RESEARCH ARTICLE

Anaerobic oxidation of dimethylsulfide and methanethiol in mangrove sediments is dominated by sulfate-reducing bacteria

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Abstract

The oxidation of dimethylsulfide and methanethiol by sulfate-reducing bacteria (SRB) was investigated in Tanzanian mangrove sediments. The rate of dimethylsulfide and methanethiol accumulation in nonamended sediment slurry (control) incubations was very low while in the presence of the inhibitors tungstate and bromoethanesulfonic acid (BES), the accumulation rates ranged from 0.02–0.34 to 0.2–0.4 nmol g FW sediment^{-1} h^{-1}, respectively. Degradation rates of methanethiol and dimethylsulfide added were 2–10-fold higher. These results point to a balance of production and degradation. Degradation was inhibited much stronger by tungstate than by BES, which implied that SRB were more important. In addition, a new species of SRB, designated strain SD1, was isolated. The isolate was a short rod able to utilize a narrow range of substrates including dimethylsulfide, methanethiol, pyruvate and butyrate. Strain SD1 oxidized dimethylsulfide and methanethiol to carbon dioxide and hydrogen sulfide with sulfate as the electron acceptor and exhibited a low specific growth rate of $0.010 \pm 0.002\ h^{-1}$, but a high affinity for its substrates. The isolated microorganism could be placed in the genus *Desulfosarcina* (the most closely related cultured species was *Desulfosarcina variabilis*, 97% identity). Strain SD1 represents a member of the dimethylsulfide/methanethiol-consuming SRB population in mangrove sediments.

Introduction

Dimethylsulfide is recognized for its pivotal role in the biological feedback mechanism of climate control. Its atmospheric oxidation products form aerosol particles and cloud condensation nuclei (Charlson *et al.*, 1987). Dimethylsulfide and methanethiol are the major volatile organic compounds in most natural ecosystems, being produced by degradation of sulfur-containing amino acids. Methanethiol and dimethylsulfide can also be produced by methylation of sulfide and methanethiol, respectively, during degradation of methoxylated aromatic compounds (Lomans *et al.*, 1997, 2002). In marine systems, they originate mainly from the degradation of dimethylsulfoniopropionate, an osmolyte found in algae and halophilic plant species (e.g. Kiene & Visscher, 1987; Kiene & Taylor, 1988; Kiene, 1990). Emission of volatile organic compounds to the atmosphere is limited because they can be degraded by both aerobic and anaerobic microorganisms.

Degradation of dimethylsulfide and methanethiol in anaerobic marine sediments and salt marshes has been ascribed to both sulfate-reducing bacteria (SRB) and methanogenic archaea (Kiene *et al.*, 1986; Kiene & Visscher, 1987; Kiene, 1988; Kiene & Capone, 1988). In inhibitor studies with bromoethanesulfonic acid (BES) and molybdate, it is generally found that at higher concentrations (mM range), dimethylsulfide and methanethiol or their precursors are mainly converted into methane. However, at lower concentrations (μM range), inhibition experiments suggested a lower contribution of methanogenic archaea compared with SRB. In ^{14}C-tracer experiments, only a small fraction of the methyl group of methylated sulfur compounds was converted to methane (Kiene *et al.*, 1986; Kiene & Visscher, 1987).

In contrast, in freshwater systems, dimethylsulfide is mainly degraded by methanogenic archaea (Lomans *et al.*, 1997). It was shown that inhibition by BES (an inhibitor of methanogenic archaea) resulted in an increased accumulation

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of methanethiol in freshwater sediments, indicating that methanogenic archaea were responsible for its degradation. Hence, methane may be the major product in freshwater sediment whereas carbon dioxide (CO_2) may be the major product in marine systems. Recently, it was also suggested that methanethiol may play an important role in anaerobic sulfate-driven methane oxidation, which involves methanotrophic archaea performing reverse methanogenesis along with SRB (Boetius *et al.*, 2000; Moran *et al.*, 2008a). Methanethiol was postulated as the compound operating as a shuttle between the two partners in the consortium. These authors also suggest that methyl sulfides (e.g. dimethylsulfide and methanethiol) were the dominant product during trace methane oxidation (Moran *et al.*, 2005, 2008b) and in pure culture incubations of the methanogenic archaea *Methanosarcina acetivorans* when carbon monoxide served as the only electron donor (Moran *et al.*, 2008a).

In spite of the important role of marine bacteria as a sink for dimethylsulfide and the assumed importance of methyl sulfides in anaerobic methane oxidation, the number of marine bacteria known to be capable of using dimethylsulfide or methanethiol as an energy source under anaerobic conditions is still small. Salt marsh sediments, especially, have been used as a model system in which the production and consumption processes of organic sulfides under anoxic conditions have been investigated in some detail (Kiene, 1988, 1990; Kiene & Capone, 1988; Kiene & Taylor, 1988). A methanogenic archaeon that can grow on dimethylsulfide was isolated from an estuarine sediment (Oremland *et al.*, 1982) and *Methanosarcina semesiae* was isolated from a mangrove sediment and was able to grow on dimethylsulfide as well as on methanethiol (Lyimo *et al.*, 2000). Until now, no pure cultures of marine dimethylsulfide-utilizing SRB have been described. Three thermophilic, dimethylsulfide-utilizing, sulfate reducers have been isolated (Tanimoto & Bak, 1994). They were all tentatively placed into the genus *Desulfotomaculum*, but this was not supported by 16S rRNA gene data. No dimethylsulfide-utilizing methanogenic archaea could be isolated from their reactor and the authors concluded that SRB were responsible for the degradation of dimethylsulfide and methanethiol in thermophilic freshwater environments. The isolation of new species capable of degrading dimethylsulfide, and the investigation of their dimethylsulfide-degrading capacities, should thus enhance our understanding of the role of microorganisms in dimethylsulfide and methanethiol consumption in the natural environment.

Preliminary data (Lyimo *et al.*, 2002a) showed that the dimethylsulfide consumption rate in anaerobic mangrove sediment slurry was affected much more by tungstate, an inhibitor of SRB, than by BES. This suggests that SRB may be the most important dimethylsulfide consumer in this anaerobic marine ecosystem. This article reports further on the involvement of SRB in dimethylsulfide and methanethiol degradation and interconversions in mangrove sediment and the enrichment, isolation and characterization of a dimethylsulfide-utilizing SRB, *Desulfosarcina* sp. strain SD1, from these sediments.

Materials and methods

Source of sediment and incubations

Sediment samples were collected from the Mtoni mangrove forest at Mzinga creek, Dar-es-Salaam, Tanzania, and handled as described before (Lyimo *et al.*, 2002a).

Measurements of the initial productions rates of dimethylsulfide and methanethiol were performed 2 days after sampling the mangrove sediment. The sediment was diluted three times with anoxic synthetic seawater (Lyimo *et al.*, 2002a) in an anaerobic cabinet. The resulting slurry was sieved (2-mm mesh) and portions of 20 mL were dispensed into 120-mL serum bottles and tightly closed with black butyl rubber stoppers and aluminum caps. The headspace was evacuated and gassed with N_2/CO_2 (80%/20% v/v at 0.5 atm. overpressure) to maintain anaerobic conditions, and this resulted in a pH of 7. Four duplicate sets were prepared: (1) blank, without additions, (2) inhibitor of methanogenic archaea, 25 mM BES, (3) inhibitor of SRB, 4 mM tungstate and (4) both inhibitors, BES and tungstate. For dimethylsulfide and methanethiol consumption rates, the sediment was diluted twice and preincubated at 30 °C for a week, after which the pH had declined to 6.7. Anaerobic stock solutions of dimethylsulfide or methanethiol were added (final concentrations 12–16 μM) with or without inhibitors in a setup similar to that described above. Incubation was carried out at 30 °C, pH 7, by slow shaking. Gray butyl rubber stoppers were used instead of black rubber because they showed less absorption of dimethylsulfide and methanethiol.

Enrichment and isolation

When the dimethylsulfide in the serum bottles with BES was consumed, new additions (starting with 20 μM and increasing to 100 μM) were performed to a total amount of about 5 mM. The initial sulfate concentration was 20 mM. Thereafter, inocula (10% v/v) were transferred to a fresh basic medium as described for most probable numbers (MPN) of SRB (Lyimo *et al.*, 2002a). After several transfers (about 20 times) into a mineral medium with dimethylsulfide (batchwise additions of 100 μM to avoid toxicity) and BES, it was assumed that BES could support the growth of some bacteria. Therefore, BES was omitted to reduce the number of contaminants. After several transfers in medium without BES, the well-growing enriched culture was serially 10-fold diluted into liquid and solid (15 g L^{-1} of purified agar

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added) media with dimethylsulfide (100 μM) in the headspace. No growth or dimethylsulfide consumption was observed on solid medium and therefore the isolation of cells utilizing dimethylsulfide was achieved by dilutions series to extinction on liquid medium (three repetitions, positive growth up to 10^7 dilution). This resulted in a pure culture strain named SD1. Purity was checked by addition of (w/v) glucose 1%, yeast extract 0.2%, tryptic soy broth 0.2%, methanol (10 mM), trimethylamine (4 mM) and BES (25 mM). In addition, cultures were routinely examined using a phase-contrast microscope.

Determination of optimal conditions

Optimum conditions for growth were determined by measuring the consumption rates of dimethylsulfide and the growth rates ($OD_{600\,nm}$). The dimethylsulfide consumption rates corresponded with density-based growth rates of the culture and this trend remained the same even after repeated transfer under the same conditions.

When tested for utilization of different substrates, the inoculum was obtained from dimethylsulfide-grown cells at the exponential phase. Incubation was performed at 30 °C, pH 7, for about a month. The substrates tested were; acetate (10 mM), lactate (10 mM), methanol (10 mM), ethanol (5 mM), propanol (5 mM), dimethylsulfoxide (5 mM), dimethyldisulfide (5 mM), methanethiol (5 mM), dimethylsulfoniopropionate (10 mM), methionine (10 mM), mercaptoethanesulfonate (5 mM), trimethylamine (5 mM), trimethoxybenzoic acid (10 mM), betaine (10 mM), H_2/CO_2 (80%/20% v/v at 0.5 atm. overpressure), pyruvate (10 mM), syringate (2 mM) and butyrate (10 mM).

Analyses of gases and calculations

Dimethylsulfide, methanethiol and hydrogen sulfide were measured by flame photometric detector GC as described previously (Lomans *et al.*, 1997). CH_4 was measured on a gas chromatograph equipped with a flame ionization detector and a column packed with Porapack Q (80/100 mesh). For calculating liquid concentrations of dimethylsulfide and methanethiol in the sediment slurry, the liquid/gas ratios were determined by measuring the headspace loss after addition of these compounds to control bottles with an anaerobically heated slurry. The values determined were 7.8 and 5.2, respectively. For calculating the total amount of methyl-sulfide groups (MSG) in a bottle, the liquid and headspace amounts of dimethylsulfide were multiplied by 2 and added to those of methanethiol.

Molecular analyses

Genomic DNA (gDNA) from strain SD1 was isolated as described by Juretschko *et al.* (1998) without the use of lytic enzymes. The 16S rRNA gene was amplified by a hot-start PCR using the primers 616F (5′-AGA GTT TGA TYM TGG CTC AG-3′) and 630R (5′-CAK AAA GGA GGT GAT CC-3′) (Juretschko *et al.*, 1998). The dsrA/dsrB genes, encoding subunits of the dissimilatory sulfite reductase, were amplified using the primers designed by Wagner *et al.* (1998). PCR products were purified from agarose gels using the QIAEX II gel extraction kit (Qiagen) and cloned using the TOPO TA cloning kit (Invitrogen). Plasmids were purified using the FlexiPrep kit (Amersham Biosciences) and sequenced with M13R, M13F and internal primers.

Phylogenetic analyses

Phylogenetic analyses were performed using the MEGA 4.0.1 package (Tamura *et al.*, 2007). A total of 1540 aligned positions were utilized for the 16S rRNA gene data set. In all cases, missing data or alignment gaps were treated as missing information. The tree was calculated using the neighbor-joining algorithm with a Kimura 2-parameter correction. For protein (DsrAB) phylogeny, we prepared a concatenated data set of the α- and β-subunit. The final data sets consisted of 514 positions.

Results and discussion

Dimethylsulfide and methanethiol oxidation by mangrove sediments slurries

Previously (Lyimo *et al.*, 2000), it was demonstrated that methanogenic archaea outcompete SRB during enrichment on dimethylsulfide in a mangrove sediment. However, it was also found that at low dimethylsulfide concentrations, the initial consumption rates in the sediment slurry were affected more by the SRB inhibitor tungstate than by BES, which specifically inhibits methanogenic archaea. Further investigations showed that the accumulation rates of dimethylsulfide and methanethiol in incubations of fresh surface sediments were strongly stimulated during the first 50 h upon inhibition of SRB with tungstate, and then degraded (Fig. 1) presumably due to the growth of methanogenic archaea. Inhibition of methanogenic archaea by addition of BES only resulted in some accumulation of methanethiol (dimethylsulfide was still around the detection limit). Addition of both inhibitors (tungstate and BES) resulted in accumulation of both methanethiol and dimethylsulfide without subsequent degradation. The maximum production of dimethylsulfide and methanethiol, in the presence of tungstate and BES, ranged from 0.02–0.34 to 0.2–0.4 nmol g^{-1} FW per sediment h^{-1}, respectively. Generally, methanethiol accumulated in higher concentrations than dimethylsulfide. These results suggest that methanogens were actively involved in the dimethylsulfide and methanethiol degradation, but the much stronger effects of

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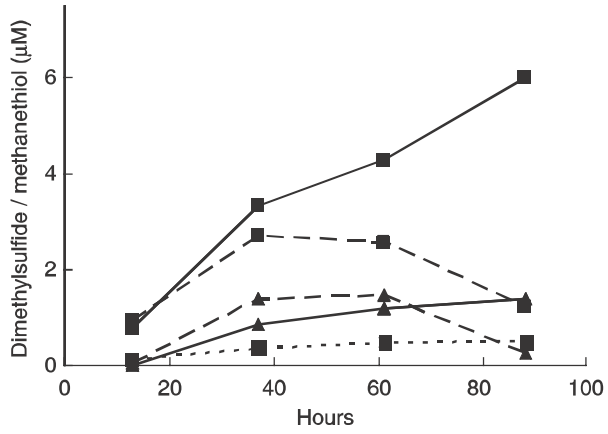


Fig. 1. Production of dimethylsulfide (▲) and methanethiol (■) from sediment slurry after the addition of inhibitors; BES (- - - - - -); tungstate (— — — —); and both BES and tungstate (———). Controls were below the detection level.

tungstate on accumulation of dimethylsulfide and methanethiol compared with BES implied that SRB were more important. The magnitude of the contribution of methanogenic archaea was still unclear and in our previous inhibitor study, we measured the initial dimethylsulfide conversion rates, but did not include measurement of the methane recovered from dimethylsulfide. Moreover, methanethiol was not measured. Interconversions between dimethylsulfide and methanethiol are well known in anoxic sediments (Kiene & Capone, 1988; Lomans *et al.*, 1999a, b) and may influence the observed consumption rates and interpretation of data. Therefore, we set up a series of sediment incubations in which dimethylsulfide, methanethiol and methane were simultaneously measured. In one series, dimethylsulfide was added as a substrate (Fig. 2), and in the other, methanethiol (Fig. 3) with or without (control) inhibitors. In the control incubations, methanethiol and dimethylsulfide conversion started immediately at high rates, but simultaneously there was a rapid production of dimethylsulfide and methanethiol, respectively (Figs 2a and 3b). In order to discriminate between consumption and interconversion the total amount of MSG in the bottles were calculated and taken as a measure for actual consumption of methanethiol or dimethylsulfide. Sediments had been preincubated for a week to reduce the background production of methylated sulfur compounds and methane by an endogenous substrate. Probably as a result of this, in the initial phase, the rates of MSG consumption were somewhat lower, but were rather constant during the following days. Therefore, the rates at 1.5–2 days are used for comparisons. When the degradation rates in uninhibited incubations (1.6–1.9 nmol MSG g FW^{-1} h^{-1}, Table 1) were compared with the methanethiol/dimethylsulfide production rates (calculated as MSG) in fresh sediment (Fig. 1), the degradation rates were generally much higher, occasionally only twofold. Although the rates were measured at concentrations higher than the *in situ* values, degradation of dimethylsulfide/methanethiol is linear down to their detection limits. Together with the fact that the net production in control slurry incubations was very low (concentrations were around the detection limits), this implies a balance between production and degradation.

Incubations with dimethylsulfide and methanethiol behaved very similarly, obviously as a result of the rapid interconversion observed. BES caused a 47–50% inhibition of MSG consumption (Figs 2b and 3b) and after the initial phase, the rates remained constant for the 4-day period. This is consistent with our previous observations (Lyimo *et al.*, 2002a). This implies that the growth of SRB-consuming MSG in this period was not significant. Tungstate caused 99% and 87% inhibition in the dimethylsulfide incubation (Fig. 2c) and methanethiol incubation (Fig. 3c), respectively. However, after 2 days low rates were observed that increased in time, obviously a result of the rapid growth of methanogenic archaea. Addition of both inhibitors blocked MSG consumption completely (> 99%) in all incubations (Figs 2d and 3d). The fact that BES inhibited MSG consumption from the start of the incubations suggested the involvement of methanogenic archaea. However, in the noninhibited sediments, the amount of methane produced was very low (Table 1). The dimethylsulfide/methanethiol added did cause an increase in methane production compared with the background rate, but this could account for only 1% of the MSG conversion observed. In the tungstate-inhibited sediment, methane production increased almost 10-fold over the background rate, but the dimethylsulfide/methanethiol addition did not result in a further increase. Hence, although BES inhibition suggests the involvement of methanogenic archaea in dimethylsulfide/methanethiol consumption in mangrove sediments, this did not result in methane production. A role for methanogenic archaea can be envisaged by assuming an almost total carbon flow (from methyl groups) to CO_2. Therefore, oxidation of methyl groups without electrons were being used for concomitant reduction of methyl groups into methane. Instead, electrons, possibly in the form of hydrogen, are lost to the vast population of SRB surrounding the methanogenic archaea. Interspecies hydrogen transfer from methanol has been shown in a coculture of *Methanosarcina barkeri* and *Desulfovibrio vulgaris* (Phelps *et al.*, 1985) in which the SRB cannot use the methanol, but consumed 42% of the available electrons. Obtaining conclusive evidence to show such an interaction in sediments will be difficult as, even with labelled substrates, conversion of MSG to CO_2 by methanogenic archaea cannot be discriminated from the conversion performed by SRB.

Interconversion of dimethylsulfide/methanethiol could be an alternative role for methanogenic archaea, but the

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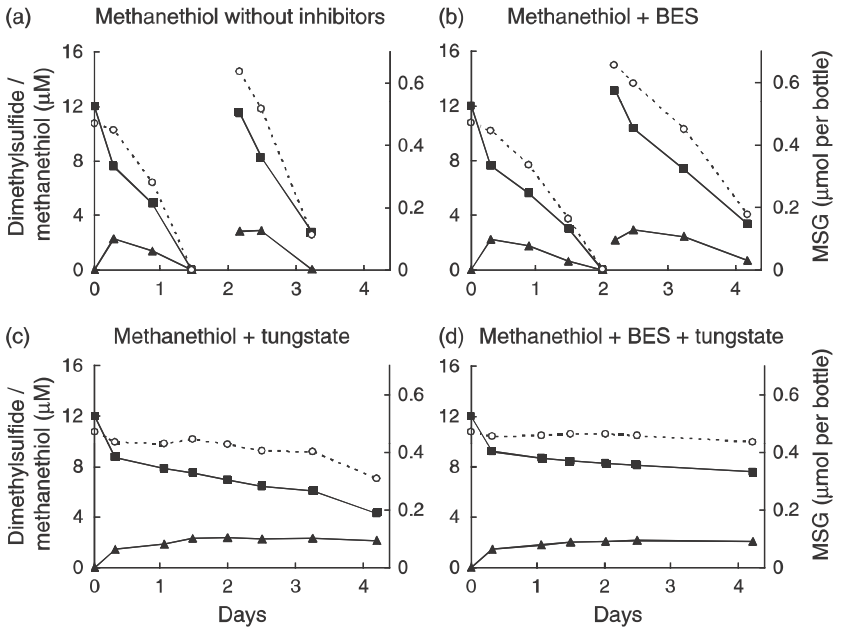


Fig. 2. Dimethylsulfide (▲) and methanethiol (■) in a sediment slurry amended with dimethylsulfide; (a) without inhibitor; (b) with BES; (c) with tungstate; (d) with both BES and tungstate. Dotted lines represent the total amount MSG in the bottles. Initial dimethylsulfide concentrations (time zero) were calculated on the basis of the amount added.

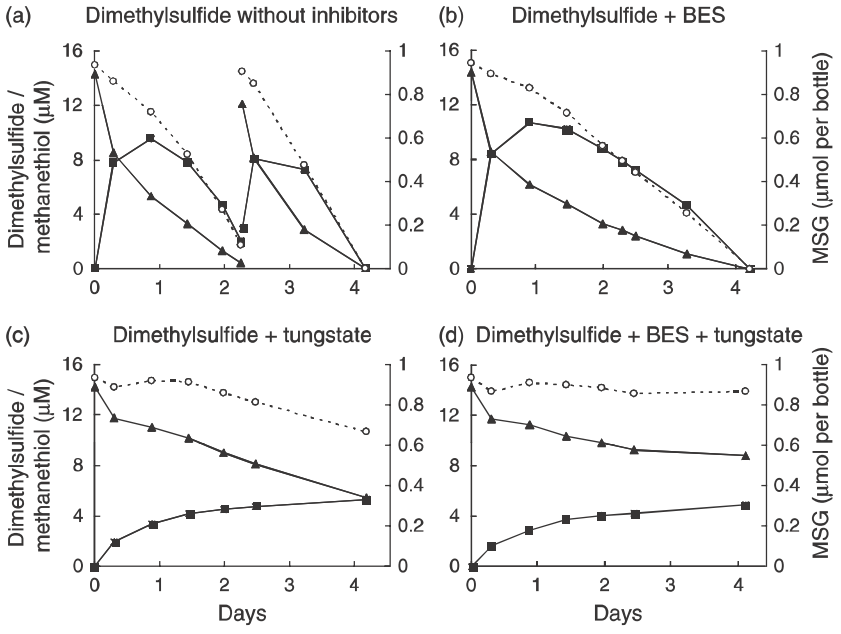


Fig 3. Dimethylsulfide (▲) and methanethiol (■) in the sediment slurry amended with methanethiol (a) without an inhibitor; (b) with BES; (c) with tungstate; and (d) with both BES and tungstate. Dotted lines represent the total amount MSG in the bottles. Initial methanethiol concentrations (time zero) were calculated on the basis of the amount added.

conversion of both dimethylsulfide and methanethiol was not significantly affected by BES (Fig. 2b). In contrast, tungstate strongly reduced dimethylsulfide conversion into methanethiol (Fig. 2c). In the control incubation, after about half a day, only a small amount of MSG was consumed, but 50% of the dimethylsulfide was already converted into methanethiol. In the incubations with BES, this was very similar, while in the incubation with tungstate, only 20% dimethylsulfide was converted, and so SRB appear to be responsible. Starting from methanethiol as a substrate, conversion rates were considerably lower and comparison of the results for methanethiol with those for dimethylsulfide suggests that these compounds are in a thermodynamic equilibrium favoring methanethiol. Although interconversions of dimethylsulfide and methanethiol are generally observed (Kiene *et al.*, 1986; Kiene, 1988; Kiene & Capone, 1988; Lomans *et al.*, 1997, 1999a, b; Lyimo *et al.*, 2000) in sediments and ascribed to SRB and methanogenic archaea, this has not been analyzed quantitatively. Relatively large abiotic losses were observed especially during the first day and hamper interpretations (Kiene *et al.*, 1986; Kiene & Capone, 1988). Our results, however, showed virtually

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Table 1. Consumption and production of MSG and methane in mangrove sediment slurry incubations

Substrate	Inhibitor	MSG consumption (nmol g FW^{-1} h^{-1})	Inhibition (%)	Methane production (nmol g FW^{-1} h^{-1})
Dimethylsulfide	None	1.6 ± 0.1		0.05 ± 0.01
Methanethiol	None	1.9 ± 0.0		0.04 ± 0.01
Dimethylsulfide	BES	0.8 ± 0.1	50 ± 5	0.01 ± 0.02
Methanethiol	BES	1.0 ± 0.2	47 ± 9	ND
Dimethylsulfide	Tungstate	ND	> 99	0.17 ± 0.02
Methanethiol	Tungstate	0.02 ± 0.02	> 97	0.17 ± 0.01
Dimethylsulfide/methanethiol	BES+tungstate	ND	> 99	ND
		MSG production (nmol g FW^{-1} h^{-1})		Methane production (nmol g FW^{-1} h^{-1})
Control	None	ND		0.02 ± 0.01
Control	BES	ND		ND
Control	Tungstate	0.2 ± 0.0		0.2 ± 0.0
Control	BES+tungstate	0.2 ± 0.0		ND

ND, not detectable; FW, fresh weight. Values are means ± SD (n = 4). Rates of MSG consumption (initial concentrations of methanethiol and dimethylsulfide 12–14 μM) were estimated from the curves after 1.5–2 days of incubation. Values represent the average of a total of four incubations; two duplicates from two different sediment cores, taken 10-m apart. Rates in duplicate incubations differed < 5%, while duplicate cores without inhibitors differed 10%. On treatment with inhibitors differences between cores increased. Because of the low methane production rates the SDs tended to be higher.

constant amounts of MSG in the BES+tungstate-inhibited incubations with dimethylsulfide or methanethiol over many days. Some background production of MSG was observed (Table 1) in the absence of added dimethylsulfide or methanethiol (about 10% compared with the consumption rate) and so, a small increase was expected in methanethiol- or dimethylsulfide-supplemented incubations with BES and tungstate. Although some incubations did show a small increase of MSG, this was considered not significant and smaller than expected. Production is possibly counter balanced by losses, but an alternative explanation is that the production of MSG was inhibited by the presence of the relatively high concentrations of dimethylsulfide or methanethiol added.

Although an important role of methanogenic archaea still cannot be ruled out, our results were in agreement with other findings (Kiene & Visscher, 1987; Kiene, 1988) that at a low concentration of dimethylsulfide, SRB are more important dimethylsulfide utilizers than methanogenic archaea in marine sediments.

Enrichment and isolation

The sediment was enriched by the addition of dimethylsulfide as the only catabolic substrate and BES as an inhibitor of methanogenic archaea. BES has been used extensively in various ecological studies especially when studying the interaction of sulfate reduction and methanogenesis (Oremland *et al.*, 1982; King, 1984; Kiene & Visscher, 1987; Lomans *et al.*, 1997). However, after several transfers of the enrichment with dimethylsulfide and BES, it was realized that BES was supporting the growth of contaminants. When BES was omitted, the predominant dimethylsulfide-utilizing bacteria could be isolated from the enrichments using the serial dilution method and was named strain SD1. The deep agar technique was attempted several times under various conditions, such as incubation temperature and pH, but in all cases no growth occurred. Presumably the agar contains some toxic compounds that inhibited the growth of this type of bacteria. The isolate was microscopically pure and no growth was observed when inocula were transferred to rich media. PCR on gDNA of strain SD1 with 16S rRNA or *dsr* gene-specific primers and subsequent cloning and sequencing resulted in both cases only in identical sequences (10 clones sequenced each).

The cells of strain SD1 are ovoid or rod-shaped, sometimes forming long chains (Fig. 4). The size of single rods

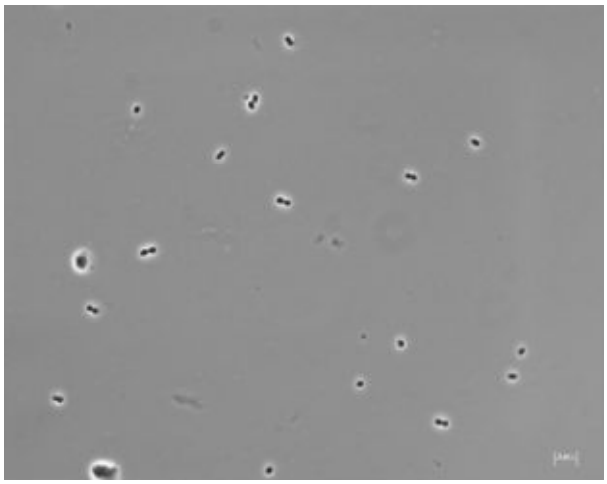


Fig. 4. Microphotograph of strain SD1 growing on sulfate and dimethylsulfide.

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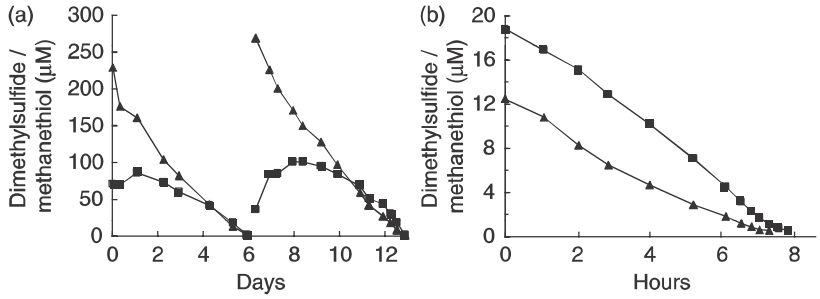


Fig. 5. Dimethylsulfide (▲) consumption and intermediate methanethiol (■) production by strain SD1 (a) during growth upon repeated addition of dimethylsulfide and (b) showing another measurement at a lower concentration during a day.

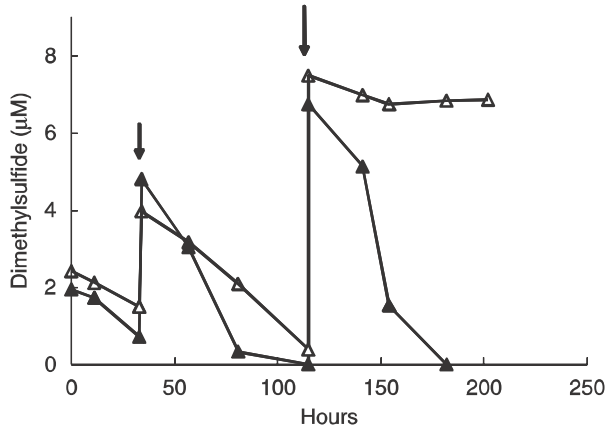


Fig. 6. Dimethylsulfide consumption by strain SD1 in a culture medium with (▲) or without (△) sulfate. The dimethylsulfide was added several times (arrows).

was 1.1 ± 0.2 μm in width and 2.2 ± 0.5 μm in length when grown on dimethylsulfide. When grown on methanethiol, the size of the cells became much smaller. On pyruvate, almost all cells were elongated and on butyrate, the cells became thicker at the ends. Single cells were motile, stained Gram positive and did not lyse upon addition of freshwater or sodium dodecyl sulfate (2% w/v).

Catabolic substrates

Strain SD1 was tested for its ability to use various substrates potentially available for SRB in marine ecosystems. These were dimethylsulfide, methanethiol, acetate, lactate, methanol, ethanol, propanol, H_2/CO_2, dimethylsulfoniopropionate, dimethylsulfoxide methionine, syringate, mercaptoethanesulfonate, trimethylamine, trimethoxybenzoate, betaine, dimethylsulfoxide and dimethyldisulfide. The isolate was able to metabolize dimethylsulfide and/or methanethiol as the only catabolic substrate. Toxicity of dimethylsulfide or the intermediate methanethiol was observed at low millimolar concentrations, but active cultures could grow up to 10 mM dimethylsulfide. During growth on dimethylsulfide, the isolate produced high amounts of methanethiol as an intermediate (Fig. 5) and vice versa, on methanethiol the isolate released dimethylsulfide. In the later phases of SD1 cultures, dimethylsulfide and methanethiol were consistently found in a ratio of 1 to 2–3; apparently, they were in an equilibrium favoring methanethiol and this is very similar to the observations made with the sediment. This also resembles observations made with dimethylsulfide/methanethiol-consuming methanogenic archaea (Finster *et al.*, 1992; Ni & Boone, 1993; Lomans *et al.*, 1999a, b; Lyimo *et al.*, 2000). Butyrate and pyruvate were also utilized, but resulted in a very slow growth. The other substrates tested were not utilized even after prolonged incubations.

Optimum growth conditions

Strain SD1 grew well in similar environmental conditions as those found in Tanzanian mangrove forests (Lyimo *et al.*, 2002b). Thus, the optimum temperature was 30–35 °C and good growth was observed between pH 6 and 8 (optimum 7.5). The fastest growth occurred at an NaCl concentration from 200 to 400 mM. The average growth rate (μ) on dimethylsulfide calculated from the increase in $OD_{600\,nm}$ under optimum conditions was $0.010 \pm 0.002\ h^{-1}$ ($n = 10$), i.e. a doubling time of about 2.5 days. Sulfate was required for growth apparently as an electron acceptor, which indicates that the isolate was a real sulfate reducer. This was clearly demonstrated by sulfide production and when sulfate was omitted, growth and dimethylsulfide consumption stopped completely after the sulfate from the inoculum was exhausted (Fig. 6). Furthermore, it was shown that addition of tungstate (4 mM), a specific inhibitor of SRB, completely inhibited dimethylsulfide consumption. Stoichiometric calculations showed that the amount of sulfide produced was about 2.3 times the amount of dimethylsulfide consumed and this corresponded with the theoretical equation:

$$C_2H_6S + 1.5SO_4^{2-} \rightarrow 2CO_2 + 2H_2O + 2.5S^{2-} + 2H^+$$

Phylogenetic analyses

Analysis of the almost complete sequence (1540 bp) of the 16S rRNA gene of strain SD1 revealed that the novel isolate grouped within the genus *Desulfosarcina* (family *Desulfobacteraceae*, class *Deltaproteobacteria*) (Fig. 7a). The

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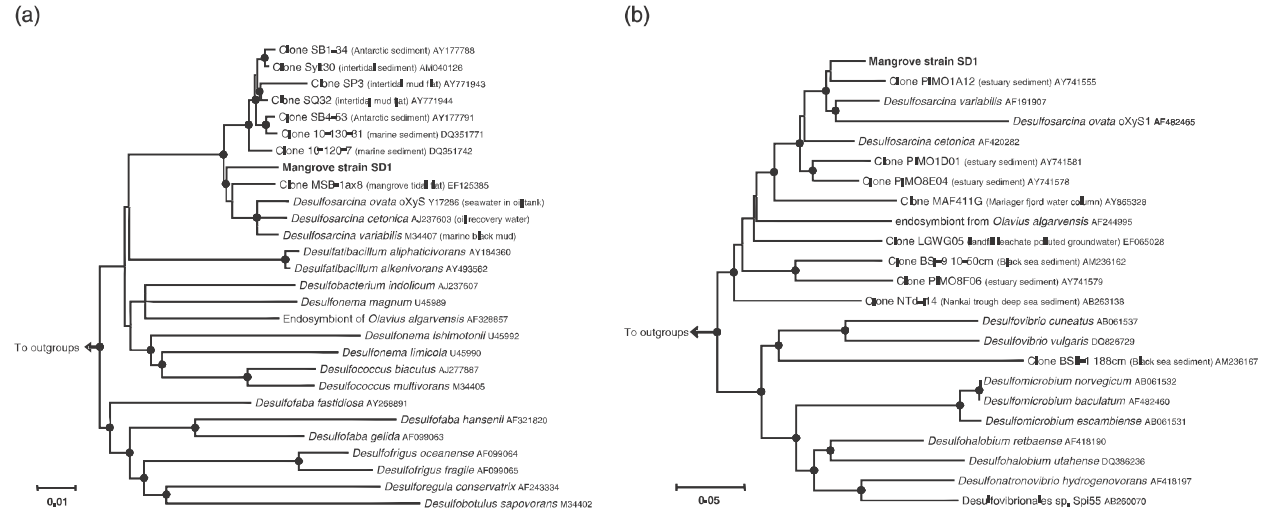


Fig. 7. Neighbor-joining phylogenetic tree of 16S rRNA genes (a) and DsrAB protein sequences (b) from strain SD1 and selected close relatives. Bootstrap consensus trees inferred from 500 replicates are shown. Branches corresponding to partitions reproduced in < 50% bootstrap replicates are collapsed. Dots indicate > 60% of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates). Evolutionary distances were computed using the maximum composite likelihood method (a) or the Dayhof matrix-based method (b) and are in the units of the number of base/amino acid substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons. There were a total of 1540 (a) and 514 (b) positions in the final datasets.

cultured species with the highest identity were *Desulfosarcina variabilis* (96.8–97.3%). Inocula for isolation of these species also had a marine origin (Widdel, 1980; Galushko & Rozanova, 1991; Harms *et al.*, 1999; Kuever *et al.*, 2005). Closely related environmental clone sequences (96.2–97.6% identity) originated from marine sediments including mangrove tidal flat sediments. Environmental studies showed a high and stable abundance of members of the *Desulfosarcina/Desulfococcus* group, both over depth and season, which suggests that these bacteria may play a more important role than previously assumed, based on low sulfate reduction rates in parallel cores (Musat *et al.*, 2006). The phylogenetic position of the strain SD1 within the genus *Desulfosarcina* was supported by a phylogenetic analysis of its concatenated DsrAB protein sequence (514 amino acids) (Fig. 7b). Also, the cell shape is in the same range as reported for *Desulfosarcina* sp. (Kuever *et al.*, 2005). We cultivated the closest relatives *Desulfosarcina cetonica* (DSM7267); *D. variabilis* (DSM2060) and *Desulfosarcina ovata* (DSM13228) on the media prescribed by DSMZ (http://www.dsmz.de). Growing cultures were transferred to the same medium. After growth continued, dimethylsulfide was added (15 μM). In all cases, no net consumption of dimethylsulfide was observed, not even at the moment the butyrate and benzoate were exhausted. Dimethylsulfide did not inhibit growth. Other sulfate reducers with the ability to utilize dimethylsulfide have been isolated from the thermophilic fermentor sludge (Tanimoto & Bak, 1994). However, no 16S rRNA or *dsr* gene data are available for these strains. Based on G+C content, morphological and physiological characteristics, it was suggested that they belong to the genus *Desulfotomaculum*. The strains described were thermophiles with a very narrow range of temperature for growth, a wide range of substrates and they formed spores.

Conclusion

The newly isolated strain SD1 is the first reported SRB from a marine environment that has the ability to grow on dimethylsulfide and methanethiol and has the characteristics to represent the dimethylsulfide/methanethiol-degrading SRB population. Its extremely slow growth rate compared with the previously isolated mangrove methanogenic archaea *M. semesiae* (0.01 and 0.07 h^{-1} on dimethylsulfide, respectively; Lyimo *et al.*, 2000) explains why such an SRB can be outcompeted easily at a higher substrate concentration (Lyimo *et al.*, 2002a) and explains the high methane recoveries in other studies with relatively high concentrations of dimethylsulfide or MSG precursors. Incubations were performed over many days and exponential increases in methane production were consistently observed. This means that the population rapidly changed in favor of the methanogenic archaea, which does not reflect their 'natural' contribution at the start, where dimethylsulfide-consuming SRB may outnumber methanogenic archaea by virtue of their higher affinity for methylated sulfur compounds, and because the energetics of sulfate reduction with dimethylsulfide will be better than reduction of CO_2 with dimethylsulfide. Although there are no data for SRB and methanogens on the affinities for these sulfur

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compound, preliminary results showed an almost constant decrease of methanethiol and dimethylsulfide in strain SD1 cultures (Fig. 4), down to detection-level concentrations (< 0.5 μM). This implies that the affinity constant (K_s) for dimethylsulfide and methanethiol is < 0.5 μM. Comparison of the dimethylsulfide consumption rates (both at the same dimethylsulfide concentration) of environmental samples with those from strain SD1 (1.4 nmol MSG × 10^6 per cell h^{-1}) showed that about 10^6 cells would be sufficient to explain the slurry activity. This amount is in good agreement with the MPN data of SRB reported before (Lyimo *et al.*, 2002a). In ecosystems such as mangrove sediments where the methylated sulfur compounds are limiting, SRB such as the new isolate *Desulfosarcina* strain SD1 are likely to dominate the conversion of dimethylsulfide and methanethiol.

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