***Methanosarcina semesiae* sp. nov., a dimethylsulfide-utilizing methanogen from mangrove sediment**

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***Methanosarcina semesiae* MD1^T (T = type strain), a novel obligately methylotrophic methanogenic archaeon is described. Strain MD1^T was isolated from an enrichment on dimethylsulfide inoculated with mangrove sediment. The cells were irregularly coccoid, non-motile, 1.4 ± 0.2 μm in diameter and stained Gram-positive. The catabolic substrates used included dimethylsulfide, methanethiol, methanol and methylated amines, but not acetate, formate, H_2/CO_2 or a combination of these substrates. When cells grown on dimethylsulfide were transferred to trimethylamine or methanol and vice versa, a lag phase was observed. The same lag phase occurred when cells grown on trimethylamine were transferred to methanol and vice versa, indicating that for each substrate different enzymes were induced. Fastest growth occurred within a temperature range of 30–35 °C and a pH of 6.5–7.5. Both Na^+ and Mg^{2+} were required for growth, with maximum growth rates at 200–600 mM Na^+ and 20–100 mM Mg^{2+} . The cells exhibited specific growth rates (h^{-1}) of 0.07 ± 0.02 , 0.15 ± 0.04 and 0.18 ± 0.05 on dimethylsulfide, methanol and trimethylamine, respectively. Analysis of the 16S rRNA gene sequence showed that strain MD1^T was phylogenetically closely related to members of the genus *Methanosarcina*, but clearly differed from all described species of this genus (94–97 % sequence similarity).**

Keywords: *Methanosarcina semesiae* sp. nov., archaea, methanogen, mangrove, dimethylsulfide

INTRODUCTION

Mangroves are characteristic of tropical coasts and are counterparts of tidal salt marshes of the temperate regions. An important characteristic of this ecosystem is that the sediment is anaerobic and highly reduced. Sulfate reduction and methanogenesis are the predominant terminal processes in the anaerobic degradation of organic matter in such environments. In marine sediment, where sulfate is readily available, sulfate reduction predominates (Crill & Martens,

1986; Holmer & Kristensen, 1994). However, methanogenesis co-exists in these environments, presumably by utilization of non-competitive substrates. Indeed, many methanogens isolated from marine sediments are obligately methylotrophic (Franklin *et al.*, 1988; Kadam *et al.*, 1994; Mohanraju *et al.*, 1997; Ni *et al.*, 1994; Oremland & Boone, 1994). The inability of these obligate methylotrophic methanogens to use hydrogen or acetate shows that they occupy niches in which there is no competition for methylated substrates like dimethylsulfide (DMS), methanethiol (MT), trimethylamine (TMA) and methanol (Oremland & Polcin, 1986; Oremland *et al.*, 1982).

Volatile methylated sulfur compounds have been intensively studied since they play an important role in the global sulfur cycle. DMS and carbonylsulfide (COS) constitute climatically relevant trace gases in

Abbreviations: COS, carbonylsulfide; DMA, dimethylamine; DMS, dimethylsulfide; MMA, monomethylamine; MT, methanethiol; TMA, trimethylamine.

The GenBank/EMBL accession number for the sequence of the 16S rRNA gene of strain MD1^T is AJ012742.

the atmosphere (Andreae & Raemdonck, 1983; Charlson *et al.*, 1987). DMS has been estimated to account for half of the biogenic input of volatile sulfur into the atmosphere with nearly 75% of it being generated in marine regions (Andreae & Raemdonck, 1983). The main precursor for DMS is dimethyl-sulfoniopropionate, an osmolyte of marine algae, phytoplankton, reef corals and some plants (Dacey & Wakeham, 1986; Hill *et al.*, 1995; Iverson *et al.*, 1989; Paquet *et al.*, 1995). Other precursors for DMS include methionine (Kiene & Visscher, 1987) and methoxylated aromatic compounds, the latter in combination with inorganic sulfide (Bak *et al.*, 1992; Kreft & Schink, 1993).

Degradation of DMS and MT in anaerobic marine sediments has been ascribed to both sulfate-reducing bacteria and methanogenic archaea (Kiene, 1988; Kiene & Capone, 1988; Kiene *et al.*, 1986). Some obligately methylotrophic methanogens able to use DMS for growth and methanogenesis have been described: *Methanohalophilus zhilinae* WeN5^T (Mathrani *et al.*, 1988); *Methanohalophilus oregonensis* WAL1^T (Liu *et al.*, 1990); *Methanobolus bombayensis* B-1^T (Kadam *et al.*, 1994); and two strains of *Methanosarcina siciliae*, T4/M^T and H1350 (Ni & Boone, 1991; Ni *et al.*, 1994). Isolations of pure cultures of methanogens from mangrove sediment are scarce. Only recently, Mohanraju *et al.* (1997) isolated a methanogenic archaeon (*Methanococcoides methylutens*) which grew on TMA and methanol. DMS utilization was not tested and no phylogenetic analysis was made. In a previous study, we have shown that addition of DMS stimulated methanogenesis in a Tanzanian mangrove sediment (T. J. Lyimo and others, unpublished). The present paper reports the isolation and characterization of a novel DMS-consuming obligately methylotrophic methanogenic archaeon, *Methanosarcina semesiae* sp. nov. strain MD1^T (T = strain), from this sediment.

METHODS

Source of inoculum. Sediment samples were taken from the Mtoni creek mangrove forest, Dar es Salaam, Tanzania (bordering the Indian Ocean) located at approximately 6°45' latitude and 39°41' longitude. Annual rainfall in the region is about 1050 mm. The major part of the area is muddy. Oceanic water covers the sediment surface from 0 to about 4 m depending on tidal variations and location (Lyimo *et al.*, 1997). Samples were taken by pressing a sampler (plastic cylinder, 5.5 cm i.d., 45 cm long) into the sediment. The sampler was tightly closed at both sides with airtight PVC plungers (3 cm high) and air was expelled through small openings in the plungers. The sampler was transported to the laboratory and opened in an anaerobic chamber.

Media and culture techniques. The sediment samples were diluted 2–3 times with anaerobic synthetic seawater (NaCl, 460 mM; MgSO₄, 40 mM; CaCl₂·2H₂O, 10 mM; and KCl, 10 mM). The resulting slurries were dispensed into serum bottles and the headspace was gassed with N₂/CO₂. Ratios used for gassing of samples with N₂/CO₂ or H₂/CO₂ were

80%/20% (v/v) at 0.5 atm overpressure. After several additions of low concentrations (0.5 mM) of DMS, the enriched slurry was transferred to the modified mineral medium of Widdel & Bak (1992). The medium contained the following compounds: NaCl, 340 mM; NH₄Cl, 4.7 mM; KH₂PO₄, 1.5 mM; NaHCO₃, 30 mM; KCl, 6.7 mM; MgCl₂·6H₂O, 15 mM; CaCl₂·2H₂O, 1 mM; Na₂S, 2 mM; Na₂S₂O₄, 0.2 mM; 1 ml trace element solution l⁻¹; and 10 ml vitamin solution l⁻¹. Trace element solution was made up of (l⁻¹): nitrilotriacetate, 15 g; FeSO₄·7H₂O, 1 g; H₃BO₃, 0.3 g; H₂SeO₃, 0.12 g; Al₂(SO₄)₃·12H₂O, 0.32 g; CuSO₄·2H₂O, 0.32 g; CoCl₂·6H₂O, 0.32 g; Na₂MoO₄·2H₂O, 0.032 g; NiCl₂·6H₂O, 0.31 g; ZnSO₄·7H₂O, 0.32 g; and MnCl₂·4H₂O, 0.32 g. Vitamin solution was made up of (l⁻¹): *p*-aminobenzoic acid, 0.1 g; riboflavin, 0.1 g; thiamin, 0.2 g; nicotinic acid, 0.2 g; pyridoxine, 0.5 g; pantothenic acid, 0.1 g; cobalamin, 0.1 g; biotin, 0.02 g; folic acid, 0.05 g; and lipoic acid, 0.05 g.

For substrate utilization experiments, anaerobic sterile stock solutions were added to the medium prior to inoculation. The substrates tested were: sodium acetate, 10 mM; DMS, 10 mM; MT, 5 mM; sodium formate, 20 mM; methanol, 20 mM; TMA/HCl, 10 mM; dimethylamine (DMA)/HCl, 10 mM; and monomethylamine (MMA)/HCl, 20 mM. Different pH values were obtained by adjusting the concentration of NaHCO₃ (for pH range 5.5–7.0) and Na₂CO₃ (for pH range 7–9) or by use of HEPES (25 mM). The pH changes (increase or decrease depending on substrate) during growth were 0.3 pH units or less. Media with different Na⁺ values were obtained by adjusting NaCl concentration only (i.e. NaHCO₃, Ca²⁺ and Mg²⁺ were kept constant). Media with various concentrations of Mg²⁺ and Ca²⁺ were prepared by adding sterile anoxic stock solutions to the prepared medium. When tested for Mg²⁺, Ca²⁺ was omitted and vice versa. The toxicity levels of DMS were tested by adding different concentrations (1–50 mM) of filter-sterilized DMS into sterile medium.

Enrichment and isolation. The DMS-utilizing methanogen was enriched by addition of low concentrations of DMS as the only catabolic substrate. However, after several transfers on DMS only, a combination of methanol (5 mM) and DMS (final concentration of 10 mM) was used. In this way, faster growth was obtained and the proportion of non-methanogenic contaminants decreased. Methanol was used because preliminary tests showed that DMS and methanol were used simultaneously. The enriched culture was serially diluted and inoculated into medium supplemented with 15 g purified agar l⁻¹ and 5 mM DMS or a combination of 5 mM methanol and 5 mM DMS. From the agar tubes containing a few colonies, some colonies were picked with a hypodermic needle (1.1 × 40 mm) and purified by repeated dilution series in liquid medium supplemented with the same substrates. The purity of isolates was checked by addition of glucose (1%, w/v), yeast extract (0.2%, w/v) and tryptic soy broth (0.2%, w/v) which would result in growth of non-methanogenic contaminants. In addition, cultures were routinely examined with a phase-contrast microscope (Leitz Wetzlar).

Growth experiments. Optimum conditions for growth were determined by measuring specific growth rates under various conditions. Growth was quantified by measuring methane formation (growth rates calculated from optical density and methane formation during preliminary experiments were similar). The specific growth rates (μ) during exponential growth were calculated (linear regression) from plots in which the logarithm of total methane accumulated was

plotted against time and expressed as h^{-1} (Powell, 1983). When the effects of environmental parameters (pH, temperature, salt concentration) were tested, growth rates were determined from cultures adapted to those conditions. Cultures were transferred at least twice under the conditions tested before growth rates were measured.

When tested for utilization of acetate, formate and H_2/CO_2 (substrates which did not support growth), inocula were pre-grown on different substrates (DMS, methanol and TMA). Cells were transferred at the exponential phase and incubated at 30 °C for a month. Combinations of substrates were also tested (Muller *et al.*, 1986; Boone & Whitman, 1988). All incubations were performed at 30 °C and pH 7 unless otherwise stated. Growth on methylotrophic substrates was stimulated by yeast extract, but cultures were routinely grown in medium without yeast extract.

Analytical techniques. Methane formation was determined by GC using a Porapak Q (80/100 mesh) column and flame-ionization detection. In experiments with added methylated sulfur compounds, DMS, MT and H_2S were measured on a GC equipped with a flame photometric detector as described previously (Lomans *et al.*, 1997).

16S rRNA gene analysis. For DNA extraction, cultures (3–6 ml) were centrifuged (2 min at 16000 g), and the cells were lysed by resuspending the pellets in 550 μl extraction buffer (Tris/HCl, 10 mM; EDTA, 0.1 mM; and SDS, 0.5%). Proteinase K (100 $\mu\text{g ml}^{-1}$) was added, and the tubes were incubated at 65 °C for 2–3 h. Then, 900 μl phenol:chloroform:isoamyl alcohol (25:24:1) was added. After gentle mixing for 10 min the mixture was centrifuged (16000 g) for 10 min. The top aqueous phase was transferred to a new sterile tube and extracted with chloroform:isoamyl alcohol (24:1). Then, 50 μl sodium acetate (3 M) and 1000 μl ethanol (96%) were added. The DNA was precipitated overnight at –20 °C and collected by centrifugation (10 min at 16000 g). The pellet was washed with 250 μl ethanol (70%). After centrifugation, the pellets were air-dried for about 20 min, dissolved in 50 μl sterile demineralized water and stored at –20 °C until use.

The gene encoding the 16S rRNA was amplified by PCR with the archaea-specific primers REV007 (5'-GTTGATC-CTGCCAGAGGYA-3') and 23S047 (5'-CCCBGGGCT-TATCGCAGCTT-3') (Raskin *et al.*, 1994). Reaction mixtures contained the following components in a final volume of 50 μl in a 200 μl PCR reaction tube: 5 μl 25 mM MgCl_2 ; 5 μl PCR buffer 10 $\times$; 2 μl 5 mM dNTP; 1 μl *Taq* polymerase (0.5 units μl^{-1}); 31 μl sterile H_2O ; 2 μl REV007 (20 pmol μl^{-1}); 2 μl 23S047 (20 pmol μl^{-1}) and 2 μl DNA template. The PCR was controlled with a thermocycler (Perkin Elmer) programmed as follows: denaturation at 94 °C for 4 min, followed by 30 cycles consisting of denaturing at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min; the last cycle was followed by an additional extension at 72 °C for 10 min and cooling to 4 °C. Amplified DNA fragments were separated by agarose gel electrophoresis (1% agarose) and stained with ethidium bromide. The PCR fragment product was directly ligated into the pGEM-T easy vector according to the manufacturer's procedure (Promega). *E. coli* XL-1 Blue competent cells were used for transformation. Plasmid DNA of clones with an insert was isolated and purified using the FlexiPrep kit (Pharmacia). Sequencing of the 16S rRNA gene was performed on a Perkin Elmer automated laser fluorescent DNA sequencer (ABI 310) with the following

primers: ARC1326 (5'-TGTGTGCAAGGAGCAGGG-AC-3'); FOR1241 (5'-ACACGCGGGCTACAATG-3'); M13 REV and M13 FORW (pGEM-T easy vector primers); ARC915; MC1109; and REV344 and REV915 (Raskin *et al.*, 1994) (REV344 and REV915 being the reverse of the sequences published).

Phylogenetic analysis. The 16S rDNA sequence of strain MD1^T was compared to sequences in the GenBank and EMBL databases using the BLAST algorithm (Dutch CAOS/CAMM Center Facility, Nijmegen, The Netherlands). A selection of genera yielding the highest similarity was made and used to make an alignment with PILEUP (GCG package; Devereux *et al.*, 1984). The alignment was checked manually and ambiguous positions were removed. Phylogenetic analysis was performed using the PHYLIP 3.5c package (Felsenstein, 1989). Bootstrap analysis was based on 100 resamplings of the dataset using the SEQBOOT program. The 16S rRNA gene sequences of the archaea used in the tree have the following GenBank/EMBL accession numbers: *Methanosaeta thermoacetophila* CALS-1 (M59141), *Methanosaeta concilii* FE-INRA (M59146), *Methanosaeta concilii* Opfikon (X16932), *Methanohalophilus oregonensis* WAL1^T (U20152), *Methanobolus taylorii* GS-16^T (U20154), *Methanobolus vulcanii* PL-12M^T (U20155), *Methanobolus bombayensis* B-1^T (U20148), *Methanobolus tindarius* Tindari3^T (M59135), *Methanococcoides methylutens* TMA-10^T (M59127), *Methanococcoides burtonii* DSM 6242^T (X65537), *Methanohalophilus* sp. SF1 (M59132), *Methanohalophilus mahii* SLP^T (M59133), *Methanohalobium evestigatum* Z-7303^T (U20149), *Methanosarcina mazei* SarPi (AF028691), *Methanosarcina mazei* S-6^T (U20151), *Methanosarcina frisia* C16^T (M59138), *Methanosarcina thermophila* TM-1^T (M59140), *Methanosarcina barkeri* Sar (AF028692), *Methanosarcina barkeri* 227 (M59144), *Methanosarcina acetivorans* C2A^T (M59137), *Methanosarcina siciliae* T4/M^T (U20153), *Methanosarcina* sp. WH1 (M59136) and *Methanospirillum hungatei* JF1 (M60880).

RESULTS AND DISCUSSION

Isolation

Previously, it was clearly demonstrated that methanogens are involved in the degradation of DMS in mangrove sediments. The most probable numbers of methanogens from these sediments were also high, up to 10^5 cells (g fresh sediment)⁻¹ (Lyimo *et al.*, 1998). To obtain pure cultures of DMS-utilizing methanogens, micro-organisms were enriched by sequential additions of low amounts of DMS to mangrove sediment. The enrichment was serially diluted and inoculated in agar medium with DMS or DMS + methanol. A DMS utilizer, strain MD1^T, was isolated from an agar tube with DMS as the only catabolic substrate. From the tubes with DMS + methanol, a second methanogenic strain was isolated, designated strain MM1. However, this strain was unable to utilize DMS. The 16S rDNA sequence from cells of strain MM1 was compared to that from strain MD1^T. Although they look morphologically identical, they were quite different phylogenetically. Strain MM1 was more closely related to *Methanococcoides* species whereas strain MD1^T was related to *Methanosarcina* species (see below). Strain MM1 has yet to be physiologically characterized.

Fig. 1. Growth of strain MD1^T after transfer from DMS, methanol or TMA-containing media to new media with different carbon sources. (a) Transfer of DMS-grown cells to DMS (◆), methanol (□) or TMA (▲). (b) Transfer of methanol-grown cells to methanol (×), MMA (▲), DMA (□) or TMA (◆). (c) Transfer of TMA-grown cells to TMA (◆), DMA (□), MMA (▲) or methanol (×). (d) Transfer of methanol-grown cells to methanol (□) or DMS (◆) and TMA-grown cells to TMA (×) or DMS (▲).

Morphology

Cells of strain MD1^T were single, non-motile and irregularly coccoid, with a diameter of 0.8–2.1 μm with a mean of 1.4 ± 0.2 μm ($n = 50$; determined from phase-contrast microphotographs). Aggregates were not found. Since the presence of flagella was not determined, the possibility of motility cannot be excluded. From microscopic observations, it was concluded that cells stained Gram-positive and lysed on addition of water or SDS (final concentration 2%, w/v) indicating that a proteinaceous cell wall was present (Boone *et al.*, 1993).

Catabolic substrates

Strain MD1^T was able to metabolize DMS, MT, methanol and methylated amines (MMA, DMA and TMA) since these substrates resulted in methane production and an increase in optical density of the

cultures. Acetate, formate or H₂/CO₂ did not result in growth or in methane production. Cells also did not utilize a combination of acetate and hydrogen, not even in the presence of methanol. The isolated archaeon was tested for toxic levels of DMS. No growth or methane production was observed at DMS concentrations of 30 mM or higher. In most cases, cells grew linearly when DMS concentrations above 20 mM were used. During growth on DMS, the isolate produced significant amounts of MT (maximum 1.5 mM) as an intermediate; on MT, the isolate released small amount of DMS. This corresponds with published data on other DMS-utilizing methanogens (Finster *et al.*, 1992; Ni & Boone, 1991).

When cells adapted to DMS were transferred to methanol and TMA a lag phase of about 5 h and 15 h, respectively, was observed (Fig. 1a). When cells adapted to methanol were transferred to methylated amines (MMA, DMA or TMA), the lag phase was about 2 h

Fig. 2. Temperature optimum (a) and pH optimum (b) for growth of strain MD1^T. The specific growth rate (μ), determined from the methane production rate during the exponential growth phase, is plotted against the incubation temperature (a) and pH (b). Growth was tested with different substrates: DMS ($\blacklozenge$), methanol ($\square$) and TMA ($\blacktriangle$). Values are expressed in h^{-1} and are the means of two independent cultures.

(Fig. 1b). Cells grown on TMA had a much longer lag phase (~ 50 h) before growth on methanol started whereas no lag phase was observed when the cells were transferred to DMA or MMA (Fig. 1c). TMA- and methanol-grown cells began to produce methane after a lag phase of 3–5 d when transferred to DMS (Fig. 1d). The ability to catabolize DMS was highly dependent on the growth phase of the methanol or TMA culture. The best results were obtained when cultures were transferred in the exponential phase. Cultures transferred at the late exponential or stationary phase were unable to utilize DMS even after incubation for 4 weeks. These results are in contrast with those showed by Ni & Boone (1991) for *Methanosarcina siciliae* H1350. In this micro-organism, the enzymes for the utilization of methanol and TMA were constitutively expressed. Our results with strain MD1^T clearly demonstrate that the enzymes involved in DMS, methanol and TMA degradation are different and have to be induced.

Fig. 3. NaCl range and magnesium requirement for growth of strain MD1^T. The specific growth rate (μ), determined from the methane production rate during the exponential growth phase, is plotted against the NaCl concentration (a) and the initial Mg^{2+} concentration (b) in the medium. Values are expressed in h^{-1} and are the means of two independent cultures.

The methanogenic conversion of methanol and methylated amines proceeds by similar mechanisms (Wasenaar *et al.*, 1996). Little information is available concerning biochemical pathways for methanogenesis from methylated thiols. Ni & Boone (1993) published some initial experiments with cell-free extracts from *Methanosarcina siciliae*. Enzymes involved in the metabolism of DMS have not been identified from typical DMS-utilizing methanogens. Recently, Tallant & Krzycki (1997) identified a 480 kDa coenzyme M-methylase from *Methanosarcina barkeri* MS, grown on acetate, as the only enzyme detectable which is capable of carrying out coenzyme M methylation from methylated thiols. However, they were unable to establish cultures of *Methanosarcina barkeri* MS with DMS as the sole substrate. Strain MD1^T is interesting for biochemical studies since it is relatively simple to handle and it has a reasonable growth rate on DMS (maximum growth rate 0.095 h^{-1}). Thus, we hope to use this organism for biochemical studies in the near future.

Fig. 4. A phylogenetic tree based on a distance matrix (EFITCH) calculated from 1288 unambiguously aligned positions of strain MD1^T and 23 other methanogens. *Methanospirillum hungatei* JF1 was defined as the outgroup. Bootstrap values are given to the left of a node; only values above 80 are given. Scale bar, the expected number of changes per sequence position along the horizontal lines of the tree.

Optimum growth conditions

Fig. 2 shows the influence of temperature and pH on growth and methanogenesis. Optimum growth took place within the temperature range 30–35 °C and at pH values between 6.5 and 7.5. Cells were able to grow and produce methane at temperatures of 18–39 °C (lower temperatures were not tested) and a pH range of 6.2–8.3 on methanol or TMA. On DMS, growth occurred within the same range of temperatures but no growth was found when the pH was above 8. The mean specific growth rates (μ) under optimum conditions were 0.07 ± 0.02 , 0.15 ± 0.04 and 0.18 ± 0.05 h⁻¹ ($n = 30$ for all values) on DMS, methanol and TMA, respectively.

Fastest growth occurred when the NaCl concentration was about 200–600 mM (Fig. 3a). Methane formation from cells inoculated into a medium without addition of NaCl (only NaHCO₃ present) was linear and no growth was observed. The optimal Mg²⁺ concentration was 20–100 mM (Fig. 3b). Mg²⁺ could be replaced by 5–10 mM of Ca²⁺ (Ca²⁺ concentrations > 10 mM resulted in a precipitate). Methane formation from a culture without Mg²⁺ or Ca²⁺ was linear and cells lysed after 2 d incubation at 35 °C. Inocula for controls

came from cultures grown with 1 mM Ca²⁺ and 15 mM Mg²⁺. These results indicate that both Na⁺ and either Mg²⁺ or Ca²⁺ were required for growth.

16S rRNA sequence characterization

The 16S rDNA sequence data were obtained for positions 27–1478 (*Methanosarcina barkeri* numbering) of the 16S rRNA gene. When compared to other sequences the 16S rRNA gene of strain MD1^T was found to be most similar to the sequences of *Methanosarcina* strains (94.7–96.0%). Highest similarity was observed with *Methanosarcina siciliae*. A phylogenetic tree based on a distance matrix (EFITCH) calculated from 1288 unambiguously aligned positions of strain MD1^T and 23 other methanogens is shown in Fig. 4. The topology of the tree was also checked with alternative methods (parsimony and bootstrap parsimony analysis). Strain MD1^T clusters within the family *Methanosarcinaceae* and appears to be most closely related to the genus *Methanosarcina*, although the position of strain MD1^T is always outside of the cluster of known representatives of this genus. Clustering is supported by high bootstrap values. Rouviere *et al.* (1992) defined a fully conserved signature sequence

for the 'methanosarcina group' of the order *Methanosarcinales*, present in a helical region, at positions 234–242 and 248–256 (*Methanosarcina barkeri* numbering). Remarkably, in strain MD1^T two bases in this region were different: C instead of T at position 237 and G instead of A at position 253.

Taxonomy

The physiological and morphological characteristics together with the phylogenetic analysis of the 16S rRNA gene sequence of strain MD1^T suggested that this organism is a member of the genus *Methanosarcina*. Like *Methanosarcina* species, strain MD1^T utilized DMS, methanol and methylated amines. On the basis of substrate utilization, strain MD1^T was most similar to *Methanosarcina siciliae*. However, morphologically these two strains differed; *Methanosarcina siciliae* forms massive aggregates with pseudosarcinae (Ni & Boone, 1993; Ni *et al.*, 1994) whereas strain MD1^T occurs as single irregular coccoid cells. Furthermore, a strain of *Methanosarcina siciliae* (C2J) was reported to utilize acetate (Elberson & Sowers, 1997) whereas strain MD1^T was an obligate methylotroph. Since our isolate was obtained from an enrichment with DMS as substrate, it is possible that we selected for an obligate methylotroph that has a mutation in the aceticlastic pathway. It cannot be excluded that enrichment on acetate would yield strains of this species which are not obligately methylotrophic. The observation that strain MD1^T occurred as single cells resembled the situation seen in some strains of *Methanosarcina frisia* and *Methanosarcina mazei* but these species are not restricted to methylotrophy (Blotevogel & Fischer, 1989; Maestrojuán *et al.*, 1992). The 16S rRNA gene sequence of strain MD1^T differed by 4.0–5.3 % from all described species of the genus *Methanosarcina*. Phylogenetic analysis of the family *Methanosarcinaceae* based on 16S rRNA and *mcr1* gene sequences revealed intergeneric distance values of 97 ± 0.65 % and distance values of 92 ± 1 % between different genera of this family (Springer *et al.*, 1995). In view of the above mentioned arguments, and supported by the deep phylogenetic division of this strain from other strains of *Methanosarcina*, it is proposed to include strain MD1^T in the genus *Methanosarcina* as a new species, *Methanosarcina semesiae* sp. nov.

Description of *Methanosarcina semesiae* sp. nov.

Methanosarcina semesiae (sem.es.i.ae. M.L. gen. n. *semesiae* of semesi, named after Adelaida K. Semesi in recognition of her many contributions to marine biology studies and for her mentorship/supervision of many postgraduate students in the field of microbiology).

Cells are coccoid bodies 1.4 ± 0.2 µm in diameter occurring as individual cells. Susceptible to hypotonic lysis. Cells stain Gram-positive. Obligate meth-

ylotroph. DMS, MT, methanol, MMA, DMA and TMA used for growth and methanogenesis. Acetate, formate and H₂/CO₂ are not utilized. The aforementioned substrate utilization, however, is based on only one strain. Cells grow optimally at 30–35 °C, pH 6.5–7.5 and in the presence of 0.2–0.6 M NaCl and 10–100 mM Mg²⁺ or 5–10 mM Ca²⁺. Habitat is marine sediments. The type strain is MD1^T, which was isolated from Indian Ocean sediments obtained from the mangrove forest at Mtoni creek, Dar es Salaam, Tanzania. *M. semesiae* strain MD1^T is deposited in the DSM culture collection (DSM 12914).

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