA sequences are consistent with the reports by Heyer et al. (2005) and Tsubota et al. (2005), suggesting that these two genera within the currently known gammaproteobacterial genera are closely related.

Because the 16S rRNA sequence of strain HTM55T was quite similar to that of *Methylothermus thermalis* MYHTT, genomic relatedness between these strains was examined by DNA–DNA hybridization (37 °C, 4 h) as described by Ezaki et al. (1989) using DNA from both strains as probes in reciprocal experiments. DNA–DNA relatedness between
these strains was <39%. Generally, strains showing <70% DNA–DNA relatedness are recognized as representing separate species (Wayne et al., 1987). According to this criterion, strain HTM55T represents a novel species of the genus Methylothermus.

Other characteristics also distinguished strain HTM55T from Methylothermus thermalis MYHTT, as shown in Table 1. The difference in the DNA G+C content between both strains was as much as 8.1 mol%. Both strains possessed C18:1 fatty acids; however, the predominant fatty acid in strain HTM55T was C18:1ω7c (11-cis-octadecenoic acid) while that of Methylothermus thermalis MYHTT is reported as C18:1ω9c (9-cis-octadecenoic acid). The optimum growth temperatures of the two strains were similar. However, strain HTM55T preferred more acidic conditions and lower salinity than Methylothermus thermalis MYHTT; the optimum pH for strain HTM55T (pH 5.8–6.3) was out of the range for Methylothermus thermalis MYHTT (pH 6.5–7.5). The strains also differed in their abilities to utilize the nitrogen sources nitrite, aspartic acid, tryptophan and formamide; strain HTM55T grew with nitrite and aspartic acid, but not with tryptophan and formamide, and vice versa for Methylothermus thermalis MYHTT.

Unlike Methylothermus thermalis MYHTT, microscopy showed that cells of strain HTM55T were motile, possessed a single flagellum and produced many intracellular inclusions (Fig. 1).

Considering these differences in morphological, physiological and chemotaxonomic characteristics between strain HTM55T and Methylothermus thermalis MYHTT, in addition to the phylogenetic analysis, it is evident that strain HTM55T represents a novel species of the genus Methylothermus, for which the name Methylothermus subterraneus sp. nov. is proposed.

### Emended description of the genus Methylothermus Tsubota et al. 2005

*Methylothermus* (Me.thy.9lo.ther.9mus. N.L. n. methyl the methyl group; N.L. masc. subst. from Gr. adj. thermos hot; Methylothermus methyl-using thermotolerant organism).

Gram-negative, motile or non-motile coccoids, 0.6–1.4 μm in diameter. Reproduction by normal cell division, with no resting stages (exospores, Azotobacter-type cysts and lipid cysts). Members possess pMMO and a typical type I intracytoplasmic membrane system, but not sMMO. Moderate thermophiles; grow at 37–67 °C (optimum 55–60 °C). Obligate methanotrophs; utilize methane or methanol via the RuMP pathway. The major fatty acids are C16:0 and C18:1. Belongs to the class Gammaproteobacteria (type I methanotrophs); the closest genus is Methylohalobius. Habitats are terrestrial hot springs. The type species is Methylothermus thermalis.

**Description of Methylothermus subterraneus sp. nov.**

*Methylothermus subterraneus* (sub.ter.ra.9ne.us. L. masc. adj. subterraneus under the earth, indicating the first isolation source).

Shows the following characteristics in addition to those given in the genus description. Motile coccoids, 0.8–1.4 μm in diameter. Grows at 37–65 °C (optimum 55–60 °C) and pH 5.2–7.5 (optimum pH 5.8–6.3). Grows well without NaCl (optimum 0–0.3 % NaCl), but not with 1.5 % NaCl. Utilizes methane and methanol via the RuMP pathway and grows on ammonia, nitrate, nitrite, Casamino acids, l-aspartic acid, l-lysine, l-glutamic acid, Tris and...
urea as nitrogen sources. The major fatty acids are C16:0 and C18:1ω7c.

The type strain, HTM55T (=JCM 13664T=DSM 19750T), was isolated from subsurface hot aquifer water in the Hishikari gold mine, Kagoshima, Japan. The DNA G+C content of the type strain is 54.4 mol%.

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References


