Characterization of Three Thermophilic Strains of *Methanotherix* (“*Methanosaeta*”) *thermophila* sp. nov. and Rejection of *Methanotherix* (“*Methanosaeta*”) *thermoacetophila*

YOICHI KAMAGATA,^{1*} HIROKO KAWASAKI,² HIROSHI OYAIZU,³ KAZUNORI NAKAMURA,¹ EIICHI MIKAMI,¹ GINRO ENDO,⁴ YOSUKE KOGA,⁵ AND KAZUhide YAMASATO²

Fermentation Research Institute, Agency of Industrial Science and Technology, Tsukuba, Ibaraki 305¹;
Institute of Applied Microbiology² and Department of Agricultural Chemistry,³ The University of
Tokyo, Tokyo 113; Department of Civil Engineering, Tohoku Gakuin University, Tagajo-shi,
Miyagi 985⁴; and Department of Chemistry, University of Occupational and
Environmental Health, Japan, Yahatanishi-ku, Kitakyushu 807,⁵ Japan

Three thermophilic *Methanotherix* (“*Methanosaeta*”) strains, strains P_T^T (= DSM 6194^T) (T = type strain), CALS-1 (= DSM 3870), and Z-517 (= DSM 4774), were characterized chemotaxonomically and compared with five mesophilic strains, *Methanotherix soehngenii* (“*Methanosaeta concilii*”) GP6 (= DSM 3671), Opfikon (= DSM 2139), FE (= DSM 3013), U_A, and P_M. These methanogens were exclusively acetotrophic and had a characteristic sheathed structure. The DNA base compositions of the strains which we studied ranged from 50.3 to 54.3 mol% guanine plus cytosine. The thermophilic strains often had phase-refractive gas vesicles inside their cells. Denaturing electrophoresis of proteins showed that the mesophilic and thermophilic *Methanotherix* strains formed two distinct groups and that there were differences in protein patterns between the groups. The difference between the thermophiles and mesophiles was also verified by comparing partial 16S rRNA sequences (ca. 30 base differences in ca. 540 bases). On the basis of our results, we propose the name *Methanotherix thermophila* for the three thermophilic strains. The type strain of *M. thermophila* is strain P_T^T (= DSM 6194). We also propose that the name *Methanotherix thermoacetophila* (“*Methanosaeta thermoacetophila*”), which was given to strain Z-517 (type strain), should be rejected because of its description, which was based on an enrichment culture, was inadequate.

Of the methanogenic microorganisms, members of the genus *Methanotherix* play a very important role as acetate consumers in anaerobic digestion of organic compounds (6). *Methanotherix* spp. are sheathed, rod-shaped, aceticlastic methanogens, and the first *Methanotherix* strain was described by Söhngen (24). Later, Barker described this organism as *Methanobacterium soehngenii* (1). However, no axenic *Methanotherix* cultures were obtained for many years because of difficulties in cultivation.

A *Methanotherix* strain was first isolated as an “acetate organism” (strain Opfikon) by Zehnder et al., and later these authors described this strain as *Methanotherix soehngenii* (9, 27). After this description, several mesophilic *Methanotherix* strains were isolated or “highly purified” (8, 10, 18, 20, 26). A thermophilic *Methanotherix* strain was first enriched from thermal lake mud by Nozhevnikova and Chudina (17), and these authors named their enriched organism (strain Z-517) *Methanotherix thermoacetophila*. Zinder et al. first obtained a pure culture of thermophilic *Methanotherix* sp. strain CALS-1 from a thermophilic anaerobic digester (28).

Recently, Patel and Sprott proposed the genus *Methanosaeta* (21). According to the proposal of these authors, *Methanosaeta concilii* is the only valid species which contains mesophiles, and the type strain is strain GP6, which was described as *Methanotherix concilii* by Patel (20). However, there is controversy concerning the correct designation because *Methanosaeta concilii*, *Methanotherix concilii*, and *Methanotherix soehngenii* are considered synonyms (3).

Patel and Sprott also proposed that *Methanotherix thermoacetophila*, which was originally described by Nozhevnikova and Chudina (17), should be reclassified as *Methanosaeta thermoacetophila* (21). However, the description of the type strain (strain Z-517) of “*Methanotherix thermoacetophila*” (“*Methanosaeta thermoacetophila*”) was not validly published because it was not based on an axenic culture (17), and the level of taxonomic relatedness between strains Z-517 and CALS-1 is not known. (Patel and Sprott have indicated that strain Z-517 has been purified and now is available as a pure culture [21], but no information about the taxonomic characteristics of the pure culture has been published.) Very recently, we isolated a new thermophilic *Methanotherix* (“*Methanosaeta*”) strain, strain P_T^T (T = type strain), from a thermophilic anaerobic digester and described its properties (11). In this study, we chemotaxonomically characterized three thermophilic *Methanotherix* strains, including our isolate, and compared them with *Methanotherix soehngenii* (“*Methanosaeta concilii*”). We propose that a new species, *Methanotherix thermophila*, should be established and that the name “*Methanotherix thermoacetophila*” (“*Methanosaeta thermoacetophila*”) should be rejected.

MATERIALS AND METHODS

Sources of microorganisms. *Methanotherix soehngenii* (“*Methanosaeta concilii*”) Opfikon (= DSM 2139), *Methanotherix soehngenii* FE (= DSM 3013), *Methanotherix soehngenii* GP6 (= DSM 3671), “*Methanotherix thermoacetophila*” (“*Methanosaeta thermoacetophila*”) Z-517 (= DSM

* Corresponding author.

TABLE 1. Properties of *Methanothrix* strains^a

Species	Strain	DSM no.	Optimum temp (°C) ^b	Growth factor(s) ^c	Guanine-plus-cytosine content (mol%) ^d
<i>Methanothrix soehngenii</i>	Opfikon ^e	2139	37	SF ^f	51.9
	FE ^e	3013	35	ND ^g	52.6
	GP6	3671	35–40	SF, vitamins ^h	50.3
	U _A ^e		37	ND	51.1
	P _M ^e		37	ND	50.9
<i>Methanothrix thermophila</i>	CALS-1	3870	60	CoM, biotin ⁱ	54.2
	Z-517	4774	55	ME, vitamins ^j	54.3
	P _T ^T	6194	55–60	SF ^f	52.7

^a The cells of all of the strains were sheathed rods, and all of the strains grew at a neutral pH (data from references 9 through 11, 17, 20, 21, and 26 through 28) and were not susceptible to lysis.

^b Data from references 9 through 11, 17, 20, 21, and 26 through 28.

^c SF, sludge fluid; CoM, coenzyme M (mercaptoethane sulfonate); ME, manure extract.

^d Data for strains Opfikon, FE, GP6, U_A, P_M, and P_T^T from references 10, 11, and 26.

^e Highly purified culture.

^f Sludge fluid had a stimulatory effect but was not required (9, 11).

^g ND, not determined.

^h Sludge fluid and vitamins had a stimulatory effect but were not required (20, 21).

ⁱ Coenzyme M had a stimulatory effect but was not required (28).

^j Manure extract and vitamins had a stimulatory effect but were not required (17, 21).

4774), and *Methanothrix* sp. strain CALS-1 (= DSM 3870) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), Braunschweig, Germany. *Methanothrix soehngenii* U_A and P_M and *Methanothrix* sp. strain P_T^T (= DSM 6194^T) were obtained in our laboratory (10, 11). The cultures of mesophilic strains Opfikon, FE, U_A, and P_M were highly purified but not axenic (10, 21, 26). However, these cultures were considered to be pure enough for analyses of protein patterns of whole cells, 16S rRNA sequences, and DNA base compositions, as well as the other physiological studies described below.

Cultivation. *Methanothrix soehngenii* Opfikon, FE, GP6, U_A, and P_M and *Methanothrix* sp. strain P_T^T were cultivated on DSM medium 334 (5). *Methanothrix* sp. strain CALS-1 and "*Methanothrix thermoacetophila*" Z-517 were cultivated on DSM medium 387 (5). Unless otherwise stated, all of the strains were cultured at 37°C (strains Opfikon, FE, GP6, U_A, and P_M) or 55°C (strains CALS-1, Z-517, and P_T^T) in 125-ml serum vials containing 30 ml of medium or in 1,000-ml bottles containing 500 ml of medium under an N₂-CO₂ (80/20, vol/vol) atmosphere. The serum vials were closed with butyl rubber stoppers that were sealed with aluminum crimps.

Electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed as described by Laemmli (12), using a gradient gel containing 4 to 20% acrylamide. Portions (approximately 5 µg) of denatured protein from whole cells were loaded on the gel, and bovine milk α-lactalbumin (molecular mass, 14 kDa), soybean trypsin inhibitor (20 kDa), bovine erythrocyte carbonic anhydrase (30 kDa), egg white ovalbumin (43 kDa), bovine serum albumin (67 kDa), and rabbit muscle phosphorylase b (94 kDa) were used as the M_r standards. The gel was stained with Coomassie brilliant blue R-250.

Determination of DNA base composition. We determined the DNA base compositions of strains Z-517 and CALS-1 in this study. DNA base composition data for the other strains were obtained from previously published papers (10, 11, 26). DNA was extracted and purified by using the methods of Beji et al. (2) and Saito and Miura (22). The purified DNA was hydrolyzed with P1 nuclease (GC kit; Yamasa Shoyu Co., Chiba, Japan) and then with alkaline phosphatase from *Escherichia coli* (Wako Pure Chemicals Industries, Ltd.,

Osaka, Japan), as described by Tamaoka and Komagata (25). Guanine-plus-cytosine contents were determined by reversed-phase high-performance liquid chromatography (Shimadzu model LC-6A system). Separation was achieved at 40°C by using a flow rate of 1 ml/min, a column of CLC-ODS (Shimadzu), and 5% methanol in 10 mM phosphate buffer (pH 3.5) as the mobile phase. Each deoxyribonucleoside was detected by determining the A₂₆₀, and an equimolar mixture of four deoxyribonucleosides was used as the standard.

16S rRNA sequencing. We determined partial 16S rRNA sequences for strains Opfikon, GP6, U_A, P_M, CALS-1, Z-517, and P_T^T. The RNA was extracted by using previously described methods (13, 19). Partial 16S rRNA sequences were determined by using the reverse transcriptase procedure (13). In this procedure, we used three nucleotide primers that were complementary to universally conserved regions, TACCGCGGCGGCTGGC (position 520, reverse direction), CAATTCCTTTAAGTTTC (position 920, reverse direction), and ACGGGCGGTGTGTGC (position 1400, reverse direction) (*E. coli* 16S rRNA numbering). These primers were made by using an ABI model 381A DNA synthesizer. The DNA sequences were determined by the dideoxynucleotide chain termination method (23).

Lipid analysis. The membrane polar lipids of strain P_T^T were extracted and analyzed by using a previously described method (16).

Other methods. Physiological characteristics were determined by using the minimal standards described by Boone and Whitman (4).

RESULTS AND DISCUSSION

Morphological and physiological characteristics. Table 1 summarizes the morphological and physiological properties of three thermophilic and five mesophilic *Methanothrix* strains. The data for optimum temperature, pH at which growth occurred, and growth factors were obtained from previous reports (9–11, 17, 20, 21, 26–28). All of the strains had characteristic sheathed structures in which a number of cells were arranged. The cells were nonmotile straight rods with flat ends and were gram negative. The mean dimensions of single cells were 0.8 to 1.3 by 2 to 6 µm, and cells were

FIG. 1. Protein patterns for three strains of *Methanotrix thermophila* and five strains of *Methanotrix soehngenii* as determined by SDS-polyacrylamide gel electrophoresis. Electrophoresis was performed with a 4 to 20% acrylamide gel. Lane ST, M_r standards.

sometimes connected to each other. The sheathed filaments of the thermophiles were generally much shorter (length, 10 to 100 μm) than those of the mesophiles (>100 μm). The cells were not susceptible to lysis by SDS or hypotonic conditions at room temperature when we used the procedures described by Boone and Whitman (4).

As described previously, one of the characteristics that differentiated the thermophiles from the mesophiles group was the fact that the thermophiles contained phase-refractive particles, probably gas vesicles, within their cells (11, 17, 28). These particles could be readily eliminated by centrifugation or sonication. However, in strain Z-517 we did not observe these particles, whereas Nozhevnikova and Chudina observed them in their original culture (17).

DNA base composition. The guanine-plus-cytosine contents of the DNAs of strains CALS-1 and Z-517 were calculated to be 54.2 and 54.3 mol%, respectively. The values for the other strains were obtained from previous reports (10, 11, 26) (Table 1).

Protein patterns of whole cells. The protein patterns for the whole cells of the three thermophiles, as determined by SDS-polyacrylamide gel electrophoresis, were almost identical (Fig. 1), and these patterns were quite distinct from those of the mesophiles.

Partial 16S rRNA sequences. Figure 2 shows an alignment of the partial 16S rRNA sequences obtained for three thermophiles and four mesophiles. Sequence information was obtained for approximately 540 bases (ca. 37% of all of the 16S rRNA bases). The alignment was optimized by using the secondary structure of *Methanobacterium formicicum* DSM 1312 (15). The sequence for strain Opfikon determined by us was identical to that determined previously by Eggen et al. (7), suggesting a high fidelity of sequencing from the RNA

template. Table 2 shows the levels of sequence homology and the numbers of base differences for the seven strains. Within the thermophilic group, the strain P_T^T sequence exhibited 100% similarity with the sequence of strain Z-517, while there were six base differences between strains P_T^T and CALS-1. Our comparative analysis of the 16S rRNAs revealed a considerable difference between the thermophiles and the mesophiles. Within the mesophilic group, the strains exhibited more than 99% similarity with each other, and this finding was in close agreement with the results of the DNA-DNA hybridization studies of Touzel et al. (26), who used strains FE, GP6, and Opfikon. However, the members of the thermophilic group exhibited less than 95% similarity (corresponding to 29 to 34 base differences) to the mesophiles, suggesting that the thermophilic group is quite distant from any of the mesophilic *Methanotrix* strains.

Nomenclature of thermophilic strains belonging to the genus *Methanotrix*. The thermophilic *Methanotrix* strains form a taxon which is phenotypically and genotypically distinguishable from the mesophilic group of the genus *Methanotrix*. However, as described above, the partial 16S rRNA sequence of strain CALS-1 was different from the sequences of strains Z-517 and P_T^T at six positions, indicating that the thermophilic *Methanotrix* strains apparently should be separated into two genogroups.

In this study, the two genogroups could not be differentiated by any of conventional methods used for characterization (Table 1). In general, a species should be established with phenotypic characteristics which apparently distinguish the species from allied species. Therefore, we are reluctant to separate the thermophilic *Methanotrix* strains into two species and propose that the two genogroups should be assigned to a single species.

In the thermophilic *Methanotrix* group, "*Methanotrix thermoacetophila*" ("*Methanosaeta thermoacetophila*") is the name that has been given to strain Z-517 (17, 21). However, the description of "*Methanotrix thermoacetophila*" was inadequate because it was based on an enriched culture (17), and the name is invalid according to the *International Code of Nomenclature of Bacteria* (Rule 31a) (14). In this paper we propose the name *Methanotrix thermophila* sp. nov. for the thermophilic strains of the genus *Methanotrix*. The type species is strain P_T (= DSM 6194). *Methanotrix thermophila* contains two genogroups which differ in their 16S rRNA sequences. Genogroup 1 consists of strains P_T^T (= DSM 6194^T) and Z-517 (= DSM 4774), and genogroup 2 contains strain CALS-1 (= DSM 3870).

Description of *Methanotrix thermophila* sp. nov. *Methanotrix thermophila* (ther. mo' phi. la. Gr. adj. *thermos*, hot; Gr. adj. *philos*, loving; Gr. adj. *thermophila*, heat loving) is a thermophilic (optimum growth occurs at 55 to 60°C), rod-shaped, sheathed, strictly anaerobic, aceticlastic methanogen that belongs to the genus *Methanotrix* ("*Methanosaeta*"). *Methanotrix thermophila* is apparently distinct from *Methanotrix soehngenii* ("*Methanosaeta concilii*") because of its thermophily and 16S rRNA sequence. Cells are nonmotile, strictly anaerobic rods with flat ends and are gram negative. The mean dimensions of single cells are 0.8 to 1.3 by 2 to 6 μm . Cells are enclosed inside an annular, striated sheathed structure and are separated by partitions that have a concentric structure. Cells grow in the form of loose sediment, and culture broth exhibits opalescent turbidity after gentle shaking. Most of the sheathed filaments are less than 100 μm long, and they do not form bundles or the cottonlike flocs which are common in *Methanotrix soeh-*

1. <i>M. formicicum</i> DSM1312	271	281	291	301	311	
2. Opfikon	GGGUUGUGAG	AGCAAGAGCC	CGGAGAUGGA	ACCUGAGACA	AGGUUCCAGG	
3. GP6	GGGUUGUGAG	AGCAAGAGCC	CGGAGAUGGA	UUCUGAGACA	CGAAUCCAGG	
4. UA	GGGUUGUGAG	AGCAAGAGCC	CGGAGAUGGA	UUCUGAGACA	CGAAUCCAGG	
5. PM	GGGUUGUGAG	AGCAAGAGCC	CGGAGAUGGA	UUCUGAGACA	CGAAUCCAGG	
6. CALS-1	GGGUUGUGAG	AGCAAGAGCC	CGGAGAUGGA	UUCUGAGACA	<u>UGAAUCCAGG</u>	
7. Z517	GGGUUGUGAG	AGCAAGAGCC	CGGAGAUGGA	UUCUGAGACA	<u>UGAAUCCAGG</u>	
8. PT	GGGUUGUGAG	AGCAAGAGCC	CGGAGAUGGA	UUCUGAGACA	<u>UGAAUCCAGG</u>	
321	331	341	351	361	371	381
1. CCCUACGGGG	CGCAGCAGGC	GCGAAA CCUC	CGCAAU GCAC	GAAAGUGCGA	CGGGGGAAAC	CCAAGUGCCA
2. CCCUACGGGG	UGCAGCAGGC	GCGAAAAACUU	UACAAUGCUG	GCAACAGCGA	UAAGGGAACC	UCGAGUGCCA
3. CCCUACGGGG	UGCAGCAGGC	GCGAAAAACUU	UACAAUGCUG	GCAACAGCGA	UAAGGGAACC	UCGAGUGCCA
4. CCCUACGGGG	UGCAGCAGGC	GCGAAAAACUU	UACAAUGCUG	GCAACAGCGA	UAAGGGAACC	UCGAGUGCCA
5. CCCUACGGGG	UGCAGCAGGC	GCGAAAAACUU	UACAAUGCUG	GCAACAGCGA	UAAGGGAACC	UCGAGUGCCA
6. CCCUACGGGG	UGCAGCAGGC	GCGAAAAACUU	UACAAUGCUG	GCAACAGCGA	UAAGGGAACC	UCGAGUGCCA
7. CCCUACGGGG	UGCAGCAGGC	GCGAAAAACUU	UACAAUGCUG	GCAACAGCGA	UAAGGGAACC	UCGAGUGCCA
8. CCCUACGGGG	UGCAGCAGGC	GCGAAAAACUU	UACAAUGCUG	GCAACAGCGA	UAAGGGAACC	UCGAGUGCCA
391	401	411	421			
1. CUCUUAACGG	GGUGGCUUUU	CUUAAGUGUA	AAAAGCUUUU			
2. G-GUUACNNA	UCUGGCUGUC	GANAUCCUA	AAAAGCAUUU			
3. G-GUUACAAA	UCUGGCUGUC	GANAUCCUA	AAAAGCAUUU			
4. G-GUUACNNA	UCUGGCUGUC	GANAUCCUA	AAAAGCAUUU			
5. G-GUUACNNA	UCUGGCUGUC	GANAUCCUA	AAAAGCAUUU			
6. G-GUUACAAA	<u>UCUGGCUUUU</u>	<u>GGG CUGCCUA</u>	<u>AAAAGCAGU C</u>			
7. G-GUUACAAA	<u>UCUGGCUGUU</u>	<u>GGG CUGCCUA</u>	<u>AAAAGCAGU C</u>			
8. G-GUUACAAA	<u>UCUGGCUGUU</u>	<u>GGG CUGCCUA</u>	<u>AAAAGCAGU C</u>			
641	651	661	671	681	691	701
1. AUAUCCCCGG	GAGGACCACC	UGUGGCGAAG	GCGGCUAACU	GGAACGGGCC	UGACGGUGAG	UAACGAAAGC
2. GUAAUCCUUG	AAGGACCACC	AGUGGCGAAG	GCGUCUCACC	AGAACGGAAC	UGACGGCAAG	GGACGAAA GC
3. GUAAUCCUUG	AAGGACCACC	AGUGGCGAAG	GCGUCUCACC	AGAACGGAAC	UGACGGCAAG	GGACGAAA GC
4. GUAAUCCUUG	AAGGACCACC	AGUGGCGAAG	GCGUCUCACC	AGAACGGAAC	UGACGGCAAG	GGACGAAA GC
5. GUAAUCCUUG	AAGGACCACC	AGUGGCGAAG	GCGUCUCACC	AGAACGGAAC	UGACGGCAAG	GGACGAAA GC
6. GUAAUCCUUG	<u>AGGGACCACC</u>	<u>AGUGGCGAAG</u>	<u>GCGUCUCACC</u>	<u>AGAACGGAUC</u>	<u>CGACGGCAAG</u>	<u>GGACGAAA GC</u>
7. GUAAUCCUUG	<u>AGGGACCACC</u>	<u>AGUGGCGAAG</u>	<u>GCGUCUCACC</u>	<u>AGAACGGAUC</u>	<u>CGACGGCAAG</u>	<u>GGACGAAA GC</u>
8. GUAAUCCUUG	<u>AGGGACCACC</u>	<u>AGUGGCGAAG</u>	<u>GCGUCUCACC</u>	<u>AGAACGGAUC</u>	<u>CGACGGCAAG</u>	<u>GGACGAAA GC</u>
711	721	731	741	751	761	771
1. CAGGGGCGCG	AACCGGAUUA	GAUACCCGGG	UAGUCCUGGC	CGUAAACGAU	GUGGACUUGG	UGUUGGGAUG
2. UAGGGGCACG	AACCGGAUUA	GAUACCCGGG	UAGUCCUAGC	CGUAAACGAU	ACUCGCUAGG	UGUCGGC - AC
3. UAGGGGCACG	AACCGGAUUA	GAUACCCGGG	UAGUCCUAGC	CGUAAACGAU	ACUCGCUAGG	UGUCGGC - AC
4. UAGGGGCACG	AACCGGAUUA	GAUACCCGGG	UAGUCCUAGC	CGUAAACGAU	ACUCGCUAGG	UGUCGGC - AC
5. UAGGGGCACG	AACCGGAUUA	GAUACCCGGG	UAGUCCUAGC	CGUAAACGAU	ACUCGCUAGG	UGUCGGC - AC
6. UAGGGGCACG	AACCGGAUUA	GAUACCCGGG	UAGUCCUAGC	CGUAAACGAU	ACUCGCUAGG	UGUCGGC - AC
7. UAGGGGCACG	AACCGGAUUA	GAUACCCGGG	UAGUCCUAGC	CGUAAACGAU	ACUCGCUAGG	UGUCGGC - AC
8. UAGGGGCACG	AACCGGAUUA	GAUACCCGGG	UAGUCCUAGC	CGUAAACGAU	ACUCGCUAGG	UGUCGGC - AC
781	791	801	811	821	831	
1. GCU C CGAGCU	G CCCCAGUGC	CGAAGGGAAG	CUGUUAAGUC	CACCGCCUGG	GAAGUACG	
2. GGU G CGACCG	UUGUCGGUGC	CGUAGGGAAG	CCGU GAAGCG	AGCCACCUGG	GAAGU	
3. GGU G CGACCG	UUGUCGGUGC	CGUAGGGAAG	CCGU GAAGCG	AGCCACCUGG	GAAGU	
4. GGU G CGACCG	UUGUCGGUGC	CGUAGGGAAG	CCGU GAAGCG	AGCCACCUGG	GAAGU	
5. GGU G CGACCG	UUGUCGGUGC	CGUAGGGAAG	CCGU GAAGCG	AGCCACCUGG	GAAGU	
6. GGU G CGACCG	UUGUCGGUGC	CGUAGGGAAG	CCGU GAAGCG	AGCCACCUGG	GAAGU	
7. GGUNNGACCG	UUGUCGGUGC	CGUAGGGAAG	CCGU GAAGCG	AGCCACCUGG	GAAGU	
8. GGUG C GACCG	UUGUCGGUGC	CGUAGGGAAG	CCGU GAAGCG	AGCCACCUGG	GAAGU	

FIG. 2. Partial 16S rRNA sequences for three strains of *Methanobacterium formicicum* and five strains of *Methanobacterium soehngenii*. The sequences are aligned with the known complete sequence for *Methanobacterium formicicum* (15). A, adenine; U, uracil; C, cytosine; G, guanine; N, ambiguous nucleotide. The dashes indicate positions that were deleted in the sequences. The underlined bases are bases that were different from those of *Methanobacterium soehngenii* Opfikon.

1141	1151	1161	1171	1181	1191	1201
1. UAGGUCCGUA	UGCCCCGAAU	CCCCU GGGCU	ACACGCGGGC	UACAAUGGUU	AGGACAAUGG	GUUCCGACAC
2. UAGGUCAGUA	UGCCCCGANN	AUCCCGGGCU	ACACGCGAGC	UACAAUGGUU	GGUACAAUGG	GUAUCUACCC
3. UAGGUCAGUA	UGCCCCGAAU	AUCCCGGGCU	ACACGCGAGC	UACAAUGGUU	GGUACAAUGG	GUAUCUACCC
4. UAGGUCAGUA	UGCCCCGANN	AUCCCGGGCU	ACACGCGAGC	UACAAUGGUU	GGUACAAUGG	GUAUCUACCC
5. UAGGUCAGUA	UGCCCCGANN	AUCCCGGGCU	ACACGCGAGC	UACAAUGGUU	GGUACAAUGG	GUAUCUACCC
6. UAGGUCAGUA	UGCCCCGANN	CCCCAGGGCU	ACACGCGGGC	UACAAUGGUU	GGUACAAUGG	GUAUCGACCC
7. UAGGUCAGUA	UGCCCCGAAU	CCCCCGGGCU	ACACGCGGGC	UACAAUGGUU	GGUACAAUGG	GUAUCGACCC
8. UAGGUCAGUA	UGCCCCGAAU	CCCCCGGGCU	ACACGCGGGC	UACAAUGGUU	GGUACAAUGG	GUAUCGACCC
1211	1221	1231	1241	1251	1261	1271
1. UGAAAGGUGG	AGGUAAUCUC	CUAAACCUUG	CCUUAGUUCG	GAUUGAGGGC	UGUAAUCUCG	CCUCAUGAAG
2. CGAAAGGGGA	CGGGAAUCUC	CUAAAACCAA	UCUUAGUUCG	GAUUGAGGGC	UGCAACUCGC	CCUCAUGAAG
3. CGAAAGGGGA	CGGGAAUCUC	CUAAAACCAA	UCUUAGUUCG	GAUUGAGGGC	UGCAACUCGC	CCUCAUGAAG
4. CGAAAGGGGA	CGGGAAUCUC	CUAAAACCAA	UCUUAGUUCG	GAUUGAGGGC	UGCAACUCGC	CCUCAUGAAG
5. CGAAAGGGGA	CGGGAAUCUC	CUAAAACCAA	UCUUAGUUCG	GAUUGAGGGC	UGCAACUCGC	CCUCAUGAAG
6. CGAGAGGGGU	AGGCAAUCC	CUAAAGCCGA	UCGUAGUUCG	GAUUGAGGGC	UGAAACUCGC	CCUCAUGAAG
7. CGAAAGGGGU	AGGCAAUCC	CUAAAACCGA	UCGUAGUUCG	GAUUGAGGGC	UGAAACUCGC	CCUCAUGAAG
8. CGAAAGGGGU	AGGCAAUCC	CUAAAACCGA	UCGUAGUUCG	GAUUGAGGGC	UGAAACUCGC	CCUCAUGAAG
1281	1291	1301	1311	1321		
1. CUGGAAUCCG	UAGUAAUCGC	GUGUCAUAAC	CGCGCGGUGA	AUACGUCCCU		
2. CUGGAAUCCG	UAGUAAUCGC	GUUUCAACAG	AACGCGGUGA	AUACGU		
3. CUGGAAUCCG	UAGUAAUCGC	GUUUCAACAG	AACGCGGUGA	AUACGU		
4. CUGGAAUCCG	UAGUAAUCGC	GUUUCAACAG	AACGCGGUGA	AUACGU		
5. CUGGAAUCCG	UAGUAAUCGC	GUUUCAACAG	AACGCGGUGA	AUACGU		
6. CUGGAAUCCG	UAGUAAUCGC	GUUUCAACAG	AACGCGGUGA	AUACGU		
7. CUGGAAUCCG	UAGUAAUCGC	GUUUCAACAG	AACGCGGUGA	AUACGU		
8. CUGGAAUCCG	UAGUAAUCGC	GUUUCAACAG	AACGCGGUGA	AUACGU		

FIG. 2—Continued.

ngenii. Colonies are sometimes observed in acetate agar medium when high cell concentrations are present (11, 28). Gas vesicles are often observed inside the cells. Cells are not susceptible to lysis by SDS or hypotonic conditions at room temperature, but cells are lysed by SDS at 60°C.

The intensity of autofluorescence (405-nm excitation) is higher than that for *Methanotrix soehngenii* because of a higher content of coenzyme F₄₂₀ (11). Coenzyme F₄₂₀ with four glutamyl residues on the side chain is more abundant than the other coenzyme F₄₂₀ types, although the total coenzyme F₄₂₀ contents are much lower than the contents in other hydrogenotrophic methanogens (11). A tetraether type

(dibiphytanediyl-diglycerol tetraether; caldarchaeol) of lipid is found in strain P_T^T.

The optimum temperature for growth and methanogenesis is 55 to 60°C, depending on the strain (11, 17, 21, 28). The optimum pH is around 7. Acetate is the only substrate used for growth and methanogenesis (11, 17, 28). Neither methanogenesis nor growth is observed when H₂-CO₂, formate, methylamines, or methanol is used for the substrate (11, 17, 28). Formate is not split into H₂ and CO₂ (11, 28). NaCl is not required for growth (11). Coenzyme M (mercaptoethane sulfonate), vitamins, manure extract, or sludge fluid stimulates growth (11, 17, 28). Chloramphenicol (5 µg/ml), bacitracin (10 µg/ml), neomycin (10 µg/ml), tetracycline (100 µg/ml), and kanamycin (100 µg/ml) inhibit growth and methanogenesis (11).

The DNA base composition ranges from 52.7 to 54.3 mol% guanine plus cytosine as determined by high-performance liquid chromatography (Table 1). The 16S rRNA sequence is quite distinct from that of *Methanotrix soehngenii* (level of similarity, less than 95%). The type strain of *Methanotrix thermophila* is strain P_T (= DSM 6194). *Methanotrix thermophila* contains two genogroups. Genogroup 1 consists of strains P_T^T (= DSM 6194^T) and Z-517 (= DSM 4774), and genogroup 2 contains strain CALS-1 (= DSM 3870). The partial 16S rRNA sequences described above have been submitted to the National Institute of Genetics, Shizuoka, Japan.

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TABLE 2. Levels of 16S rRNA sequence homology for four mesophilic and three thermophilic *Methanotrix* strains

Strain	No. of base differences or % homology with strain ^a :						
	Opfikon	GP6	U _A	P _M	CALS-1	Z-517	P _T ^T
Opfikon (= DSM 2139)		0	1	0	33	29	29
GP6 (= DSM 3671)	100		1	0	33	29	29
U _A	>99	>99		1	34	30	30
P _M	100	100	>99		33	29	29
CALS-1 (= DSM 3870)	94	94	94	94		6	6
Z-517 (= DSM 4774)	95	95	94	95	99		0
P _T ^T (= DSM 6194 ^T)	95	95	94	95	99	100	

^a The values on the upper right are the numbers of base differences, and the values on the lower left are the percentages of homology (calculated from the data in Fig. 2).

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