NOTES

Methanolobus taylorii sp. nov., a New Methylophilic, Estuarine Methanogen

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Previously published phylogenetic studies of 16S rRNA showed that methylophilic, slightly halophilic, methanogenic strain GS-16^T (T = type strain) represents a new species of bacterium. We propose the name *Methanolobus taylorii* for this species; strain GS-16 is the type strain.

Strain GS-16^T (T = type strain) is a methylophilic methanogen that was isolated from estuarine sediments from San Francisco Bay (4) and has been deposited in the Oregon Collection of Methanogens (Oregon Graduate Institute, Portland) as strain OCM 58^T. This strain was isolated by using dimethyl sulfide as the catabolic substrate (4), but it can also grow on methylamines (13) and methanethiol (8, 9) and grew when it was inoculated into MSHA medium (6) supplemented with 20 mM methanol as the sole catabolic substrate. Strain GS-16^T cells form methane from methylmercury (12) and dimethylselenide (16), although they cannot grow on these substrates, and form traces of ethane from diethyl sulfide (15). Methanogenesis from trimethylamine is inhibited by methyl fluoride (11) and methyl bromide (14), but not by dimethyl ether (11).

Strain GS-16^T cells did not grow in a mineral medium (MMSHA medium, which was MSHA medium with Trypticase peptones, yeast extract, and coenzyme M deleted and the concentration of Na₂S · 9H₂O increased to 0.5 g/liter) when 20 mM trimethylamine was added as the sole catabolic substrate, but grew well in this medium when biotin was added.

The cells are mesophilic. In the original characterization of strain GS-16^T, Oremland et al. (13) reported that this organism grew poorly at temperatures greater than 28°C in mineral medium containing a catabolic substrate (dimethylsulfide) and vitamins as the only organic compounds and grew fastest at 37°C in medium containing Trypticase peptones and yeast extract. However, during our studies of the minimum organic requirements for growth, strain GS-16^T cells grew well at 37°C in defined MMSHA medium containing biotin and a catabolic substrate (20 mM trimethylamine). It is possible that since its original isolation strain GS-16^T has acquired the ability to grow at 37°C. Similarly, the initial description of *Methanobrevibacter tindarius* Tindari 3^T indicated that the optimum growth temperature for this organism was 25°C (5), but cultures of this strain now grow fastest at 37°C (18).

Strain GS-16^T is slightly halophilic, growing well in the presence of sodium concentrations between 0.2 and 1.0 M and in the presence of Mg²⁺ concentrations of 15 mM or greater (4). It grows fastest at pH values between 7.0 and 8.8 (4). W. B. Whitman (University of Georgia, Athens) found that the

guanine-plus-cytosine content of the DNA is 40.8 ± 0.3 mol% by performing a liquid chromatographic analysis of the bases (7).

The 16S rRNA sequence of strain GS-16^T was compared with the 16S rRNA sequences of many other methanogens (3), and the results indicated that strain GS-16^T is most closely related to *Methanohalophilus oregonensis* WAL1^T. However, the evolutionary distances are great enough that the two strains belong in separate species; these distances are as great as distances between other distinct species (3). The 16S rRNA sequences of both of these strains were similar to the sequence of *Methanobrevibacter tindarius* Tindari 3^T, suggesting that all three species should be assigned to the genus *Methanobrevibacter*, as separate species (3). The results of a comparison of the whole-cell proteins of strain GS-16^T, *Methanococcoides methylutens* TMA-10^T, *Methanobrevibacter vulcani* PL-12/M^T, and *Methanobrevibacter siciliae* T4/M^T and HI350 (5) support the proposed that strain GS-16^T should be placed in a new species of the genus *Methanobrevibacter*. The results of a DNA hybridization study (1) confirmed that strain GS-16^T does not belong in the genus *Methanohalophilus* or in the genus *Methanohalobium*.

Strain GS-16^T is also physiologically distinct from species of the genus *Methanobrevibacter*. In 1989, in *Bergey's Manual of Systematic Bacteriology*, Stetter recognized three *Methanobrevibacter* species: *Methanobrevibacter tindarius*, *Methanobrevibacter siciliae*, and *Methanobrevibacter vulcani* (18). Since then, *Methanobrevibacter siciliae* has been transferred to the genus *Methanosarcina* as *Methanosarcina siciliae* (10). The two remaining species group phylogenetically with *Methanohalophilus oregonensis* (6), which should be transferred to the genus *Methanobrevibacter* (3). Although the proposal for transfer of *Methanohalophilus oregonensis* has not been formalized, we believe that *Methanobrevibacter tindarius*, *Methanobrevibacter vulcani*, and *Methanohalophilus oregonensis* are three currently recognized species whose phylogenies indicate that they should be classified as *Methanobrevibacter* species. Strain GS-16^T differs physiologically from these three species (Table 1), although *Methanobrevibacter vulcani* has not been well characterized (18) relative to minimal standards (2). Strain GS-16^T is most similar to *Methanohalophilus oregonensis* WAL1^T, but it has a lower pH range (pH 6.8 to 9.0 [4]) than *Methanohalophilus oregonensis* WAL1^T (pH 7.7 to 9.5 [6]) and it is less halophilic (strain GS-16^T does not grow in the presence of NaCl concentrations greater than 1.2 M NaCl [4], whereas *Methanohalophilus oregonensis* WAL1^T grows in the presence of 1.6 M NaCl [6]). Because of the phylogenetic and physiological differences of strain GS-16^T, we propose that this

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TABLE 1. Comparison of characteristics of some halophilic type strains

Species	Type strain	G+C content (mol%)	Temp (°C)	pH	Salinity (M)	Growth requirements		Catabolic substrates		Reference ^e
						Minimal growth	Good growth	Methanol, methyl amines	Dimethylsulfide	
<i>Methanobolus tindarius</i>	Tindari 3 (= OCM 150 = DSM 2278)	45.9	25 or 37 ^b	6.5	0.49	None	None	+	— ^c	5
<i>Methanobolus vulcani</i>	PL-12/M (= OCM 157 = DSM 3029)	39	37	ND ^d	0.5	None	None	+	— ^c	18
<i>Methanohalophilus oregonensis</i>	WAL1 (= OCM 99 = DSM 5435)	41	35	8.6	0.48	Biotin, thiamine ^e	Biotin, thiamine ^e	+	+	6
<i>Methanobolus taylorii</i>	GS-16 (= OCM 58 = DSM 9005)	40.8	37	8	0.5	Biotin	Biotin	+ ^f	+ ^g	This study
<i>Methanosarcina siciliae</i>	T4/M (= OCM 156 = DSM 3028)	41.5	37 or 40 ^h	5.6–7.0 ⁱ	0.3–0.6 ⁱ	None	Vitamins, yeast extract ⁱ	+	+ ⁱ	18
<i>Methanococcoides methylutens</i>	TMA-10 (= OCM 158 = DSM 2657 = ATCC 33938)	42	35	7.0–7.5	0.22	Biotin	Biotin	+	— ^c	17

^a Source of data except as indicated below.^b The growth temperature of 37°C is from reference 18.^c Data from reference 9.^d ND, not determined.^e Unpublished data.^f Data from reference 4.^g Data from reference 13.^h The growth temperature of 40°C is from reference 8.ⁱ Data from reference 8.

organism should be placed in a new species, *Methanobolus taylorii*. Strain GS-16 is the type strain of *Methanobolus taylorii*.

Methanobolus taylorii sp. nov. *Methanobolus taylorii* (tay'lor. i.i. N. L. gen. masc. n. *taylorii*, of Taylor; named for Barrie F. Taylor for his contributions to our understanding of the marine organosulfur cycle and for his mentorship of many students and postdoctoral associates in the field of marine microbiology).

Cells are coccoid bodies 0.5 to 1 µm in diameter and sometimes grow in large clumps. Lysed by detergents. Gram negative. Nonmotile.

Cells grow by forming methane from methylamines, methylsulfides, and methanol. They form methane from dimethylselenide and methylmercury and ethane from diethylsulfide, but they cannot grow on any of these substrates, nor can they grow on methionine, dimethyl disulfide, formate, H₂-CO₂, or acetate.

Cells grow well at 37°C, at pH 7.2 to 8.8, and in the presence of 0.2 to 1.2 M NaCl. Very strictly anaerobic. Biotin and catabolic substrate are the only organic compounds required for growth. The guanine-plus-cytosine content of the DNA of the type strain is 40.8 ± 0.3 mol% (as determined by a chromatographic analysis of bases).

The habitat is estuarine sediments.

The type strain is GS-16 (= OCM 58 = DSM 9005).

This work was supported by grant 14-08-001-G2098 from the U.S. Geological Survey.

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