Aerobic and Anaerobic Degradation of a Range of Alkyl Sulfides by a Denitrifying Marine Bacterium

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A pure culture of a bacterium was obtained from a marine microbial mat by using an anoxic medium containing dimethyl sulfide (DMS) and nitrate. The isolate grew aerobically or anaerobically as a denitrifier on alkyl sulfides, including DMS, dimethyl disulfide, diethyl sulfide (DES), ethyl methyl sulfide, dipropyl sulfide, dibutyl sulfide, and dibutyl disulfide. Cells grown on an alkyl sulfide or disulfide also oxidized the corresponding thiols, namely, methanethiol, ethanethiol, propanethiol, or butanethiol. Alkyl sulfides were metabolized by induced or derepressed cells with oxygen, nitrate, or nitrite as electron acceptor. Cells grown on DMS immediately metabolized DMS but there was a lag before DES was consumed; with DES-grown cells, DES was immediately used but DMS was used only after a lag. Chloramphenicol prevented the eventual use of DES by DMS-grown cells and DMS use by DES-grown cells, respectively, indicating separate enzymes for the metabolism of methyl and ethyl groups. Growth was rapid on formate, acetate, propionate, and butyrate but slow on methanol. The organism also grew chemolithotrophically on thiosulfate with a decrease in pH; growth required carbonate in the medium. Growth on sulfide was also carbonate dependent but slow. The isolate was identified as a Thiothrix sp. and designated strain ASN-1. It may have utility for removing alkyl sulfides, and also nitrate, nitrite, and sulfide, from wastewaters.

A variety of alkyl sulfides are found in the environment: methanethiol, dimethyl sulfide (DMS), and dimethyl sulfide (DMDS) typically occur as natural products in pelagic regions (3, 11, 18) and sediments (17, 34, 40). Higher alkyl sulfides such as alkyl thiols and dialkyl sulfides and polysulfides are present in animals (20, 24, 46), animal manure (25), and kerogens (27, 30) or are associated with sewage treatment plants (21) and the activities of the wood-pulping industry (4, 22).

DMS has received special attention because it is linked to atmospheric sulfate production, which results in cloud formation (2). Metabolism of organic sulfur compounds has been studied in pure cultures of hypomicrobia (5, 36), thiobacilli (12, 31, 32, 40), a pseudomonad (47), methanogens (8, 19, 28), and anoxygenic phototrophs (41, 43). Evidence from slurry experiments showed that sulfate reducers were potential DMS consumers as well (17).

Anaerobic organic-sulfur metabolism is of interest because relatively high concentrations of methanethiol, DMS, and DMDS have been reported for anoxic marine sediments (17, 34, 40). Recently, it was shown that polysulfides, which occur in high concentrations in some marine sediments (23, 39), react rapidly with organic matter to form organic polysulfides (38).

Metabolism of alkylsulfides other than methylated compounds has not been studied extensively. Kelly and Smith (15) mentioned the isolation of an organism that grew on diethyl sulfide (DES) and oxidized DMS, DMDS, DES, diethyl disulfide (DEDS), ethanethiol, and CS2 to sulfate and CO2; butanethiol and dipropyl sulfide (DPS) were also partially oxidized. Ethanethiol is a substrate for methanethiol oxidase, purified from Thiothrix thioparus TK-m (10), T. thioparus E6 (32), and Hyphomicrobiurn strain EG (36). Biological production of ethane from ethanethiol and DES in aquatic sediments was reported (29). Inhibitor studies of slurried sediments from Mono Lake, Calif., showed that methanogens were involved in alkyl sulfide metabolism. The aim of the present study was to isolate an organism capable of growth on alkyl sulfides under oxic and anoxic conditions.

MATERIALS AND METHODS

Site description and isolation. Sediment cores were taken from a developing microbial mat near a tidal creek in a Spartina-dominated salt marsh on Sapelo Island, Ga. Chlorophyll a content in the cyanobacterial layer (1- to 4-mm depth) was 98 μg·cm−2·day−1 and a black reduced layer containing FeS (0.2 μmol·cm−2·day−1) extended from a depth of 8 to 25 mm. Dilution series were prepared from depth horizons of 0 to 5, 5 to 10, and 10 to 20 mm in 13-ml Hungate-type screw-cap tubes and sealed with a butyl rubber septum (Belco, Vineland, N.J.). The tubes contained filtered and autoclaved seawater and carbonate-buffered (19 mM) synthetic medium (1:1, vol/vol).

Medium and growth conditions. The medium contained (in grams per liter) NaCl (25.0), NH4Cl (0.2), CaCl2·2H2O (0.225), KCl (0.2), MgCl2·6H2O (0.2), KH2PO4 (0.02), and Na2CO3 (2.0) and was supplemented with FeSO4·7H2O (1 mg/liter), 1 ml of the trace element solution of Widdel and Pfennig per liter (45), and vitamin B12 (20 μg/liter). DMS was added as growth substrate to a final concentration of 500 μmol/liter (37 μl/liter), and nitrate (KNO3) (0.505 g/liter) was added as electron acceptor. The pH of the medium was adjusted to 7.5 after autoclaving with sterile HCl or NaOH. After 2 weeks of incubation at room temperature in the dark, the DMS concentration was measured in the headspace of tubes which developed a small gas phase. Samples were taken aseptically through the butyl rubber septum with a sterile syringe. Tubes in which DMS had disappeared or

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decreased to low levels (<5 μM) were replaced with 6.5 μmol of DMS and 65 μmol of NO₃⁻. This procedure was repeated three times, after which a sample from one tube was streaked on agar plates containing DMS (0.37 ml/liter) and KNO₃ (2.02 g/liter) and incubated under an oxygen-free CO₂ atmosphere (GasPak; Becton, Dickinson & Co., Cockeysville, Md.). After repeated streaking of isolated colonies, a single colony was transferred to liquid medium. The culture was maintained on plates containing acetate (CH₃COONa·3H₂O, 0.68 g/liter) and thiourea (Na₂S₃O₃·5H₂O, 1.24 g/liter) and incubated under anaerobic conditions. Growth on alkyl sulfides was tested in static 250-ml Erlenmeyer flasks containing 100-ml portions of medium or in completely filled 150-ml screw-cap bottles. Anaerobic-incubation cultures contained nitrate (KNO₃, 2.02 g/liter) or nitrite (NaNO₂, 0.345 g/liter). Anaerobic media were autoclaved, immediately after which the bottles were closed to prevent oxygen diffusion. Prior to inoculation, the complete medium was stored for at least 1 h, when traces of oxygen were probably removed by chemical reaction with the organic sulfur compounds. Stock solutions of liquid alkyl sulfides were made by standard gravimetric dilution in sterilized Milli-Q water and were filter sterilized (0.2-μm pores). Organic sulfur compounds (1, 5, and 10 mM) tested were DMS, ethyl methyl sulfide (EMS), DES, DMS, dibutylsulfide (DBS), DMDs, DEDs, dibutyldisulfide (DBDS), ethanethiol, 1-propanethiol, 1-butanethiol, mercaptoethanol, mercaptoacetate, and 3-mercaptopyrrolionate. Dimethylsulfoniopropionate and S-methylcysteine were added at 2 mM only. Growth on formate, acetate, propionate, butyrate, methanol, methylamine, and trimethylamine was tested at 1, 5, and 10 mM concentrations. Growth was confirmed by three to five subsequent transfers on a compound, and rates and yields typically showed less than 5% variation between individual experiments.

**Cell suspension experiments.** Cells growing in the late-exponential phase were harvested by centrifugation (10 min at 10,000 × g at 5°C) and washed twice in medium lacking the growth substrate. Cell suspensions were used to examine growth with nitrate or nitrite, in anaerobic experiments, the associated oxygen consumption. Oxygen uptake was measured in cell suspensions (5 ml) at 30°C with a Clark-type electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio) and a chart recorder. Substrates were added after endogenous rates were determined. Substrate consumption rates were determined in cell suspensions (2 ml) in 14-ml serum bottles with butyl rubber stoppers and aluminum crimp seals. Anaerobic incubations were flamed for 10 min with oxygen-free N₂ prior to addition of the substrate. Chloramphenicol (75 μg/ml) was used as an inhibitor in experiments with DMS, DES, and EMS. When anaerobic consumption was measured with nitrate or nitrite, the suspensions were flushed with N₂ for 10 to 15 min prior to addition of substrates. Sorption of thiols to the stopper and glass, as well as chemical oxidation to disulfides, was measured in experiments with killed cells. All experiments were repeated and usually showed less than 5% variation between individual experiments.

**Analytical methods.** Protein was measured colorimetrically with bicinchoninic acid (33) after sulfur extraction with methanol when necessary. Chlorophyll a in sediment and microbial-mat samples was measured spectrophotometrically after methanol extraction (35). Acid volatile sulfur and field samples and sulfide in laboratory samples was measured by the methylene blue method (37). Polythionates and sulfides were determined colorimetrically after cyanalysis (14). Sulfate was measured by anion-exchange ion chromatography with a Dionex Ionpac AS4A column (4-mm diameter, 250 mm long) and conductivity detection. The eluent buffer consisted of 1.8 mM Na₂CO₃ and 2.1 mM NaHCO₃, and the flow rate was 1.5 ml/min. Volatile organic sulfur compounds were measured by headspace analysis on a gas chromatograph. A Shimadzu model GC-14A gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector and a column (1.4 m by 3 mm [internal diameter]) of Carbopak B HT 100 (Supelco Inc., Bellefonte, Pa.) was used, with a Shimadzu CR601 integrator to record peak areas. The flow rate of the carrier gas (N₂) was 60 ml min⁻¹ with a column temperature of 110°C. Retention times were as follows (in minutes): methanethiol, 0.7; DMS, 1.3; ethanol, 1.4; I-propanethiol, 3.5; EMS, 3.7; butanethiol, 6.5; DMDS, 7.7; DES, 12.5; DBS, 15.0; DEDs, 23.5; and DBDS, 28.0. In some experiments, the alkyl sulfides with long retention times the column temperature was increased to 130°C and the flow rate of the carrier gas was raised to 66 ml min⁻¹. Under these conditions, the retention times were decreased about 70%. Methanethiol and butanethiol were prepared by reduction of the disulfides DMDS and DBDS by 0.5 mM tributylphosphine (26).

**Chemicals.** Organic sulfides were obtained from Aldrich Chemical Company, Milwaukee, Wis.

**RESULTS**

DMS concentrations decreased after 2 weeks in several enrichment cultures which were obtained by direct dilutions of sediment samples. A pure culture of a gram-negative, motile organism was obtained from the 5- to 10-mm depth zone. The isolate grew on DMS or thiosulfate with oxygen and nitrate (Fig. 1). In addition, nitrite was also used as electron acceptor. Growth rates on thiosulfate and DMS were 0.18 and 0.10 h⁻¹ for aerobic conditions and 0.09 and 0.08 h⁻¹ with nitrate as electron acceptor. Anaerobic growth required nitrate or nitrite, and bubbles of gas were evolved when the screw caps were loosened on tubes or bottles of fully grown cultures. The gas evolved was DMDS (it did not dissolve in alkal) and was probably N₂. DMS and thiosulfate were stoichiometrically converted to sulfate (Table 1). Anaerobic growth on thiosulfate required carbonate and occurred with a drop in pH of the medium to 3.5 to 4.0. No polythionates were detected during growth on thiosulfate. The isolate catabolized a range of alkyl sulfides with either oxygen, NO₃⁻, or NO₂⁻ as the electron acceptor for growth. In addition to DMS, organic sulfur compounds which supported growth when used as the sole substrate were DMDS, DES, EMS, DPS, DBS, and DBDS. The aerobic yield on sulfur compounds was approximately 50% higher than the yield with either nitrate or nitrite as electron acceptor (Table 1). Increasing the chain lengths of carbon and sulfur of the substrates resulted in higher cell yields. Formate, acetate, propionate, and butyrate also supported growth. Slow growth was observed on methanol and sulfate and, as for thiosulfate, anaerobic development on sulfate depended on a carbonate buffer. Trimethylamine, methylamine, dimethylsulfoniopropionate, S-methylcysteine, 3-mercaptopropionate, and mercaptoethanol did not support growth. Optimum growth occurred at a pH range of 7.0 to 8.0, and growth did not occur when the initial pH was below 4.5. Growth was severely retarded when NaCl was omitted from the medium.

Cell suspension experiments showed that utilization of alkyl sulfides required induction or derepression of enzymes. When Thiobacillus strain ASN-1 was grown on DMS, cell
ALKYL SULFIDE DEGRADATION

FIG. 1. Growth of *Thiobacillus* strain ASN-1 on thiosulfate (10 mM) (A) and on DMS (5 mM) (B). ——, oxic incubation; ----, anoxic incubation (20 mM NO₃⁻); ⋄, protein; ■, S₂O₃²⁻ (A) or DMS (B); ▲, SO₄²⁻.

suspensions immediately oxidized DMS but only used DES after about 1 h (Fig. 2); conversely, cells grown on DES immediately consumed DES but only used DMS after a lag phase of about an hour (Fig. 2). Chloramphenicol (75 µg/ml) blocked the eventual use of DES by DMS-grown cells and the eventual use of DMS by DES-grown cells (Fig. 2). Furthermore, DES-grown cells consumed EMS with the accumulation of methanethiol, which was metabolized after about 60 min; the subsequent metabolism of methanethiol was prevented by chloramphenicol (Fig. 3). In contrast, cells grown on EMS consumed EMS immediately, but without the accumulation of either methanethiol or ethanethiol (Fig. 3). Substrate oxidation rates were highest for butyl sulfides and lowest for asymmetrical EMS (Table 2). Thiols were used immediately by cells grown on the equivalent sulfide or disulfide (Table 2). Oxidation rates, corrected for abiotic losses, were similar for methanethiol, DMS, and DMDS, as well as for butanethiol, DBS, and DBDS, but not for ethanethiol, DES, and DEDS. Cells grown on either DMDS or DMS used methanethiol immediately. Similarly, DES- or DEDS-grown cells and DBS-grown organisms used ethanethiol and butanethiol, respectively, without lag. In general, substrate oxidation rates were higher with oxygen

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TABLE 1. Growth yield (protein) and sulfate formation of *Thiobacillus* strain ASN-1 on sulfur compounds

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Yield (mg of protein/mmol) with electron acceptor</th>
<th>% SO₄²⁻ recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS</td>
<td>16.6</td>
<td>94</td>
</tr>
<tr>
<td>DMDS</td>
<td>22.7</td>
<td>NT*</td>
</tr>
<tr>
<td>DES</td>
<td>23.4</td>
<td>83</td>
</tr>
<tr>
<td>DEDS</td>
<td>27.9</td>
<td>NT</td>
</tr>
<tr>
<td>DPS</td>
<td>25.1</td>
<td>NT</td>
</tr>
<tr>
<td>DBS</td>
<td>29.0</td>
<td>87</td>
</tr>
<tr>
<td>DBDS</td>
<td>34.8</td>
<td>NT</td>
</tr>
<tr>
<td>Thiosulfate</td>
<td>6.2</td>
<td>99</td>
</tr>
</tbody>
</table>

* NT, not tested.
Hyphomicrobium. Growth of with acid of thiosulfate, requirement for in DMS-grown cells DMDS, DES, oxygen increase organic the showed 3) cultures. acetate-grown EMS; ml-') strain Thiobacillus 3. FIG. 3. EMS consumption in the presence of nitrate (20 mM) by Thiobacillus strain ASN-1. Cells were grown on EMS (151 μg of protein ml-1) (A) and DES (219 μg of protein ml-1) (B). Symbols: -□-, EMS; -▲-, EMS plus chloramphenicol (75 μg · ml-1); -●-, EMS minus NO₃⁻ (no electron acceptor); -■-, methanethiol; -▲-, methanethiol plus chloramphenicol (75 μg · ml-1).

than in the presence of nitrate. Oxygen uptake rates (Table 3) showed that thiosulfate-grown cells did not oxidize any of the organic sulfides tested. Addition of alkyl sulfides did not increase oxygen uptake rates above the endogenous level in suspensions of thiosulfate-grown cells. Thiosulfate was not oxidized in cell suspensions from DMS-, DMDS-, DES-, or acetate-grown cultures. Cells grown on acetate, DMS, DMDS, DES, and thiosulfate all used acetate and butyrate; DMS-grown cells oxidized DMDS, and DMDS-grown cells used DMS.

DISCUSSION

The autotrophic character of our isolate, as shown by the requirement for carbonate to allow anaerobic growth on thiosulfate, eliminates the possibility of its being a species of Hyphomicrobium. Growth of the bacterium on thiosulfate with acid production and its rod-shaped cells are characteristics of thiobacilli rather than Thiomicrospira or Thi-

sphaera spp., and so it is identified as a Thiobacillus sp. and designated strain ASN-1.

Metabolism of DMS under oxic conditions has been described for a variety of organisms. However, anaerobic consumption by marine isolates has only been reported for methanogens and anoxygenic phototrophs. Furthermore, anoxygenic phototrophs merely convert DMS to its sulfoxide without catabolism of the methyl groups. Although there is evidence for DMS consumption by sulfate reducers, isolates that use DMS are presently lacking. Similarly, nitrate respirers that use methylated sulfides have not been described. Methanogens or anoxygenic phototrophs are not generally abundant in marine sediments, but relatively high concentrations of DMS and its rapid turnover in anoxic marine sediment slurries suggest microbial consumption (17, 44). Thiobacilli and hyphomicrobium catabolize DMS in the presence of oxygen (12, 32, 36). The enzyme involved in DMS degradation is a monooxygenase, which attacks the methyl group, and hence metabolism only proceeds in the presence of oxygen. In this study, we isolated from a microbial mat an organism which catabolizes DMS either aerobically or under anoxic conditions when nitrate is present. Inhibitor studies indicate that DMS is catabolized via a methyltransferase instead of via DMS monooxygenase and methanethiol oxidase (42). Diel fluctuations in oxygen profiles are characteristic for microbial mats (6), and microbes in the upper 10 mm encounter high concentrations of

<table>
<thead>
<tr>
<th>Test substrate</th>
<th>Growth substrate</th>
<th>Oxidation rate (nmol · mg of protein⁻¹ · min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanethiol</td>
<td>DMS</td>
<td>58, 70, 72</td>
</tr>
<tr>
<td>DMS</td>
<td>DMS</td>
<td>63, 52, 60</td>
</tr>
<tr>
<td>DMDS</td>
<td>DMDS</td>
<td>67, 46</td>
</tr>
<tr>
<td>EMS</td>
<td>EMS</td>
<td>58, 55</td>
</tr>
<tr>
<td>Ethanethiol</td>
<td>Ethanethiol</td>
<td>40</td>
</tr>
<tr>
<td>DES</td>
<td>DES</td>
<td>101, 93</td>
</tr>
<tr>
<td>DEDS</td>
<td>DEDS</td>
<td>89</td>
</tr>
<tr>
<td>DPS</td>
<td>DPS</td>
<td>53</td>
</tr>
<tr>
<td>Butanethiol</td>
<td>DBDS</td>
<td>119</td>
</tr>
<tr>
<td>DBS</td>
<td>DBS</td>
<td>131</td>
</tr>
<tr>
<td>DBDS</td>
<td>DBDS</td>
<td>124</td>
</tr>
</tbody>
</table>

* Values are for O₂ as electron acceptor; when given, the second and third values are for NO₃⁻ and NO₂⁻ as electron acceptors, respectively.

Table 2. Consumption rates of alkyl sulfides by cell suspensions of Thiobacillus strain ASN-1

<table>
<thead>
<tr>
<th>Test substrate</th>
<th>O₂ uptake rate (nmol · min⁻¹ · mg of protein⁻¹) with growth substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate</td>
</tr>
<tr>
<td>Acetate</td>
<td>154</td>
</tr>
<tr>
<td>S₂O₃²⁻</td>
<td>0</td>
</tr>
<tr>
<td>DMS</td>
<td>0</td>
</tr>
<tr>
<td>DMDS</td>
<td>0</td>
</tr>
<tr>
<td>DES</td>
<td>0</td>
</tr>
<tr>
<td>DBS</td>
<td>0</td>
</tr>
<tr>
<td>Butyrate</td>
<td>165</td>
</tr>
</tbody>
</table>

* Endogenous rates have been subtracted.

b NT, not tested.

Table 3. Oxygen uptake rates by cell suspensions of Thiobacillus strain ASN-1

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oxygen during the day and anoxic conditions in the dark. Under these conditions, the facultative anaerobes such as *Thiobacillus* strain ASN-1 could use the same metabolic route for DMS catabolism with either oxygen or nitrate as electron acceptor.

Alkyl sulfides, other than methylated ones, have been reported in a variety of environments. In general concentrations are relatively low, but measurements are sparse. It was shown that polysulfides, which contribute significantly to the total sulfur pool in marine sediments (1, 39), react rapidly to form organic polysulfides (33, 38). *Thiobacillus* strain ASN-1 used a range of alkyl sulfides, and specific enzyme systems appear to be required for metabolism of different chain lengths. Interestingly, only a few of the other C₃ compounds tested supported growth in *Thiobacillus* strain ASN-1: methanol and formate were used but not methylamine or trimethylamine, providing indication of an autotrophic rather than a methylo trophic nature. Growth on methanol may even be autotrophic, as shown for *Thiobacillus versutus* (16) and *Thio pheraea pantotropha* GB17M (7). C₃ sulfur compounds other than DMS and methanethiol, such as S-methyl cysteine and dimethylsulfoniopropionate, were not catabolized by *Thiobacillus* strain ASN-1. Also, organic sulfur compounds with carboxyl and hydroxyl groups were not used: neither mercaptoethanol, mercaptoacetate, nor 3-mercaptopropionate sustained growth, which suggests that the enzyme systems involved are specific for alkyl groups.

The presence and removal of alkyl sulfides have been studied in wastewater (4, 21, 22). Le Cloirec et al. (22) reported anaerobic removal of DMDS in a biofilter from wastewater to which nitrate was added as electron acceptor. The autochthonous bacterial population removed 65% of the DMDS fed to the system, with a maximum rate of 1.3 g of DMDS liter⁻¹ · day⁻¹, which equals approximately 9,400 nmol · liter⁻¹ · min⁻¹. However, the biofilter could not sustain DMDS sorption and degradation for more than 2 weeks. *Thiobacillus* strain ASN-1 consumed 55 nmol of DMDS · mg of protein⁻¹ · min⁻¹ in the presence of nitrate (Table 2), which implies that a culture of 171 mg · liter⁻¹ is required to remove the same amount as that reported by Le Cloirec et al. (22). Kanagawa and Mikami (13) reported aerobic removal rates of 3.6 g of DMDS, 4.0 g of DMS, and 2.0 g of methanethiol per g (dry weight) per day for *T. thioparus* TK-m. Assuming that protein constitutes 50% of the dry weight (9), these values correspond to 53 nmol of DMDS · mg of protein⁻¹ · min⁻¹, 90 nmol of DMS · mg of protein⁻¹ · min⁻¹, and 57 nmol · mg of protein⁻¹ · min⁻¹ for methanethiol. These results are similar to those obtained for *Thiobacillus* strain ASN-1 in this study. Cell extracts of *Pseudomonas acidovorans* DM-11 oxidized DMS to dimethyl sulfoxide and had a much slower removal rate of 0.03 nmol of DMS · mg of protein⁻¹ · min⁻¹ (47). In addition, with the higher removal rates for methylated sulfides, *Thiobacillus* strain ASN-1 degrades a range of alkyl sulfides which makes it an attractive candidate for possible wastewater treatment. However, research is required on the expression of multiple catabolic pathways for the destruction of mixtures of alkyl sulfides, perhaps in the presence of sulfate and/or thiosulfate.

Previously studied species of aerobic bacteria have strikingly similar growth yields on DMS (Table 4). The molar yield on DMS for three *Thiobacillus* species and one *Hyphomicrobiun* sp. ranges from about 12 to 13 g of protein. The molar yield of about 17 g of protein for aerobic growth of *Thiobacillus* strain ASN-1 on DMS is significantly higher than this range. Two different mechanisms operate for the aerobic catabolism of DMS (42), and a methyltransferase system is conceivably more energy efficient than DMS monooxygenase, which initially consumes reduced pyridine nucleotide. Although the yield is in part determined by respiratory requirements, the higher yield of *Thiobacillus* strain ASN-1 suggest that the methyltransferase is more efficient. The yields under anoxic conditions of a methanogen and an anoxygenic phototroph were, as expected, much lower than values found for aerobic metabolism. In contrast, the yield of *Thiobacillus* strain ASN-1 growing under anoxic conditions with nitrate as electron acceptor was similar to values found for *Hyphomicrobiun* strain EG and the other thiobacilli growing aerobically.

In addition to having high yields on DMS and thiosulfate, *Thiobacillus* strain ASN-1 obtained high yields on all alkyl sulfides tested in this study. This strain was able to catabolize these compounds underoxic and anoxic conditions by utilizing inducible or derepressible enzyme systems, as the experiments with DMS-, DES-, and EMS-grown cells showed. The use of alkyl sulfides by other organisms has not been studied well, so that it is difficult to assess the competitive position of our isolate in the environment. Also, in order to evaluate the consumption of alkyl sulfides of *Thiobacillus* strain ASN-1 in situ, i.e., at low substrate concentrations, the kinetic parameters half-saturation constant (K₅) and μₘₐₓ need to be determined in continuous culture. However, the metabolic capability of this organism could make it an important contributor to consumption of DMS and other organic compounds under fluctuating conditions, as often encountered in tidal sediments.

### TABLE 4. Bacterial growth rates and yields on DMS

<table>
<thead>
<tr>
<th>Organism</th>
<th>Origin</th>
<th>Growth conditions</th>
<th>Growth rate (h⁻¹)</th>
<th>Yield (mg of protein · mmol of DMS⁻¹)</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thiobacillus</em> strain ASN-1</td>
<td>Marine microbial mat</td>
<td>Oxic</td>
<td>0.10ᵃ</td>
<td>16.6</td>
<td>This study</td>
</tr>
<tr>
<td><em>Thiobacillus</em> thioparus TK-m</td>
<td>Activated sludge</td>
<td>Anoxic</td>
<td>0.08ᵇ</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td><em>Thiobacillus</em> thioparus T5</td>
<td>Marine microbial mat</td>
<td>Oxic</td>
<td>0.06ᵇ</td>
<td>12–13ᵇ</td>
<td>12</td>
</tr>
<tr>
<td><em>Thiobacillus</em> thioparus E6</td>
<td>Freshwater pond</td>
<td>Oxic</td>
<td>0.08ᵇ</td>
<td>12.0</td>
<td>40</td>
</tr>
<tr>
<td><em>Hyphomicrobiun</em> strain EG</td>
<td>Peat biofilter</td>
<td>Anoxic</td>
<td>0.08ᵇ</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td><em>Thiobacillus</em> roseopersicina M1</td>
<td>Marine microbial mat</td>
<td>(An)oxic</td>
<td>0.07ᵇ</td>
<td>11.8</td>
<td>36</td>
</tr>
<tr>
<td><em>Methanosarcina</em> strain MPT4</td>
<td>Salt marsh sediment</td>
<td>Anoxic</td>
<td>0.01ᵃ</td>
<td>4.3</td>
<td>44</td>
</tr>
</tbody>
</table>

ᵃ Growth determined in Batch cultures.
ᵇ Calculated from dry weight or TOC data, assuming dry weight is 50% TOC or 50% protein (9).
ᶜ Growth determined in chemostat cultures.
ACKNOWLEDGMENTS

Ron Kiene kindly provided laboratory space during the isolation at Sapelo Island.

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