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Description of an Estuarine Methylotrophic Methanogen Which Grows on Dimethyl Sulfide

RONALD S. OREMLAND,[1]* RONALD P. KIENE,[2] INDRA MATHRANI,[3] MICHAEL J. WHITICAR,[4] AND DAVID R. BOONE[5]

United States Geological Survey, 345 Middlefield Road, Menlo Park, California 94025[1]; University of Georgia Marine Institute, Sapelo Island, Georgia 31327[2]; School of Public Health, University of California, Los Angeles, Los Angeles, California 90024[3]; Federal Institute for Geosciences and Natural Resources, D-3000 Hannover 51, Hannover, Federal Republic of Germany[4]; and Department of Environmental Science and Engineering, Oregon Graduate Center, Beaverton, Oregon 97006-1999[5]

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Characteristics of an obligately methylotrophic coccoid methanogen (strain GS-16) previously isolated from estuarine sediment are described. Growth was demonstrated on dimethyl sulfide (DMS) or trimethylamine (TMA), but not on methane thiol, methane thiol plus hydrogen, dimethyl disulfide, or methionine. DMS-grown cells were able to metabolize DMS and TMA simultaneously when inoculated into media containing substrate levels of these compounds. However, TMA-grown cells could not metabolize [^{14}C]DMS to $^{14}CH_4$, although they could convert [^{14}C]methanol to $^{14}CH_4$. These results suggest that metabolism of DMS proceeds along a somewhat different route than that of TMA and perhaps also that of methanol. The organism exhibited doubling times of 23 and 32 h for growth (25°C) in mineral media on TMA and DMS, respectively. Doubling times were more rapid (~6 h) when the organisms were grown on TMA in complex broth. In mineral media, the fastest growth on DMS occurred between pH levels of 7.0 and 8.7, at 29°C, and with 0.2 to 0.4 M Na^+ and 0.04 M Mg^{2+}. Somewhat different results occurred for growth on TMA in complex broth. Cells had a moles percent G+C value of 44.5% for their DNA. Growth on DMS, TMA, and methanol yielded stable carbon isotope fractionation factors of 1.044, 1.037, and 1.063, respectively. Fractionation factors for hydrogen were 1.203 (DMS) and 1.183 (TMA).

The inability of certain species of methanogens to grow on hydrogen plus carbon dioxide was first noted for obligately acetotrophic organisms such as *Methanosarcina thermophila* (54) and *Methanothrix soehngenii* (15, 50). Another group of nonhydrogen-utilizing methanogens grows only on one-carbon compounds like methanol (MeOH) or methylated amines. *Methanolobus tindarius* and *Methanococcoides methylutens* were the first examples of such obligately methylotrophic methanogens (22, 44), and both were isolated from coastal marine sediments. Subsequently, four isolates representative of either moderate or extremely halophilic methanogens were identified as obligate methylotrophs (26, 29, 34, 51, 52). The inability of these obligately methylotrophic methanogens to use hydrogen or acetate allows them to occupy niches in which they avoid competition with sulfate reducers for these substrates (30, 32). About half of the culturable methanogen population of saltmarsh sediments is made up of obligate methylotrophs (10).

Osmoregulatory solutes like glycine-betaine can be the ultimate precursors of trimethylamine (TMA) in saline environments (19–21). Likewise, dimethylsulfoniopropionate is an osmoregulatory solute of marine plants and algae (24, 46) that upon decomposition forms dimethyl sulfide (DMS) and acrylic acid (6, 14, 17). Earlier studies demonstrated that lake sediments converted DMS to methane and carbon dioxide (53). More recently, this was shown to be carried out by methanogens in a diversity of sediment types (marine, alkaline-hypersaline, freshwater), and an obligately methylotrophic methanogen was isolated from estuarine sediments which grew on DMS, TMA, or MeOH (16). This organism can also metabolize traces of dimethylselenide to methane and carbon dioxide (33). We report here a further characterization of this organism and its ability to fractionate the stable isotopic composition of methane formed from TMA and DMS.

* Corresponding author.

MATERIALS AND METHODS

Organism and media. A DMS-metabolizing enrichment culture was recovered from estuarine sediments, purified by antibiotic treatment, serial dilution, and colony isolation, and checked for purity as described previously (16). An estuarine basal salts medium supplemented with vitamins (EBSV) was employed for culture and consisted of (in grams per liter): NaCl (23.0), $MgSO_4 \cdot 7H_2O$ (0.5), K_2HPO_4 (0.225), KH_2PO_4 (0.225), $CaCl_2 \cdot 2H_2O$ (0.06), $(NH_4)_2SO_4$ (0.225), Na_2CO_3 (2.0), cysteine hydrochloride (0.25), $Na_2S \cdot 9H_2O$ (0.25), HS-coenzyme M (0.15), resazurin (0.002), hemin (0.001), the trace elements solution (9 ml) and vitamin solution (10 ml) of Wolin et al. (48), the fatty acid mixture (0.3 ml) of Caldwell and Bryant (5), and DL-methylbutyric acid (1.0 ml). The medium was dispensed anaerobically into serum bottles (liquid volume, 20 ml) or Balch culture tubes (liquid volume, 10 ml), sealed under N_2-CO_2 (80:20), and autoclaved (16). Substrates (DMS or TMA) were added to individual tubes or bottles prior to autoclaving. Unless stated otherwise, the final concentrations of DMS, MeOH and TMA were 10 mM. The final pH value of the EBSV medium was ~7.7. Magnesium- and sodium-free EBSV media were prepared by substituting calcium or potassium salts, respectively. The pH value of the medium was adjusted with either HCl or NaOH to poise the cultures in order to test the spectrum of pH tolerance. The culture was also grown in a rich marine broth (MB) medium which

was composed of (in grams per liter): K_2HPO_4 (0.4), KCl (0.5), $MgCl_2 \cdot 7H_2O$ (3.0), $CaCl_2 \cdot 2H_2O$ (0.25), NH_4Cl (1.0), NaCl (30.0), cysteine (0.5), yeast extract (2.0), Trypticase peptone (BBL Microbiology Systems) (2.0), $NaHCO_3$ (4.0), and TMA (1.91; 20 mM). Cultures were incubated under N_2, and the final pH value was 8.2.

Determination of growth. Acridine orange direct counts were made to determine cell densities during growth in EBSV media (13). A sterile syringe was used to remove 0.2 ml of culture, which was added to 5 ml of filtered (0.2 μm) NaCl solution (23 g/liter) in a filter tower. This volume was reduced to 1.0 ml by gentle filtration onto a prestained (black) Nuclepore filter, which was then stained with 200 μl of 1% acridine orange. The remaining volume was filtered, and the preparation was counted by epifluoresence microscopy with a Leitz Dialux 20 epifluorescence microscope. Protein in whole cells was analyzed with a Bio-Rad dye microassay with chicken lysozyme (Sigma Chemical Co., St. Louis, Mo.) as the standard. Samples (1.0 ml) were removed, placed in test tubes containing 2.0 ml of 0.05 N NaOH, boiled for 10 min, and, after cooling, treated with the dye procedure; the optical density at 595 nm was determined with a Spectronic 21 colorimeter. Methane, methanethiol (MSH), and dimethyl sulfide in the headspace of cultures were determined by flame ionization gas chromatography (16, 32). Metabolism of [^{14}C]DMS (6 μCi/10 ml of media) and [^{14}C]MeOH (6 μCi/10 ml of media) to $^{14}CH_4$ by cells inoculated into fresh media containing TMA (10 mM) or DMS (7 mM) was measured by radio-gas chromatography (8).

Determination of moles percent guanine plus cytosine. The moles percent guanine plus cytosine was determined from the buoyant density of whole-cell DNA. DNA was isolated according to the method of Marmur (25) from late-exponential-phase cells grown in MB plus TMA as the substrate. The density of the DNA was determined by centrifugation in CsCl gradients immobilized after centrifugation by polymerization of acrylamide (35), with *Clostridium perfringens* (ρ, 1.691 kb liter^{-1}) and *Micrococcus lysodeikticus* (ρ, 1.731 kg liter^{-1}) as internal standards. The moles percent guanine plus cytosine was calculated from the density gradient by using the formula of Schildkraut et al. (40).

Preparation of scanning electron micrographs. The procedures outlined by Smith et al. (43) were used.

Determination of stable carbon and hydrogen isotope fractionation. Balch tubes containing either MeOH (10 mM), TMA (10 mM), or dimethyl sulfide (7 mM) were inoculated with a late-exponential-phase culture. In order to prevent carry-over of substrate, the inoculum was diluted into substrate-free media just prior to inoculation of the experimental tubes. Final dilution of any carry-over substrate was >1,000-fold. Tubes were incubated at 20°C, and methane in the headspaces was monitored. When the quantity of methane formed represented between 3 and 10% conversion of the substrate, the headspaces were removed by displacement with injected water. Exiting gases were captured in syringes which were next injected into water-filled, capped serum vials (10 ml). After the water was displaced, the vials were stored at −20°C until mass spectral analyses of the methane could be performed.

The methods used for determination of $^{13}C/^{12}C$ and D/H ratios in methane have been described previously (7, 9, 31, 41, 47). Values are reported in standard delta notation: dR = (Ra/Rb sample − Ra/Rb standard)/(Ra/Rb standard) × 1,000, where Ra/Rb is the $^{13}C/^{12}C$ or D/H ratio of either the sample or the standards (PeeDee Belemnite for carbon; standard mean ocean water for hydrogen).

Reagents and chemicals. Dimethyl sulfide was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. (purity, >99%). MSH was obtained from Matheson Gas Products, Inc., Secaucus, N.J. (purity, >99%). All other chemicals were standard reagent grade. Radioisotopes were obtained from Amersham Corp., Arlington Heights, Ill. Specific activities were 59 Ci/mol for [^{14}C]MeOH and 22 Ci/mol for [^{14}C]DMS.

RESULTS

Morphological characteristics. The isolated coccus (designated strain GS-16) stained gram negative and grew in multicellular aggregates or clumps when cultured on DMS or TMA in EBSV media. These clusters could be disrupted by vortexing, which enabled us to perform cell counts. Clumping was not observed during growth in the MB media. Scanning electron micrographs revealed the cells to be regular, smooth-shaped cocci, each with a diameter of about 0.5 μm (Fig. 1). Lysis, as determined by turbidity decreases and confirmed by microscopy, occurred when mid-log-phase cells were exposed for 15 min to 50 mg of either sodium dodecyl sulfate or Triton X-100 per liter, indicating a cell wall consisting of protein subunits.

Growth on methylated substrates. Growth of GS-16 in EBSV media with TMA or DMS occurred as indicated by increases of methane, cell counts, and cell protein with time (Fig. 2). Better growth and higher cell yields were obtained with TMA as a substrate, and doubling times were generally faster for TMA (23 h at 25°C) than they were for DMS (32 h). More rapid doubling times (~6 h) were observed for growth on TMA in MB media. Concentrations of DMS >~10 mM inhibited growth, and no growth was observed at 20 mM DMS, even when supplemented with a noninhibitory concentration (20 mM) of TMA (data not shown).

Growth on DMS in EBSV media resulted in the formation of MSH. Approximately 0.67 mol of MSH was formed per mol of methane, and these two parameters had a high correlation coefficient (r^2, 0.985 [Fig. 3]). However, the amount of MSH detected was probably less than the total amount produced due to loss from subsequent metabolic reactions as well as by physical absorption into the rubber stopper (16). Growth of GS-16 in media which contained both DMS and TMA indicated that cells could metabolize DMS (as indicated by formation of MSH and loss of DMS) concurrently with TMA (Fig. 4). However, cell lines carried for over 1 year on TMA had to adapt over a 3- to 4-week period before they were capable of observable growth on DMS (data not shown).

Inoculation of a TMA-grown cell line (taken through several previous transfers on TMA) into TMA media containing [^{14}C]MeOH resulted in the formation of $^{14}CH_4$ (Table 1). By contrast, inoculation into TMA media with [^{14}C]DMS produced neglible traces of $^{14}CH_4$. In addition, whereas production of $^{14}CH_4$ from [^{14}C]MeOH continued for 48 h, no detectable levels of $^{14}CH_4$ were found in the [^{14}C]DMS tube at 24 or 48 h, despite the fact that CH_4 levels increased. Assuming a 3:1 conversion ratio of MeOH or DMS to methane, the percentages of isotope metabolized by 4 h were 0.45 and 0.09 for [^{14}C]MeOH and [^{14}C]DMS, respectively. By 48 h, about 29% of the [^{14}C]MeOH had reacted, but there were no observable counts in the TMA tube containing [^{14}C]DMS. At 120 h, a small quantity of $^{14}CH_4$ (~23×10^3 dpm) was observed in the TMA plus [^{14}C]DMS tube (data not shown). When another TMA plus [^{14}C]DMS tube was inoculated, essentially the same results were obtained (data

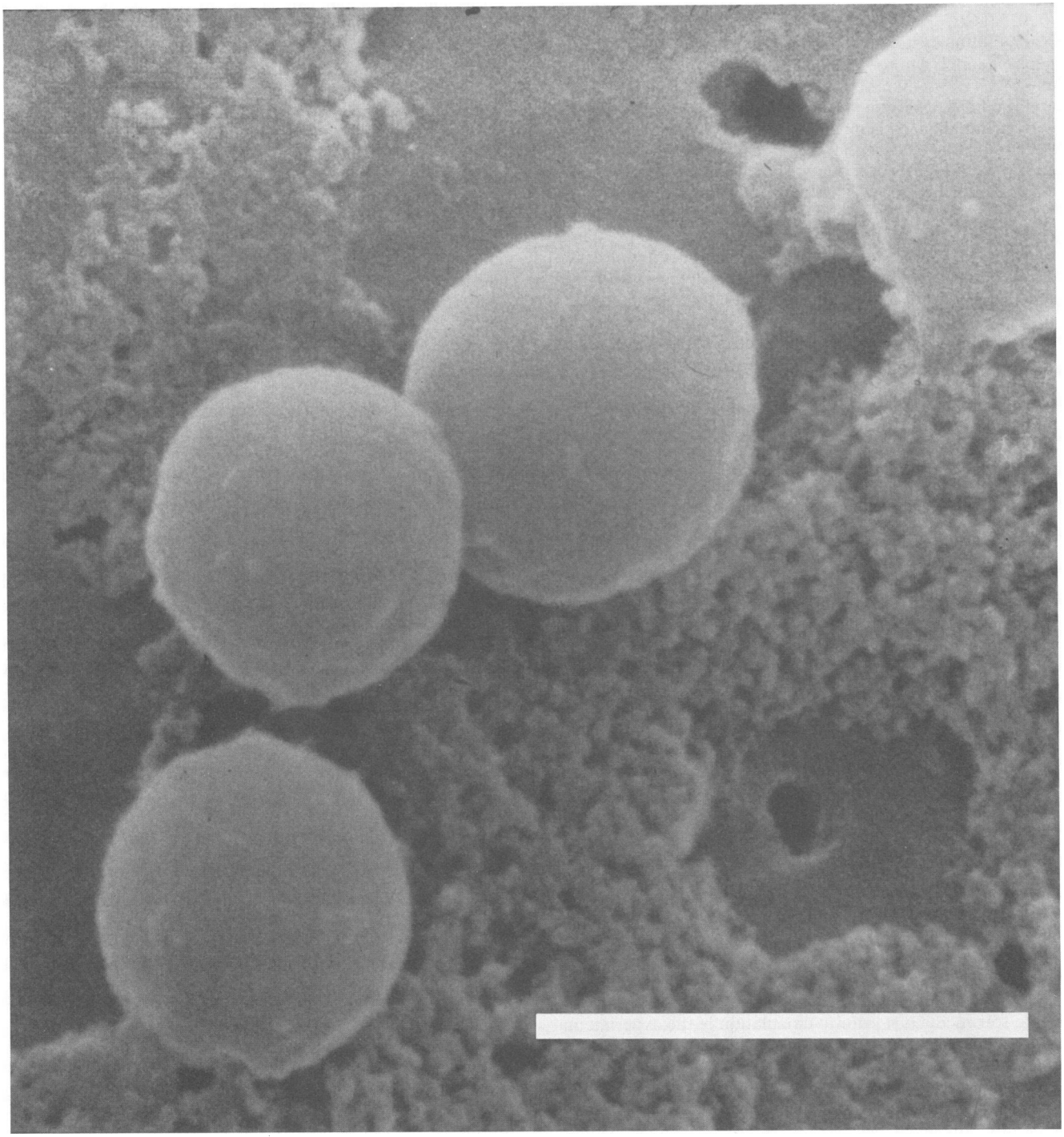

FIG. 1. Scanning electron micrograph of GS-16. Bar, 1 μm.

not shown). The absence of the initially observed $^{14}CH_4$ in the headspace of the [^{14}C]DMS tube after 24 h may have been due to loss of some of this small quantity (to below detection limits, ~8,000 dpm per tube) through the stopper coupled with no further production. In contrast, a control cell line grown on DMS was capable of continued $^{14}CH_4$ formation from [^{14}C]DMS, which represented a conversion of about 1.9% of the isotope to $^{14}CH_4$ by 72 h (Table 1).

GS-16 did not grow in media containing methionine, dimethyl disulfide, MSH, or MSH plus 20% H_2 as the substrate (Table 2). A significant amount of growth was observed when dimethyl sulfoxide was the substrate; however, this was found to be an experimental artifact. It was later determined that a chemical reduction of dimethyl sulfoxide to DMS occurred in sterile media. Similar observations have been made by other investigators (S. Zinder, personal communication). GS-16 grew well on MeOH as evidenced by several successive transfers in EBSV plus

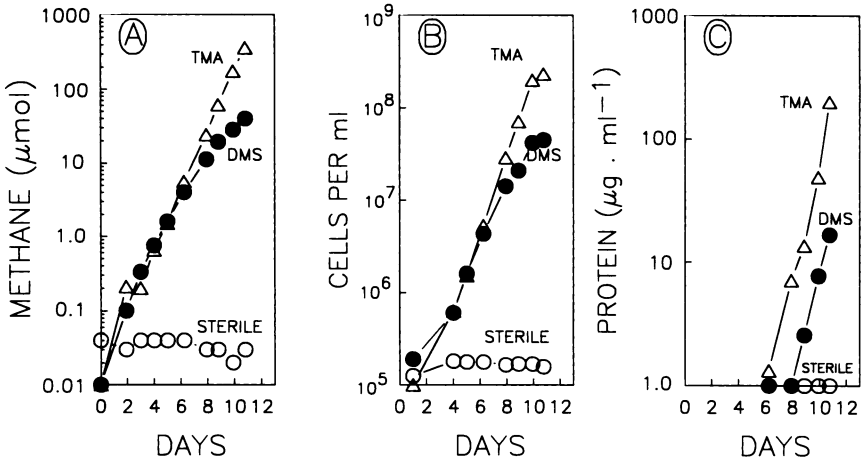

FIG. 2. Growth of GS-16 in EBSV medium at 20°C on TMA or DMS as measured by (A) methane, (B) cell counts, or (C) protein in whole cells.

MeOH medium with concomitant methane formation (data not shown).

Growth optima for temperature, pH level, Na^+, and Mg^{2+}. Growth of GS-16 in MB medium plus TMA exhibited pronounced optima for temperature, pH level, and Na^+ concentration, while in EBSV medium plus DMS, optima occurred over broader ranges and at lower growth rates (Fig. 5 through 8). Growth in EBSV medium on DMS was optimal at a temperature of 29°C, with only slight growth at 4°C or above 36°C (Fig. 5). This experiment was repeated with either DMS or TMA in EBSV medium with essentially the same results. Growth rates (μ) at 29°C were 0.069 for DMS and 0.063 for TMA; at 32°C, they were 0.018 for DMS and 0.017 for TMA. There was no growth at 40°C. The temperature optimum for growth on TMA in MB was 37°C, with significant growth occurring at 40°C but not at 45°C. These results were reproducible. We did not attempt a growth-versus-temperature experiment in MB plus DMS; however, we did observe growth at 37°C under these conditions. The pH range for growth in EBSV plus DMS was 5.7 to 9.2, with a broad optimum between 7.0 and 8.7 (Fig. 6). EBSV medium precipitated at pH levels above 9.2. The pH curve for growth in MB medium plus TMA was somewhat different, exhibiting a range from 7.0 to 8.6 and having a marked optimum at 8.0. The Na^+ optimum in EBSV medium plus DMS was 0.4 M but was 0.6 M in MB plus TMA; growth occurred in MB medium at 1.2 M, while no growth occurred in EBSV medium at 1.2 M (Fig. 7). The optimum Mg^{2+} concentration for growth (EBSV medium plus DMS) was 0.04 M (Fig. 8). The culture was successfully transferred sequentially 10 times (1% [vol/vol] inocula) into EBSV medium plus DMS that did not contain HS-coenzyme M, without any noticeable influence on growth (data not shown).

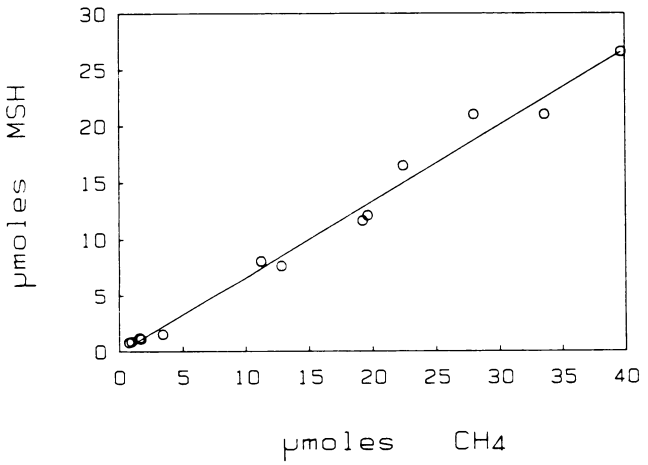

FIG. 3. Relationship between methane (CH_4) formed and MSH produced during growth of the isolate on DMS in EBSV medium.

Colony characteristics. Cell lines cultured in EBSV plus DMS or MB plus TMA for over 1 year were inoculated into agar roll tubes of MB plus TMA medium. After 2 weeks, subsurface colonies (0.5 to 1.0 mm in diameter) were evident. Colonies from both substrate culture lines were identical. Colonies were lenslike in cross section, had entire edges, and were tan in color with grainy interiors.

Results of G+C ratios. The moles percent guanine plus cytosine was 44.5%.

Fractionation of stable isotopes. Methane in the gas phases of the culture tubes was recovered for isotopic analysis after 5 days of incubation with DMS and after 8 days of incubation with MeOH or TMA. The quantity of methane formed in these experiments was less than 10% of the available substrate (Table 3). There was no significant difference in the fractionations found in the two TMA tubes which represented conversions of 3.1 and 9.6% of the substrate. Therefore, a significant closed system effect was avoided. Fractionation factors for carbon were 1.044, 1.037, and 1.063 for DMS, TMA, and MeOH, respectively. Fractionation factors for hydrogen were 1.203 and 1.183 for DMS and TMA, respectively. Insufficient methane was formed in the MeOH incubation to allow for determination of its D/H ratio.

DISCUSSION

Strain GS-16 is a typical marine strain as indicated by its optimal growth rates in EBSV media for Na^+ (0.4 to 0.6 M) and Mg^{2+} (0.04 M) and by its pH level of ~8.0 (Fig. 6–8). These chemical values are characteristic of seawater (36). Optimal growth was also reported for these Na^+ and Mg^{2+} ranges for other marine strains of methanogens, although the methylotrophs *M. methylutens*, *Methanococcus frisius*, *M. tindarius*, and *Methanosarcina acetivorans* all have pH

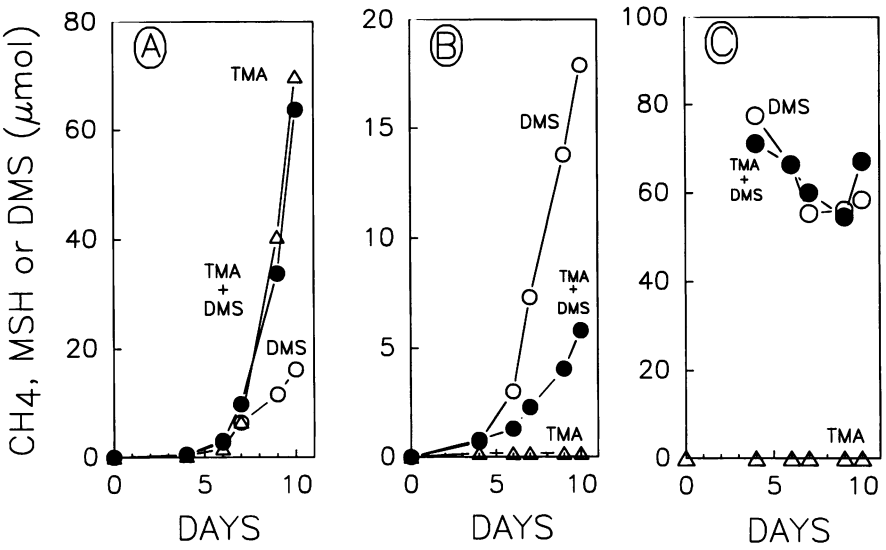

FIG. 4. Formation of methane (A) and MSH (B) and loss of DMS (C) during growth of cultures on DMS (○), DMS plus TMA (●), and TMA (△).

optima (6.5 to 7.5) that are lower than what we observed (3, 22, 44, 45). The Na^+ optimum for strain GS-16 was well below that reported for moderately or extremely halophilic methanogens (26, 27, 34, 52).

It is noteworthy that somewhat different results were obtained when the isolate was grown on TMA in a Trypticase peptone plus yeast extract broth (MB medium), as opposed to EBSV medium on DMS. For Na^+ and pH level, the ranges were generally similar (Fig. 6 and 7). However, a more striking difference was observed in the temperature ranges, with an optimum at 29°C in EBSV but an optimum at 37°C in MB (Fig. 5). These results were repeated and reproducible for either DMS or TMA when cells were grown in EBSV medium. Cells grown in EBSV medium did not grow at 35°C, while we consistently observed maximal growth in MB at 37°C on TMA and growth on DMS in MB also occurred at 37°C. The purity of the culture was ascertained by the uniformity of cultures arising from either the EBSV plus DMS or the MB plus TMA cell lines when inoculated into roll tubes of MB plus TMA. We conclude that this difference in temperature optimum was due to the different compositions of the two media. These points underscore the fact that different media can produce different physiological responses (especially with regard to temperature), thereby illustrating the limitations of physiological characterizations. Most reports of novel isolations employ only one medium.

We observed clumping when cells were grown in EBSV media, but not in the MB media. Flocculation has been found to be related to the divalent cation content of media for growth of both *Methanosarcina barkeri* and *Methanosarcina mazei* (4, 39, 49). This suggests that the aggregation we observed in EBSV media may have been related to a deficiency of calcium or magnesium ions and that no such deficiency occurred during growth in MB media. EBSV medium contains both Mg^{2+} (2 mM) and Ca^{2+} (0.4 mM) ions; however, these levels could have been too low to avoid clumping (4).

Cells grew better on TMA than they did on DMS when both these substrates were present at similar concentrations (Fig. 2). Higher levels of DMS (>10 mM) proved inhibitory to growth, and no growth occurred at 20 mM DMS, even when TMA was present (data not shown). The poor growth on DMS, as well as its inhibitory action at high concentrations, may be a consequence of its toxicity, the accumulation of toxic end products like H_2S or of the MSH intermediate, or all of these factors (16). Formation of MSH was clearly associated with growth on DMS (Fig. 3). However, we did not observe growth on MSH itself, even when we included H_2 in the gas phase (Table 2). Previous work demonstrated that MSH is an intermediate in the metabolism of DMS but that MSH fermentation to CH_4, CO_2, and H_2S provides insufficient energy for growth unless linked to DMS metabolism (16). Therefore, H_2-linked MSH metabolism does not

TABLE 1. Production of $^{14}CH_4$ from [^{14}C]DMS or [^{14}C]MeOH after inoculation of GS-16 into 10 ml of EBSV medium with either TMA or DMS as substrate

Substrate (mM)	Isotope (μM)	Amount[a] of CH_4 or $^{14}CH_4$ produced in indicated no. of h							
		4		24		48		72	
		CH_4	$^{14}CH_4$	CH_4	$^{14}CH_4$	CH_4	$^{14}CH_4$	CH_4	$^{14}CH_4$
TMA (10)	MeOH (9)	127	43	523	885	814	2,888	ND[b]	ND
TMA (7)	DMS (24)	151	9	581	0	830	0	ND	ND
DMS (7)	DMS (24)	50[c]	24	ND	ND	ND	ND	874	256

[a] Amount of CH_4 determined as nanomoles per 10-ml culture. Amount of $^{14}CH_4$ determined as number of disintegrations per minute × 10^3 per 10-ml culture; detection limit, ≅ 8 × 10^3/10 ml.

[b] ND, Not determined.

[c] Sampled 1 h postinoculation.

TABLE 2. Methane production by strain GS-16 from various methylated organosulfur compounds[a]

Substrate	Concn (mM)	Days of incubation	Methane produced (μmol)
None	0	11	0.14
DMS	10	11	4.43
DMDS[b]	10	11	0.10
DMSO[c]	10	11	1.51
Methionine	10	11	0.12
MSH	1	20	0.17
MSH + 20% H_2	2	11	0.19

[a] Results are for 10 ml of culture incubated at 22 to 25°C under N_2-CO_2 (80:20) unless indicated otherwise.
[b] DMDS, Dimethyl disulfide.
[c] DMSO (dimethyl sulfoxide) utilization was found to be an artifact of its chemical reduction to DMS (see text).

occur in strain GS-16 as H_2-linked MeOH reduction occurs in *Methanosphaera* species (2, 28). Apparently, GS-16 lacks hydrogenase. Neither dimethyl disulfide nor methionine was capable of sustaining growth (Table 2), and no growth was observed on diethyl sulfide, even though GS-16 could form traces of ethane from this ethylated analog of DMS (32a).

We did, however, observe the formation of MSH and the loss of DMS when DMS-grown cells were grown on TMA plus DMS (Fig. 4). Greater methane production in the tubes containing TMA or TMA plus DMS than in the DMS tube (Fig. 4A) implied that the TMA was being metabolized as well. These results suggest that the organism can metabolize DMS and TMA simultaneously, provided that the cell line originates from DMS media. Thus, in this instance diauxie did not occur as in the case of MeOH and acetate-grown *Methanosarcina* spp. (42). TMA-grown cells had the ability to continuously metabolize traces of [^{14}C]MeOH in the TMA medium for 48 h, but they were incapable of sustained metabolism of [^{14}C]DMS (Table 1). This suggests that a different enzyme(s) is involved in DMS metabolism which is not constitutive in TMA-grown cells. This is reinforced by the observation that cell lines kept in TMA for >1 year had a long lag time (~3 to 4 weeks) before they demonstrated growth on DMS.

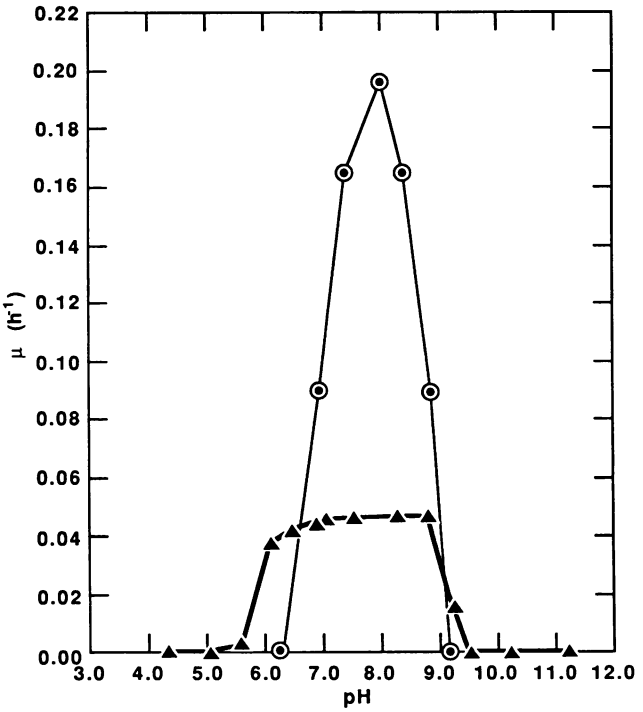

FIG. 6. Effect of pH level on specific growth rate (μ) in TMA plus MB medium (⊙) and DMS plus EBSV medium (▲).

The ability of methylotrophic metanogens to grow on DMS does not appear to be widespread in the available stock cultures. No growth occurred when *M. barkeri* 227, *M. mazei* S-6, *M. thermophila* TM-1, the moderate halophile strain SF1 (26), or the moderate halophile strain SD-1 (I. Mathrani, D. Boone, and R. A. Mah, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, I85, p. 160) was grown in 5 mM DMS. Only *Methanohalophilus zhilinae* demonstrated growth on DMS (27). This implies that a unique enzyme system for DMS metabolism is distributed among only a few species of methanogens currently available in culture collec-

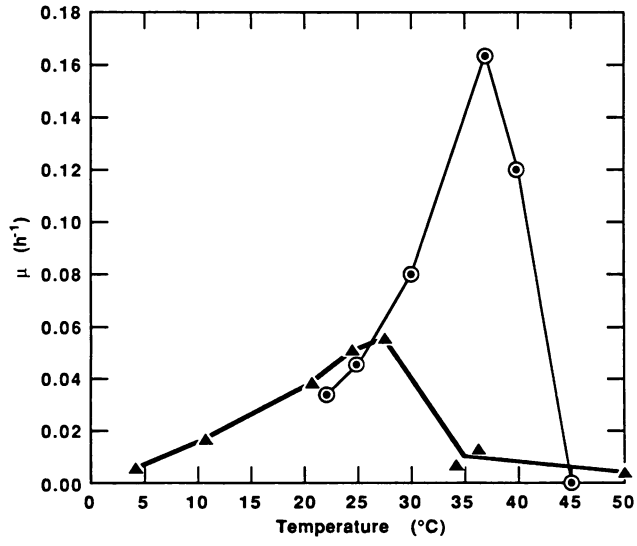

FIG. 5. Effect of temperature on specific growth rate (μ) in TMA plus MB medium (⊙) and DMS plus EBSV medium (▲).

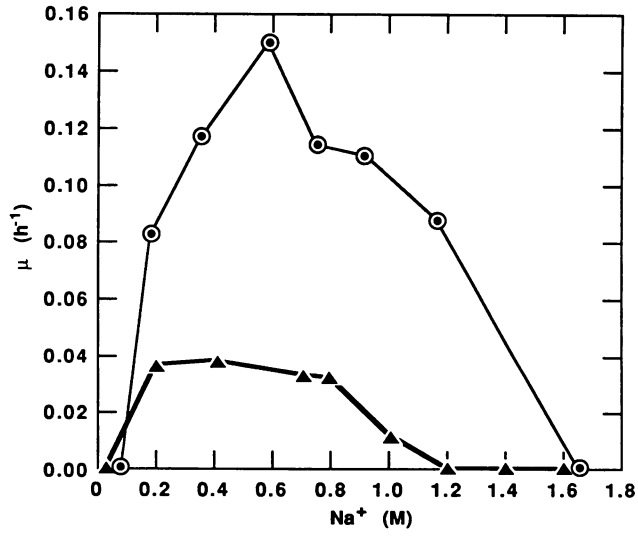

FIG. 7. Effect of Na^+ on specific growth rate (μ) in TMA plus MB medium (⊙) and DMS plus EBSV medium (▲).

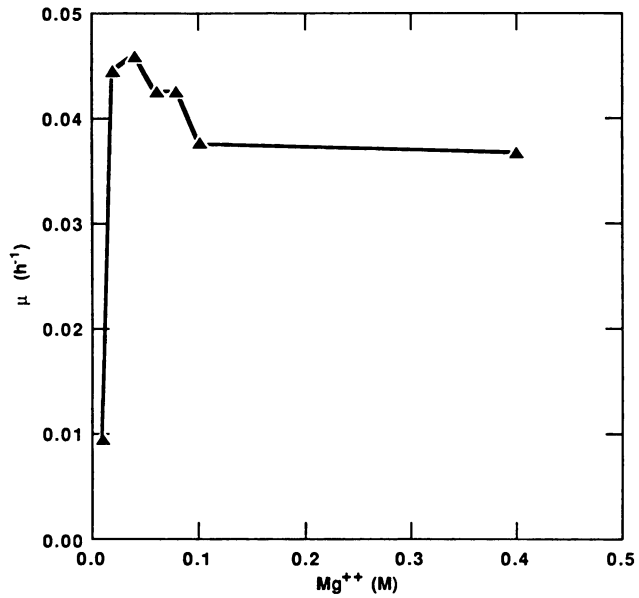

FIG. 8. Effect of Mg^{2+} on specific growth rate (μ) in DMS plus EBSV medium (▲).

tions. However, successful DMS enrichment cultures have been obtained from alkaline (pH 9.8) Mono Lake (Oremland, unpublished data), and DMS conversion to methane has been noted in freshwater sediments (16, 53). This indicates that other DMS-utilizing strains exist in nature.

The stable carbon isotope enrichment factors for methane produced from DMS, TMA, and MeOH were 1.043, 1.037, and 1.063, respectively, when cells were grown in the temperature range of 22 to 25°C (Table 3). The value for MeOH compares favorably with other reports which range from 1.065 (38) to 1.075 (23). Values for TMA and DMS were 0.017 to 0.023 less than that observed for MeOH in our experiments. This suggests that metabolism of TMA and that of DMS have similar kinetics which differ from that of MeOH. This is the first report of carbon isotope fractionation from TMA or DMS in pure culture, although a fractionation factor of 1.040 was reported for methane formation by DMS-amended sediments (32a). Therefore, comparisons can only be made to H_2 plus CO_2, MeOH, or acetate. In the case of acetate, only a small fractionation (1.021) was observed for *M. barkeri* (23). Reported fractionations for H_2 plus CO_2 vary widely depending on species and temperature. Thus, *Methanobacterium thermoautotrophicum* fractionation factors of 1.025 to 1.034 have been reported at 65°C (11, 12), while greater values (1.061) occur at 40°C (12). Fractionation factors of ~1.075 occur for *M. barkeri* at 37°C (23), while those for *Methanobacterium* sp. strain Ivanov are ~1.051 at 46°C and 1.033 at 37°C (1). Therefore, our values for TMA and DMS fall in the lower range of that observed for H_2 plus CO_2 but considerably above the value for acetate. With respect to fractionation of hydrogen, very large enrichment factors (1.190 to 1.203) were observed for growth on DMS and TMA (Table 2). Such a large hydrogen isotope fractionation has been predicted in the case of methanogenesis from methylated substrates (47).

TABLE 3. Stable carbon and hydrogen isotope fractionation by the isolate

Substrate (mM)	δ^{13}C-subst. (‰)[a]	δD-subst. (‰)[b]	% Reacted[c]	$\delta^{13}CH_4$ (‰)	δDCH_4 (‰)
DMS (7)	−34.8	−112	5.5	−79.2	−315
TMA (10)	−40.2	−111	9.6	−77.2	−301
TMA (10)	−40.2	−111	3.1	−77.1	−288
MeOH (10)	−45.9	−59	5.3	−108.8	ND[d]

[a] Substrate-$^{13}C/^{12}C$ ratio.
[b] Substrate-D/H ratio.
[c] Based on stoichiometry of 1 DMS → 1.5 CH_4, 1 TMA → 2.25 CH_4, and 1 MeOH → 0.75 CH_4. Amounts in micromoles of CH_4 formed were 5.8 (DMS), 21.7 (TMA-1), 6.9 (TMA-2), and 4.0 (MeOH).
[d] ND, Not determined.

The ecological niche occupied by GS-16 is not entirely clear. Although DMS-consuming methanogens are active in a variety of anoxic sediments (16), they do not consume the bulk of the available DMS present in sulfate-containing sediments. Inhibitor studies indicated that sulfate-respiring bacteria were responsible for much of the DMS metabolized in sediments of this type (16, 18). Despite the fact that methanogens consumed less than 20% of the DMS in salt-marsh sediments, DMS could still be responsible for up to 30% of the methane released from such environments (18). Therefore, DMS may contribute significantly to the pool of methylotrophic substrates present in anoxic, sulfate-rich sediments. However, the reported ability of certain clostridia to convert MeSH and other S-methylated compounds to methane (37) requires that care be taken in such investigations, and routine employment of specific inhibitors of methanogenesis (e.g., 2-bromoethanesulfonic acid, which blocks methanogenesis from DMS in GS-16 [33]) is to be encouraged. Future research devoted to isolating new strains of DMS-utilizing methanogens and characterizing their genotypes and the biochemistry of DMS metabolism should ultimately reveal the phylogenetic significance of these organisms.

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