Taxonomy and Halotolerance of Mesophilic *Methanosarcina* Strains, Assignment of Strains to Species, and Synonymy of *Methanosarcina mazei* and *Methanosarcina frisia*

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We examined 22 previously described and newly isolated *Methanosarcina* strains by performing denaturing gel electrophoresis of whole-cell proteins and assigned these strains to previously described species. *Methanosarcina mazei* S-6^T (T = type strain) and *Methanosarcina frisia* C 16^T were very similar in terms of the electrophoresis patterns of their proteins and in their DNA sequences (the results of reassociation experiments indicated that there was 77% sequence similarity). Thus, *M. frisia* is a junior subjective synonym of *M. mazei*, and strain C 16 is a reference strain of *M. mazei*. *M. mazei* C 16 was similar to *M. mazei* in other characteristics that have not been reported previously, including the ability to catabolize acetate and a lack of halophily. All of the *Methanosarcina* strains examined, including the marine strains *M. mazei* C 16 (= *M. frisia* C 16^T) and *Methanosarcina acetivorans* C2A^T, were slightly halotolerant (rather than halophilic, as originally described). *Methanosarcina* sp. strain FR-1, which has gas vesicles, was more similar to *Methanosarcina barkeri* MS^T than to *Methanosarcina vacuolata* Z-761^T in both its protein patterns and its DNA sequence (80% similarity to *M. barkeri* MS^T and 38% similarity to *M. vacuolata* Z-761^T). Thus, the presence of gas vesicles is not an adequate taxonomic characteristic for assigning *Methanosarcina* strains to *M. vacuolata*.

MATERIALS AND METHODS

The five commonly recognized mesophilic *Methanosarcina* species (*Methanosarcina barkeri*, *Methanosarcina mazei*, *Methanosarcina vacuolata*, *Methanosarcina frisia*, and *Methanosarcina acetivorans*) are found in marine and freshwater environments (24, 33, 34). The first three of these species are considered to be freshwater species, and their type strains were isolated from freshwater environments. However, early strains of *M. barkeri* were isolated from marine or brackish mud (36, 42), and the current type strains of *M. barkeri* and *M. mazei* thrive at marine salinities (40). It has been postulated that *Methanosarcina* species evolved in marine habitats and adapted to freshwater environments by developing a heteropolysaccharide matrix or capsule (41). When *Methanosarcina* strains are grown in the presence of marine concentrations of Na⁺ and Mg²⁺, they have a protein cell wall, are sensitive to lysis by detergents, and occur as individual cells (35, 40, 46). When these organisms are grown in the presence of low concentrations of these cations, a heteropolysaccharide matrix is often formed, and cells may occur as aggregates, "cysts," or lamina (27, 40, 46).

The other two mesophilic *Methanosarcina* species, *M. frisia* and *M. acetivorans*, are considered to be marine species because all of the known strains (*M. frisia* C 16^T [T = type strain] and *M. acetivorans* C2A^T and C2E) were isolated from marine sediments (4, 39). The cells of these strains are sensitive to lysis by detergent, and their cell walls are protein (4, 39).

In this paper we describe some of the physiological characteristics, especially halotolerance, of some *Methanosarcina* strains, the results of a phylogenetic analysis, and taxonomic assignments for strains and propose that *M. mazei* and *M. frisia* are synonymous.

Source of strains. All of the strains that were not isolated in the course of this study were obtained from the Oregon Collection of Methanogens. The accession numbers and sources of the strains are listed in Table 1.

Media and culture techniques. We used the anaerobic techniques of Hungate (15) with syringes and serum tubes (27 ml, containing 5 ml of medium) or serum vials (171 ml, containing 50 ml of medium, or 2 liters, containing 1 liter of medium) (22). The basal culture medium was MS medium (6), which contained yeast extract, peptones, minerals, coenzyme M and sulfide as reducing agents, and a bicarbonate buffer system. The pH was 7.2 (with 0.3 atm [30.39 kPa] of CO₂ in the headspace) or, for growth of *M. barkeri* MS^T and *M. vacuolata* Z-761^T, 6.7 (with 1 atm [101.29 kPa] of CO₂ in the headspace). The medium was dispensed, the tubes or vials were sealed, and the preparations were sterilized by autoclaving. When H₂ was added as a catabolic substrate, it was added by pressurizing the tubes or vials with pure H₂ to a level that was 101 kPa above atmospheric pressure after inoculation. These H₂-containing cultures were incubated on a shaker and were frequently repressurized (to a level that was 101 kPa above atmospheric pressure) with a mixture of H₂ and CO₂ (3:1). Other catabolic substrates were added from sterile, anoxic stock solutions (final concentrations, 100 mM methanol, 50 mM acetate, and 15 mM trimethylamine). Media containing various Na⁺ concentrations were prepared by adding NaCl to the medium during preparation or by adding a sterile, anoxic stock solution of NaCl to the prepared medium. MS mineral medium was the same as MS medium except that yeast extract, Trypticase peptones, and coenzyme M were omitted.

Enrichment and isolation of new *Methanosarcina* strains. We isolated new *Methanosarcina* strains from laboratory

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TABLE 1. Sources and taxonomy of *Methanosarcina* strains

Strain	Previous taxonomic designation		Proposed change in species designation	Other designations ^a			Reference(s) in which isolation was described
	Taxon	Reference(s)		OCM no.	DSM no.	ATCC no.	
S-6 ^T	<i>M. mazei</i> ^b	25		26 ^T	2053 ^T	43572 ^T	23
LYC	<i>M. mazei</i>	19		34	4556	43573	19
KCl-2S	<i>M. mazei</i>	14		25			14
MS ^T	<i>M. barkeri</i>	9		38 ^T	800 ^T	43569 ^T	9
UBS	<i>M. barkeri</i>	45	<i>M. mazei</i>	27	1311		10
227	<i>M. barkeri</i>	2		35	1538	43241	26
227BM ^c	<i>M. barkeri</i>			40			37
227BES ^{rc}	<i>M. barkeri</i>			29			37
Jülich	<i>M. barkeri</i>	35		86	2948		35
FR-1	<i>M. barkeri</i>	1	<i>M. barkeri</i>	93	2256		1
	<i>M. vacuolata</i>	49					
Fusaro	<i>M. barkeri</i>	17		83	804	29787	17
S-3	<i>M. barkeri</i>	38		37			38
3	<i>M. barkeri</i>	17		84	805	29786	17
Wiesmoor	<i>M. barkeri</i>	35		89			35
Z-761 ^T	<i>M. vacuolata</i>	49, 50		85 ^T	1232 ^T		47, 49
C2A ^T	<i>M. acetivorans</i>	39		95 ^T	2834 ^T	35395 ^T	39
C 16 ^T	<i>M. frisia</i> ^d	3	<i>M. mazei</i>	98 ^T	3318 ^T	43340 ^T	4
Z-558 (= biotype 3)	<i>M. mazei</i>	12	<i>M. mazei</i>	92	2244		48
	<i>Methanosarcina</i> sp.	13					
CW3A	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	20			28
G1	<i>Methanosarcina</i> sp.	12	<i>M. mazei</i>	87	338		
G61	<i>Methanosarcina</i> sp.	12	<i>M. mazei</i>	88	3647		11
MCMB703	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	213			32
GA3 ^e	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	75			This study
GA4 ^e	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	76			This study
GA5 ^e	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	77			This study
GA6 ^e	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	78			This study
GA8 ^e	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	79			This study
GA12 ^e	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	80			This study
GA14 ^e	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	53			This study
GA15 ^e	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	81			This study
Equi ^f	<i>Methanosarcina</i> sp.	30	<i>M. mazei</i>	61			This study

^a OCM, Oregon Collection of Methanogens, Beaverton; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; ATCC, American Type Culture Collection, Rockville, Md.

^b Previously *Methanococcus mazei* (23).

^c Mutant of strain 227 (also *M. barkeri*).

^d Previously *Methanococcus frisia* (4).

^e Isolated from one of four laboratory scale methanogenic reactors.

^f Isolated from the cecum of a horse.

scale anaerobic digestors that were degrading a mixture of short-chain fatty acids (originally inoculated from a swine lagoon); one strain (strain Equi [= OCM 61]) was isolated from a horse cecum. Samples were collected and were immediately serially diluted in MS enrichment medium (similar to MS medium but with decreased concentrations of organic compounds [22]) supplemented with 100 mM methanol as the substrate. Cultures at the highest dilution which produced methane were selected and maintained by periodic transfers to fresh medium. We obtained axenic cultures by serially diluting the cultures and inoculating them into roll tube medium (MS medium supplemented with 20 g of purified agar per liter and 100 mM methanol). The incubated cultures formed colonies, and the epifluorescent colonies were removed with modified Pasteur pipets (15). Each colony was dispersed in sterile medium, and the resulting preparations were diluted and reinoculated into roll tube medium. We repeated this procedure until we had a pure culture (as determined by the presence of a single colony type, by the lack of growth in medium without substrate, and by microscopic observation) and then one additional time to ensure purity.

Determination of specific growth rates and organic nutrient requirements. Specific growth rates under various environmental conditions were determined from the specific rates of methane formation (21) by cultures adapted to those conditions, taking into account the methane produced by the inoculum prior to transfer (31). The levels of methane were determined by gas chromatography (21). Requirements for organic nutrients were investigated by cultivating the organisms for at least three transfers (5%, vol/vol) in medium containing a catabolic substrate as the sole organic addition.

Electrophoretic analysis of proteins. Cell pellets were collected by centrifugation at 10,000 × g for 20 min, washed in 30 ml of 1% (wt/vol) NaCl, and suspended in 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.5). The cells were then broken by passage through a French pressure cell (8,000 kPa). Several passages were required to break *M. barkeri* MS^T, 227, 227BM, 227BES^r, Jülich, FR-1, and Fusaro. *M. vacuolata* Z-761^T, which resisted breakage by this method, was broken by suspending 0.1 g (wet weight) of cells in 0.5 ml of 10 mM HEPES buffer (pH 7.5), adding 0.5 g of glass beads, and vortexing the preparation four times (1 min each). The

proteins in the lysates were analyzed by using denaturing gel electrophoresis (18).

DNA isolation and reassociation. Cell pellets were collected by centrifugation at $10,000 \times g$ for 20 min and washed in 30 ml of a 1% (wt/vol) NaCl solution. Cells without rigid walls (strains C 16^T, S-6^T, and C2A^T) were lysed by resuspending them in TE buffer (10 mM Tris, 1 mM EDTA, 0.15 M NaCl; adjusted to pH 8 with NaOH) and adding sodium dodecyl sulfate (0.1 g/liter). DNA was harvested from these lysates by using modifications of the chloroform-phenol method (16). Washed cell pellets of other strains were lyophilized and ground with a mortar and pestle (29). This method worked well and yielded high-molecular-weight DNA from *Methanosarcina* strains which resisted breakage by other methods. This DNA was harvested by using the modifications of Oakley et al. (29) of the technique of Summerton et al. (43), except that we washed the initial DNA pellet with 70% (vol/vol) ethanol and extracted carbohydrates after the RNase step with chloroform-isoamyl alcohol (24:1). DNAs for interstrain reassociation experiments were sheared by sonication (16) to lengths of about 200 to 1,500 bases. DNA size was estimated by using agarose gel electrophoresis of denatured DNA and comparison with an *Hind*III digest of λ phage DNA. DNA was labeled by using DNA polymerase (Amersham, Chicago, Ill.), random primers (Amersham), and [α -³²P]dCTP (NEN Research Products, DuPont, Boston, Mass.). The sizes of some, but not all, of the labeled DNA preparations were determined and were found to be 150 to 800 bases. The concentration of labeled DNA was adjusted to 0.6 mg/liter, and labeled DNA was not used unless its specific activity was at least 10^{11} Bq/g. The reassociation buffer was 10 mM Tris buffer containing 1 mM EDTA and 0.88 g of NaCl per liter (pH 7). Reassociation was done at 61.5°C in all experiments; this temperature was 25°C below the calculated melting point of the DNA (16). After reassociation (20 to 24 h), the remaining single-stranded DNA was digested with S1 nuclease (Pharmacia, Pleasant Hill, Calif.). Undigested DNA was precipitated with trichloroacetic acid and collected on glass fiber filters (16). The reassociation experiments always included four replicates of the negative control (sheared salmon sperm DNA), four replicates of the positive (homologous) control, and three replicates of DNA from each strain that was being tested. All of the relative sequence similarity values were based on reciprocal determinations unless stated otherwise. The standard deviations of the relative sequence similarity values were calculated by using the standard deviations of the radioactive counts for positive controls, negative controls, and sample DNA.

RESULTS

Isolation and characterization of new *Methanosarcina* strains. Several new *Methanosarcina* strains were isolated from four laboratory scale methanogenic reactors, and one strain was isolated from the cecum of a horse (Table 1). These strains grew in MS mineral medium supplemented with methanol as the sole organic substrate. They also grew in MS medium containing acetate, trimethylamine, methanol, or H₂-CO₂ as the sole catabolic substrate. These organisms grew in MS medium as individual cells except when they were switched from one substrate to another or after several months of storage without transfer. Under these conditions, aggregates often formed, but these aggregates broke up into individual cells after one or two transfers in

MS medium. All of these strains grew best between pH 7.0 and 7.5 at 37 to 40°C.

Electrophoretic analysis of *Methanosarcina* strains. Denaturing polyacrylamide gels of whole-cell proteins have been recognized as valuable taxonomic indicators at the species level (8). Most of the strains that previously have been assigned to the species *M. barkeri* (Table 1) produced protein patterns that were more similar to the protein patterns of *M. barkeri* MS^T than to the protein patterns of *M. mazei* S-6^T, *M. vacuolata* Z-761^T, or *M. acetivorans* C2A^T (Fig. 1A), supporting the assignment of these strains to *M. barkeri*. An exception was strain UBS, which appeared to be more similar to *M. mazei* S-6^T than to *M. barkeri* MS^T (Fig. 1C). The morphology of strain UBS was also similar to the morphology of *M. mazei* S-6^T because strain UBS usually grew as individual coccoid cells in MS medium and occasionally formed large aggregates. Thus, strain UBS should be considered a reference strain of *M. mazei*. In contrast to previous findings (44), our protein analysis indicated that *M. barkeri* MS^T and 227 are similar. This similarity is supported by the level of sequence similarity (97%) determined by DNA reassociation (41) and by the results of the rRNA cataloging studies of Balch et al. (2), which indicated that these strains are nearly identical.

Strain FR-1 was initially assigned to *M. barkeri* (1). Later, Zhilina and Zavarzin proposed that the possession of gas vesicles by *Methanosarcina* strains is an exclusive property of the species *M. vacuolata* (49). However, despite the presence of gas vesicles in strain FR-1, the results of an electrophoretic analysis of the whole-cell proteins suggested that this organism should be assigned to *M. barkeri* (Fig. 1A).

All of the *Methanosarcina* strains that were newly isolated in this study (strains GA3, GA4, GA5, GA6, GA8, GA12, GA14, GA15, and Equi) produced protein patterns that were more similar to those of *M. mazei* S-6^T than to those of *M. barkeri* MS^T, *M. vacuolata* Z-761^T, or *M. acetivorans* C2A^T (Fig. 1B). Thus, these new strains are reference strains of *M. mazei*.

Our study of whole-cell proteins was initially hampered by incomplete breakage of cells by the French pressure cell, especially incomplete breakage of *M. vacuolata* Z-761^T cells. Low yields of proteins suggested that cell breakage was not complete, so the proteins obtained may not have been representative. When breakage was incomplete, few protein bands were observed, and those bands which did appear were at low molecular weights and were not sharp. Another electrophoretic analysis (44) which relied on breakage by a French pressure cell revealed profound differences between *M. vacuolata* Z-761^T and other *Methanosarcina* strains; these results also may have been due to incomplete breakage of *M. vacuolata* cells. When we broke *M. vacuolata* Z-761^T cells by vortexing them with glass beads, the extracted proteins appeared to be more similar to the proteins of *M. barkeri* MS^T, although some differences remained (Fig. 1).

The results of our protein analysis (Fig. 1C) also suggested that *M. frisia* C 16^T is similar to *M. mazei* S-6^T.

DNA reassociation of *Methanosarcina* sp. strain FR-1 with *M. barkeri* MS^T and *M. vacuolata* Z-761^T. To confirm that strain FR-1 is more closely related to *M. barkeri* than to *M. vacuolata*, we labeled the DNA of strain FR-1 and hybridized it with DNAs from *M. barkeri* MS^T, *M. vacuolata* Z-761^T, and *M. mazei* S-6^T. The level of sequence similarity between *M. barkeri* FR-1 and *M. barkeri* MS^T was 80% ($\pm 7\%$), but the levels of sequence similarity between strain

FR-1 and the type strains of *M. vacuolata* and *M. mazei* were less than 40%. These are the average results of two separate experiments, but reciprocal analyses (with labeled DNAs from *M. mazei* S-6^T and *M. barkeri* MS^T) were not performed.

Reassociation of DNAs from *M. frisia* C 16^T, *M. mazei* S-6^T, and *M. acetivorans* C2A^T. DNA reassociation data indicated that *M. frisia* C 16^T exhibited 77 ± 11% sequence similarity with *M. mazei* S-6^T but only 31 ± 4% sequence similarity with *M. acetivorans* C2A^T. The level of sequence similarity between *M. mazei* S-6^T and *M. acetivorans* C2A^T was 26 ± 12%. These data support the hypothesis that *M. acetivorans* is a separate *Methanosarcina* species but suggest that *M. mazei* and *M. frisia* are subjective synonyms.

Effect of salinity on the growth of *Methanosarcina* strains. A major previously reported physiological difference between *M. frisia* C 16^T and *M. mazei* S-6^T is the effect of salinity on growth. *M. frisia* C 16^T was isolated from a marine source, and this strain has been reported to be slightly halophilic (4). The source of *M. mazei* was an anaerobic digester (23), and this strain is slightly halotolerant (21). However, a careful comparison of the effects of salinity on the growth of these two strains has not been described previously, so we tested the growth of *M. frisia* C 16^T and found that, like *M. mazei* (21), *M. frisia* C 16^T was slightly halotolerant but not halophilic (Fig. 2A). Furthermore, we found that a wide range of newly isolated strains of *M. mazei* were also slightly halotolerant, growing well at salinities of 0.75 to 1.0 M Na⁺ (Fig. 2B and C). Likewise, the other marine *Methanosarcina* species, *M. acetivorans*, was not halophilic but was slightly halotolerant (Fig. 2A); this species grew better in MS medium with a low osmolarity (ca. 220 mosM) than in medium containing marine concentrations of salt.

The two marine isolates, *M. frisia* C 16^T and *M. acetivorans* C2A^T, were more halotolerant when they were grown on trimethylamine than when they were grown on methanol (Fig. 2A).

Catabolism of acetate by *M. frisia* C 16^T. A second major difference between the previously reported characteristics of *M. frisia* C 16^T and *M. mazei* S-6^T is the inability of the former strain to catabolize acetate, although some strains of *M. mazei*, such as strain LYC, cannot catabolize acetate. However, *M. frisia* C 16^T grew in MS medium containing 50 mM acetate. Like other *Methanosarcina* strains (7), methanol-grown *M. frisia* C 16^T had a 2-month lag before growth began in acetate-containing medium.

DISCUSSION

Taxonomy of *Methanosarcina* sp. strain FR-1. On the basis of characteristics other than possession of vesicles, *Methanosarcina* sp. strain FR-1 might be placed in either *M. barkeri* or *M. vacuolata*. Archer and King (1) considered strain FR-1 and other *Methanosarcina* strains with vesicles to be members of *M. barkeri*. However, DNA reassociation studies (20) have shown that *M. barkeri* MS^T and *M. vacuolata* Z-761^T exhibit only 61% DNA sequence similarity, suggesting that they should be different species. The

results of an antigenic analysis (5) also support the distinction between *M. barkeri* and *M. vacuolata*. Zhilina and Zavarzin proposed that the possession of gas vesicles by *Methanosarcina* strains is an important characteristic which indicates that the strains should be assigned to the species *M. vacuolata* (49). Although the physiological differences between *M. barkeri* MS^T and *M. vacuolata* Z-761^T are limited, one recognized difference is the inability of the latter strain to grow well on H₂-CO₂. This organism grows poorly on H₂-CO₂ in complex medium and not at all in mineral medium (21). The results of our protein analysis and DNA reassociation studies indicated that strain FR-1 is more similar to *M. barkeri* MS^T than to *M. vacuolata* Z-761^T, supporting placement of strain FR-1 in *M. barkeri* as suggested by Archer and King. Also, strain FR-1, like *M. barkeri* MS^T but unlike *M. vacuolata* Z-761^T (21), grows in mineral medium supplemented with H₂-CO₂ (1). Thus, strain FR-1 is a reference strain of *M. barkeri*, and gas vesicles are not a characteristic that occurs exclusively in *M. vacuolata* strains. We propose that the species description of *M. barkeri* should be emended to include strains with gas vesicles.

Rejection of *M. frisia* as a junior subjective synonym of *M. mazei*. *M. frisia* C 16^T is physiologically similar *M. mazei* S-6^T. Previously, the major reported differences between these two species were (i) that *M. frisia* is slightly halophilic and *M. mazei* is not and (ii) that *M. frisia* cannot catabolize acetate and *M. mazei* usually can. However, under the conditions used in this study, the type strains of these two species were not halophilic but were halotolerant, and they could catabolize acetate. Furthermore, the level of sequence similarity between the DNAs of these two strains was 77%. Thus, *M. frisia* is a subjective synonym of *M. mazei*, and strain C 16 is a reference strain of *M. mazei*.

Halotolerance of *Methanosarcina* strains. The *Methanosarcina* strains which we studied, including the two strains that previously have been reported to be halophilic, were not halophilic, but rather were halotolerant. Even the two *Methanosarcina* strains that were isolated from marine sources (strains C 16 and C2A^T) grew faster in 220 mosM medium than they did in media which had marine osmolarities (ca. 1,000 mosM). When a low-osmolarity culture medium is inoculated with a higher-osmolarity culture, hypotonic lysis of cells may result. Thus, care should be taken when the effect of salinity on growth is determined by transferring cultures to media with lower osmolarities in small steps.

Use of whole-cell protein electrophoresis in assignment of strains to species. The assignment of reference strains to *Methanosarcina* species, including our proposed changes (Table 1), produces a taxonomy with significant agreement with the assignments based on the results of electrophoresis of whole-cell proteins (Fig. 1). The resulting assignments are consistent with previous assignments except for (i) strains FR-1 and C 16, for which our assignments were confirmed by the results of DNA reassociation experiments, and (ii) strain UBS (which was previously assigned to *M. barkeri* [45]), for which our assignment was supported by morphological data.

FIG. 1. Denaturing gel electrophoresis of whole-cell proteins of *Methanosarcina* strains and molecular weight standards. The standards had molecular weights of 180,000, 116,000, 84,000, 58,000, 48,500, 36,500, and 26,600. (A) Molecular weight standards, *M. acetivorans* C2A^T, *M. vacuolata* Z-761^T, *M. mazei* S-6^T, *M. barkeri* MS^T, 227-BM, 227-Bes^T, Jülich, 227, S-3, Wiesmor, Fusaro, 3, and FR-1, and *M. vacuolata* Z-761^T. (B) Molecular weight standards, *M. acetivorans* C2A^T, *M. vacuolata* Z-761^T, *M. barkeri* MS^T, and *M. mazei* S-6^T, Equi, GA3, GA4, GA5, GA6, GA8, GA12, GA14, and GA15. (C) Molecular weight standards, *M. acetivorans* C2A^T, *M. vacuolata* Z-761^T, *M. barkeri* MS^T, and *M. mazei* S-6^T, UBS, C 16, LYC, CW3A, Göl, G1, KCl-2S, MCMB703, and Z-558.

FIG. 2. Influence of salinity on the specific growth rates of *Methanosarcina* strains grown on methanol or on trimethylamine. The growth substrate was methanol unless indicated otherwise. The standard deviation for each data point is indicated by a vertical bar; the data points without vertical bars had standard deviations that were smaller than the sizes of the symbols.

The proteins of *M. vacuolata* Z-761^T and *M. acetivorans* C2A^T were clearly different, which should allow accurate assignment of gas vesicle-containing *Methanosarcina* strains to the appropriate species in the future. Assignment of reference strains is important taxonomically because it al-

lows workers to determine whether characteristics are rare or common among members of a species. The taxonomy of many methanogenic species is hampered by the lack of reference strains, and many descriptions are based on a single isolate. For the *Methanosarcina* species included in this study, denaturing electrophoresis of whole-cell proteins is an easy, rapid method for assigning strains to species.

When cells are prepared for electrophoresis, incomplete breakage may lead to low yields and anomalous results. *Methanosarcina* cells are often particularly difficult to break, but we found that vortexing the cells with glass beads readily broke *M. vacuolata* cells.

Habitat of *M. mazei*. The habitats of reference strains of *M. mazei* now include anaerobic digestors, marine and freshwater sediments, and the gastrointestinal tracts of animals.

ACKNOWLEDGMENTS

We thank Mary Taylor (Portland State University) for help in disrupting cells and Jim Clegg (Oregon Graduate Institute) for helpful discussions and suggestions.

This work was supported by grants 480-323-4023 and IFAS-GRI-FIA-MCS 2171 from the Gas Research Institute as part of the Program on Methane from Biomass in collaboration with the Institute of Food and Agricultural Sciences, University of Florida, and by the Consejo Superior de Investigaciones Científicas, Madrid, Spain.

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