Physiological Characterization and Emended Description of *Methanolobus vulcani*

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***Methanolobus vulcani* PL-12/M^T** (T = type strain) grew on methylamines and methanol but not on dimethyl sulfide, formate, acetate, or H₂-CO₂. The cells grew rapidly at mesophilic temperatures, at neutral pH values (pH 6 to 7.5), and in medium supplemented with 0.1 to 1.2 M NaCl and 13 mM Mg²⁺. The cells grew in mineral medium containing biotin and a catabolic substrate, but growth was stimulated by yeast extract and peptones. *M. vulcani* was physiologically similar to *Methanolobus tindarius* and had a similar 16S rRNA sequence, although the results of DNA-DNA hybridization experiments indicated that these organisms should be considered separate species.

The genus *Methanolobus* is a genus that contains slightly halophilic, methylotrophic methanogens. In 1989, Stetter (16) recognized three species in this genus, *Methanolobus tindarius* (6), *Methanolobus vulcani*, and *Methanolobus siciliae*, but since then *Methanolobus siciliae* has been transferred to the genus *Methanosarcina* as *Methanosarcina siciliae* (10). In addition to *Methanolobus vulcani* and *Methanolobus tindarius*, three other species should be classified as members of the genus *Methanobolus* on the basis of phylogeny as determined by 16S rRNA sequences. These species are *Methanobolus taylorii* (11), *Methanobolus bombayensis* (5), and *Methanohalophilus oregonensis* (2, 8). However, there has been no formal proposal to transfer the latter species to the genus *Methanobolus* (2). The results of a comparison of partial sequences of the methyl-coenzyme M-methylreductase genes confirmed that this group of five species is a phylogenetically coherent group (15). All five species have been characterized phenotypically (2, 5, 6, 10–12); however, the previously published description of *Methanobolus vulcani* (16) was rudimentary.

Methanobolus vulcani PL-12/M^T (= OCM 157^T) (T = type strain) (16) was obtained from the Oregon Collection of Methanogens. The cells of this strain were irregular coccoids with

diameters of 1.0 to 1.25 µm when the organism was grown in MSHCO2 medium containing 50 mM methanol. MSHCO2 medium was the basal medium used for growth throughout this study (9). The cells lysed when 0.1 g of sodium dodecyl sulfate per liter was added, indicating that a proteinaceous cell wall was present. *Methanobolus vulcani* catabolized methanol and trimethylamine. Cultures of this organism did not catabolize H₂ either in the presence or in the absence of 50 mM methanol. Cultures also did not grow on formate, acetate, or dimethyl sulfide. The specific growth rate was determined by measuring the amount of methane formed during growth (1). The specific growth rate during exponential growth was determined by using the software package TableCurve (Jandel Scientific, San Rafael, Calif.) to fit data for the total amount of methane accumulated versus time to the Gompertz equation (4, 17). The methane produced by the inoculum was included in this calculation (13). Cells were slightly halophilic and grew fastest in the presence of 0.1 to 1.2 M NaCl (Fig. 1A). *Methanobolus vulcani* grew fastest at neutral pH values (Fig. 1B) and did not grow at pH values greater than 7.5. In contrast, most other species belonging to this genus are alkaliphilic or

FIG. 1. Effects of salinity (A), pH (B), and temperature (C) on the specific growth rate of *Methanobolus vulcani*.

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TABLE 1. Characteristics of *Methanobolus* species and *Methanohalophilus oregonensis*

Species	Type strain	Other designations ^a	G+C content (mol%)	Use of dimethyl sulfide	Growth factor(s)	T_{min} (°C) ^b	T_{opt} (°C) ^b	T_{max} (°C) ^b	b^b	c^b	Optimum pH	pH range ^c	Optimum Na ⁺ concn (mM)	Na ⁺ concn range (mM) ^c	Optimum Mg ²⁺ concn (mM)	Mg ²⁺ concn range (mM) ^c	% 16S rRNA differences ^d	Reference(s)
<i>Methanobolus tindarius</i>	Tindari 3	OCM 150, DSM 2278	46	—	None	7	28	50	0.264	0.0039	6.5	5.5–8.0	490	60–1,270	ND	ND	1.61	6
<i>Methanobolus vulcani</i>	PL-12/M	OCM 157, DSM 3029	39	—	Biotin	13	40	45	0.0167	0.487	7.2	6.0–7.5	500	100–1,200	13	0.5–85	16; this study	
<i>Methanobolus taylorii</i>	GS-16	OCM 58, DSM 9005	41	+	Biotin	11	39	40	0.0157	3.01	8	7.0–8.6	600	200–1,200	40	13–40	2.28	11
<i>Methanobolus bombayensis</i>	B-1	OCM 438, DSM 7082	39	+	None	13	37	46	0.0208	0.182	7.2	6.5–8.0	500	300–2,000	33	30–80	2.13	5
<i>Methanohalophilus oregonensis</i>	WAL1	OCM 99, DSM 5435	41	+	Biotin, thiamine	7	34	44	–0.0120	0.182	8.6	7.4–9.5	500	100–1,600	40	13–220	3.13	8

^a OCM, Oregon Collection of Methanogens, Oregon Graduate Institute of Science & Technology, Portland; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany.

^b T_{min} , T_{opt} , and T_{max} are the minimum, optimum, and maximum growth temperatures, respectively, and b and c are the two other fitting parameters used in the square-root equation (when the units for the specific growth rate are hour^{–1}).

^c Range at which the specific growth rate was at least 10% of the growth rate under optimal conditions.

^d Calculated by comparing 16S rRNA sequences with the *Methanobolus vulcani* 16S rRNA sequence. Sequence data were obtained from the Ribosomal Database Project (7).

alkali tolerant and grow well at pH values of more than 7.5 (Table 1).

Specific growth rates at various temperatures were determined (Fig. 1C) and were fitted (by using the TableCurve software) to the square-root equation (14):

$$\mu = \{b(T - T_{min}) \cdot c[1 + \exp(T_{max} - T)]\}^2,$$

where μ is the specific growth rate, T is the temperature, T_{min} is the minimum temperature for growth, T_{max} is the maximum temperature for growth, and b and c are constants. The fitted curve obtained for *Methanobolus vulcani* is shown in Fig. 1C, and the fitting parameters (T_{min} , T_{max} , b , and c) are shown in Table 1. Although the T_{min} of *Methanobolus vulcani* was 13°C, we were not able to detect growth at temperatures even as high as 20°C (Fig. 1C). The temperature at which growth is most rapid was determined graphically. We obtained growth rates at various temperatures from previously published descriptions of related species, and we fitted the data obtained in this way to the square-root equation. The results of those analyses are shown in Table 1.

Cells grew well in the presence of 13 mM Mg²⁺ but grew poorly at Mg²⁺ concentrations less than 0.5 mM or more than 80 mM.

Methanobolus vulcani did not grow in MSHCO2 mineral medium (MSHCO2 medium lacking organic constituents) supplemented with methanol, but it grew in this medium when biotin was added (specific growth rate, 0.09 h^{–1}). Growth was fastest (0.13 h^{–1}) when 2 g of yeast extract per liter and 2 g of peptone per liter were added.

Taxonomy. Placement of *Methanobolus vulcani* in the genus *Methanobolus* together with *Methanobolus tindarius*, *Methanobolus taylorii*, *Methanobolus bombayensis*, and *Methanohalophilus oregonensis* is consistent with the phylogenetic relationships and phenotypic characteristics of these organisms (Table 1). The level of differences between the 16S rRNA sequences of *Methanobolus tindarius* and *Methanobolus vulcani* is 1.6% (Table 1); this level of differences is comparable to the levels found between pairs of closely related species or between strains classified together in a single species (2, 3). On the basis of the physiological and phylogenetic similarities described above, some workers might consider these two species synonymous. However, the stability of taxonomy should be preserved unless changes are well supported, and we consider these organisms separate species. The low DNA-DNA reassociation value (29%) between these two species (16) also indicates that these species should remain separate.

Methanobolus vulcani differs more phylogenetically and phenotypically from the other organisms listed in Table 1. The levels of differences between the 16S rRNA sequences of *Methanobolus vulcani* and these species are 2% or more (Table 1). *Methanobolus vulcani* differs from *Methanohalophilus oregonensis* and *Methanobolus taylorii* by lacking alkali tolerance and by not catabolizing dimethyl sulfide. *Methanobolus vulcani* differs from *Methanobolus bombayensis* in its ability to grow without high concentrations of divalent cations.

We propose the following emended description of *Methanobolus vulcani*.

Emended description of *Methanobolus vulcani*. *Methanobolus vulcani* Stetter, König, and Thomm 1989 (vul.ca'ni. L. gen. n. *vulcani*, from Vulcan, the god after whom Vulcano Island [Insula Vulcani] was named). Irregularly coccoid, gram negative, nonmotile. Lysed by 0.1 g of sodium dodecyl sulfate per liter. Grows on methanol or methylamines, but not on dimethyl sulfide, formate, acetate, or H₂-CO₂. Biotin and a catabolic substrate are the only organic compounds required for growth;

yeast extract and peptone stimulate growth. Grows well at 37°C, at pH 7.2, and in the presence of 0.5 M NaCl and 13 mM Mg²⁺. The guanine-plus-cytosine content of the DNA is 39 mol%. The type strain is PL-12/M (= OCM 157).

Habitat: sea sediments near Vulcano Island, Italy.

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