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Demethylation of Dimethylsulfoniopropionate to 3-S-Methylmercaptocapropropionate by Marine Sulfate-Reducing Bacteria

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The initial step in the anaerobic degradation of the algal osmolyte dimethylsulfoniopropionate (DMSP) in anoxic marine sediments involves either a cleavage to dimethylsulfide and acrylate or a demethylation to 3-S-methylmercaptocapropropionate. Thus far, only one anaerobic bacterial strain has been shown to carry out the demethylation, namely, Desulfobacterium sp. strain PM4. The aims of the present work were to study how common this property is among certain groups of anaerobic bacteria and to obtain information on the affinities for DMSP of DMSP-demethylating strains. Screening of several pure cultures of sulfate-reducing and acetogenic bacteria showed that Desulfobacterium vacuolatum DSM 3385 and Desulfovibrio nacii DSM 2059 are also able to demethylate DMSP; a very slow demethylation of DMSP was observed with a salt-tolerant strain of Eubacterium limosum. From a 10⁷ dilution of intertidal sediment a new marine DMSP-demethylating sulfate-reducing bacterium (strain WN) was isolated. Strain WN was a short, gram-negative, nonmotile rod that grew on betaine, sarcosine, palmitate, H₂ plus CO₂, and several alcohols, organic acids, and amino acids. Extracts of betaine-grown cells had hydrogenase, formate dehydrogenase, and CO dehydrogenase activities but no α-ketoglutarate oxidoreductase activity, indicating the presence of the acetyl coenzyme A-Co dehydrogenase pathway. Analysis of the 16S rRNA gene sequence of strain WN revealed a close relationship with Desulfofacter hydrogenophilus, Desulfobacter latus, and Desulfofacula tolerolica. Strain PM4 was shown to group with Desulfofacter nacii. The K_m of strain WN for DMSP, as derived from substrate progress curves in cell suspensions, was approximately 10 μM. A similar value was found for D. nacii PM4.

The algal osmolyte dimethylsulfoniopropionate (DMSP) is the major precursor of dimethylsulfide (DMS), which is the most abundant volatile sulfur compound in the marine ecosystem (5). It has been suggested that DMS plays an important role in the global sulfur cycle (22) and in the formation of cloud condensation nuclei through its atmospheric oxidation products (5). Because of the formation of cloud condensation nuclei, DMS might have an effect on the global climate and even partly counteract global warming. The possible impact of the natural production of DMS on the global climate has resulted in considerable attention for this compound and its precursors over the past 10 years.

DMS is a structural and functional analog of glycine betaine, a widely used compatible solute (43). The microbial degradation of glycine betaine has been studied extensively, and a variety of aerobic bacteria as well as anaerobic glycine betaine-degrading bacteria have been isolated (see, for example, references 13 and 14). DMS is degraded by bacteria via either an initial cleavage to DMS and acrylate (18) or a demethylation to 3-S-methylmercaptocapropropionate (MMPA) and subsequently to 3-mercaptoacrylate (MPA) (17, 32). A number of aerobic marine bacteria have been isolated that degrade DMS by an initial cleavage to DMS and acrylate (7, 21) or by an initial demethylation to MMPA followed by methanethiol production (32) or a further demethylation to MPA (39). Little is known about the nature of the organisms involved in the demethylation of DMS. Even less is known about the anaerobic bacteria which are involved in the demethylation pathway of DMS in anoxic marine sediments. Kiene and Taylor (17) speculated that acetogenic bacteria similar to Eubacterium limosum could be responsible for the observed sequential demethylation of DMS to MPA in sediment slurry experiments. The first steps in understanding the microbial demethylation of DMS came with the observation of the degradation of DMSP to MMPA by Desulfofacter sp. strain PM4 (36), an organism which had been isolated on glycine betaine (14), and the conversion of MMPA to MPA by certain marine Methanosarcina species (35).

The work with strain PM4 combined with the observations of Kiene and Taylor (17) raised the following questions which we address in this paper. (i) Can DMSP-demethylating enrichment cultures of anaerobes be obtained, and how widespread is the ability to demethylate DMSP to MMPA among sulfate-reducing and acetogenic bacteria? (ii) Do DMSP-demethylating anaerobes have K_m values for their substrate that are similar to environmental DMSP concentrations?

MATERIALS AND METHODS

Microorganisms, media, and cultivation. The bicarbonate-buffered medium of Brysch et al. (3) for Desulfofacter autotrophicum was used for dilution or enrichment cultures and growth of strain WN, with the addition of yeast extract (50 to 100 mg/liter). Incubations were at 28°C in 120-ml bottles filled with 50 ml of medium (for growth curves) or 16-ml tubes with 10 ml of medium (for substrate utilization experiments); the headspace consisted of 80% N₂–20% CO₂. Substrates were added from sterile 1 M stock solutions; DMS was neutralized with 1 M NaHCO₃ and filtered sterilized.

The following strains were grown in media as described in the 1993 catalog of strains of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and, unless otherwise indicated, precultured at 30°C.
on the substrates shown in parentheses: Desulfobacterium vacuolatum BfRM (DSM 3385; 10 mM betaine), Desulfobacterium autotrophicum HRM2 (DSM 3382; 10 mM betaine, 28°C), Desulfobacterium nacieni NAV1 (DSM 2059; 10 mM betaine), Desulfococcus multivorans Göttingen 1bc1 (DSM 2059; 5 mM benzoate and Desulfobacter postgutaii Dengast 2c9 (DSM 2034; 20 mM acetate), Desulfobulbus sphaeroides (DSM 2638; 20 mM lactate), Desulfocapna halophilus SL 8903 (DSM 5663; 20 mM lactate), Desulfosarcina variabilis Montpellier 3bc13 (DSM 2060; 5 mM benzoate), Desulfobulbus propionicus Lindhorst 1pr2 (DSM 2032; 20 mM lactate), Desulfobulbus sp. strain 3pr10 (DSM 2056; 20 mM propionate), and Desulfobulbus propionicus kuznetsovii (DSM 6115; 20 mM lactate, 60°C). Desulfobulbus propionicus strain PM4 was cultivated as described previously (14). Desulfobacterales sulcicaterae Toiz (DSM 7467) was grown on 5 mM benzoate at 30°C in medium described in reference 27. The following strains were grown as described by Hensgens et al. (12); Acetobacterium woodii (DSM 2638; 0.5 mM betaine), Sporomusa ovata (DSM 2062; 50 mM methanol), Sporomusa ovata var. utilis Montpellier 3bc13 (DSM 2060; 5 mM benzoate), Desulfotomaculum kuznetsovii (DSM 6315; 20 mM lactate, 60°C). Desulfobacterium propionicum strain PM4 was cultivated as described previously (14). Desulfobacterales sulcicaterae Toiz (DSM 7467) was grown on 5 mM benzoate at 30°C in medium described in reference 27. The following strains were grown as described by Hensgens et al. (12). Acetobacterium woodii (DSM 2638; 0.5 mM betaine), Sporomusa ovata (DSM 2062; 50 mM methanol), Sporomusa ovata var. utilis Montpellier 3bc13 (DSM 2060; 5 mM benzoate), Desulfotomaculum kuznetsovii (DSM 6315; 20 mM lactate, 60°C). Sediment sampling and dilution series. Sediment was collected from the intertidal zone of the Wadden Sea near Westermeland, The Netherlands, and from the intertidal zone of the Wadden Sea near Torrevieja, The Netherlands. The sediment consisted of black sulfide-rich mud covered by a thin oxidized layer. Samples were collected as described previously (35). Per gram (wet weight) of sediment, 2 ml of dilution buffer was added. The dilution buffer contained 25 mM potassium phosphate pH 7.1, 21 g of NaCl per liter, and 3 g of MgCl₂ - 6 H₂O per liter and was made anoxic by sparging with oxygen-free N₂ and adding 0.2 mM sodium dithionite. Subsequent dilutions were made from this suspension: 0.5 ml of each dilution was added to 50 ml of the basal medium of Brysch et al. (3) supplemented with 50 mg of yeast extract per liter and approximately 4 mM DMS unless otherwise indicated. Incubations were at 28°C.

Enzyme measurements. Extracts of cells of strain WN grown on 20 mM betaine were prepared anaerobically as described by Hensgens et al. (15). Enzyme activities were measured anaerobically by bubbling the assay mixture in a cuvette with oxygen-free N₂ for at least 3 min and then closing the cuvette with a grey butyl rubber stopper. Reactions were started by addition of cell extract with an N₂-flushed microsyringe. Carbon monoxide dehydrogenase, hydrogene

zyme, formate dehydrogenase, and α-ketoglutarate oxidoreductase were assayed in accordance with the method of Schauder et al. (30).

Utilization of DMS by washed-cell suspensions. Cells were grown to the late exponential phase on 20 mM DMS and harvested anaerobically by centrifugation at 16,000 × g for 10 min at 4°C. All of the following steps were performed under anaerobic conditions by keeping the cells under a constant flow of oxygen-free N₂. After the pellet was washed twice with anoxic 25 mM potassium phosphate buffer (pH 7.1), 21 g of NaCl per liter, and 3 g of MgCl₂ - 6 H₂O per liter and was made anoxic by sparging with oxygen-free N₂, the resuspended buffer containing 21 g of NaCl and 3 g of MgCl₂ - 6 H₂O per liter, 20 mM Na₂S₂O₄, and 0.2 mM sodium dithionite, it was resuspended in N₂-sparged buffer containing 21 g of NaCl and 3 g of MgCl₂ - 6 H₂O per liter, 20 mM Na₂S₂O₄, and 0.2 mM sodium dithionite to an optical density at 660 nm of 0.25 to 0.30. This suspension (40 ml) was transferred to a 13-ml vial containing a 6 ml of mineral medium, and the experiment was started by the addition of 50 mM anoxic DMS after flushing the headspace with oxygen-free N₂ for 5 min and sealing the bottle with a butyl rubber stopper. A 1-ml sample was taken through the stopper with an N₂-flushed 1-ml syringe and transferred to a 13-ml vial containing 5 ml of 6 M NaOH, which was scaled with a Teflon-coated rubber stopper. The amount of DMS in the vial was measured after 24 h. The apparent Km value was estimated from the substate progress curve by the method described by Dalgaard and Baek for determining the Km value for NO₃⁻ or SO₄²⁻ uptake by Desulfovibrio desulfuricans (6).

DNA analysis. The moles percent G + C of the DNA was determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen by high-performance liquid chromatography (HPLC) based on the method of Mesbah et al. (34). DNA analysis. The moles percent G + C of the DNA was determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen by high-performance liquid chromatography (HPLC) based on the method of Mesbah et al. (34). The DNA of strain WN was synthesized from acrylic acid and DMS by the method of Chambers et al. (4). MMPA was obtained by alkaline hydrolysis of its methyl ester (C₇H₁₆O₃, Steinheim, Germany) as described by Wackett et al. (46). The identity and purity of the product were checked by hydrogen nuclear magnetic resonance; the DMS or MMPA content was estimated by total carbon analysis (13) and gas chromatography or HPLC analysis (35, 36).

Results S Demethylation of dimethylsulfoxonipropionate. Addition of 2.5 ml of suspended anoxic intertidal sediment to mineral medium supplemented with sulfate, DMS, and yeast extract always resulted in the formation of DMS; MMPA was not detected in these incubations. To avoid a possible rapid DMS cleavage by free DMS lyase from the sediment or by DMS-lyase-deficient bacteria, triplicate 10⁴, 10⁵, and 10⁶ dilutions of the sediment were made to study the conversion of DMS (Table 1). In the absence of sulfate, up to 50% of the DMS was degraded and converted to DMS after 26 days of incubation. No MMPA, methane, or methanethiol was detected in these incubations. However, when sulfate was present, the DMS was degraded. When the highest dilution, DMS was converted almost stoichiometrically to MMPA; in the first two dilutions, the concentration of MMPA was lower than the amount of DMS that was added. Only traces of methane and/or methanethiol were detected (Table 1).

The 10⁴ and 10⁵ dilutions contained small rod-shaped cells, some oval- to rod-shaped cells that were slightly refractile, and vibrios. A pure culture of a rod-shaped DMS-demethylation bacterium (strain WN) was obtained by three successive transfers of colonies on agar plates with DMS-containing medium which were incubated under anaerobic conditions.

Characteristics of strain WN. Cells of strain WN were short, nonmotile rods with a length of 1.0 to 2.5 μm and a width of 1.0 to 1.5 μm (Fig. 1); colonies were beige and smooth. Strain WN was able to use the following growth substrates: formate, acetate, propionate, isobutyrate (5 mM), butyrate (5 mM), valerate (5 mM), lactate, fumarate, pyruvate, succinate, propionate, butanol, ethanol, glycine, glutamine, glutamate, glutamine, glutamate, glutamine, glutamate, glutamine, glutamate, glutamine, glutamate, glutamine, glutamate, glutamine, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamate, glutamat
presence of 20 mM sulfate unless otherwise indicated. Fermentative growth occurred only on pyruvate; no growth occurred on betaine and sulfate underoxic conditions. Toluene, which was supplied via an inert hexadecane phase (1 ml per 50 ml of medium, containing 100 mM toluene), could not be utilized by strain WN. This concentration of toluene was not inhibitory for a culture growing with betaine. Thiosulfate and sulfite were used as alternative electron acceptors; nitrate, nitrite, and elemental sulfur were not. Growth on 10 mM betaine was observed in medium with biotin, p-aminobenzoate, and nicotinate as the only vitamin supplements. The fastest growth was observed between pH 6.6 and 7.0 (6.4 to 8.5 tested), at a temperature of 28°C (4 to 45°C tested; no growth at 37°C) and as a salinity of 3.0% (0.1% to 4.0% tested; no growth at 1.2% and 4.0%). Desulfoviridin could not be detected in cell extracts of strain WN. The moles percent G+C was 49.8%.

During growth on 7.5 mM DMSP, strain WN converted DMSP stoichiometrically to MMPA and produced 5.6 mM sulfide (Fig. 2). Growth was exponential only in the early growth phase, with a maximum specific growth rate of 0.04 h⁻¹ (doubling time, 17 h). Betaine was converted to dimethylglycine.

**Cellular fatty acid composition.** The fatty acid profile of cells of strain WN grown on 20 mM ethanol and 20 mM sulfate was similar to those described for Desulfobacterium autotrophicum and Desulfobacter hydrogenophilus (Table 2). The 17:0 cis-11 fatty acid, which is typical for Desulfobacterium species, was clearly present in strain WN (4.6%) and Desulfobacula toluolica (4.1%), while the cyclopropane 17:0 fatty acid, which is a biomarker fatty acid of Desulfobacter curvatus (33), was absent in strain WN as well as Desulfobacula toluolica. Strain WN did not contain the 10-methyl 16:0 fatty acid, which was present in Desulfobacter curvatus, Desulfobacula toluolica, and Desulfobacterium autotrophicum.

**Enzyme activities.** In extracts of strain WN grown on 20 mM betaine and 20 mM sulfate, the following enzyme activities were detected: carbon monoxide dehydrogenase (5.3 μmol/min/mg of protein), formate dehydrogenase (0.6 μmol/min/mg of protein), and hydrogenase (1.5 μmol/min/mg of protein). No α-ketoglutarate oxidoreductase activity was found; this activity was detected in Desulfobacter hydrogenophilus (0.2 μmol/min/mg of protein) grown on 20 mM acetate and 20 mM sulfate.

**Phylogenetic analysis.** Initially, strain WN was identified as a strain of the genus Desulfobacterium on the basis of a positive hybridization (data not shown) of its RNA with the Desulfobacterium-specific 16S rRNA probe of Devereux et al. (8). However, a study of the relationship of strain WN to other sulfate-reducing bacteria based on 16S rRNA gene sequence analysis (Fig. 3) showed that strain WN is related to Desulfobacter hydrogenophilus (94.7% similarity), Desulfobacter latus (93.1% similarity), and Desulfobacula toluolica (92.2% similarity). We also determined the 16S rRNA gene sequence of strain PM4 and the relationship of this strain to other Desulfobacterium species. Strain PM4 is closely related to Desulfobacterium nia-
Comparison of the 16S rRNA gene sequence of strain WN with the Desulfobacterium-specific probe sequence (8) shows that there is a T instead of a C at position 226 (5'-TTTGAAGATGAGTC-3'). However, there is also one mismatch with the Desulfobacter-specific probe (8), i.e., an A instead of a G at position 141 (5'-AATCTACTTTC-3'). The nucleotide sequences of strains WN and PM4 were deposited with GenBank under accession numbers U51844 and U51845, respectively.

**Kinetics of DMSP conversion.** Initial experiments with washed-cell suspensions of strain WN grown on DMSP indicated that after the addition of 0.2 mM DMSP the concentration of DMSP decreased linearly in time to about 10 μM. Experiments to determine the $K_m$ values of strains WN and PM4 for DMSP were therefore done with a DMSP concentration of approximately 50 μM and cell suspensions with optical densities (at 660 nm) of 0.2 to 0.4. Figure 4 shows progress curves for DMSP conversion by strain PM4 (Fig. 4A) and strain WN (Fig. 4B). The lines represent the theoretical progress curve for the plot that gives the best approximation of the measured values. By using this method, maximum average $K_m$ values of 10 and 9 μM DMSP were obtained for strains WN and PM4, respectively. Strain WN degraded DMSP to a level below the detection limit (0.2 μM), but in suspensions of strain PM4 about 2 μM DMSP was left unconverted. We do not have an explanation for this difference.

**Conversion of DMSP by pure cultures of sulfate-reducing and acetogenic bacteria.** A wide variety of sulfate-reducing bacteria from marine as well as nonmarine habitats were tested for their ability to demethylate DMSP (for a complete list, see Materials and Methods). Two Desulfobacterium strains were found to be able to demethylate DMSP: Desulfobacterium vacuolatum DSM 3385 and Desulfobacterium niacini DSM 2650. Interestingly, Desulfobacterium autotrophicum DSM 3382, which is able to demethylate glycine betaine to N,N-dimethylglycine (14), was not able to grow on DMSP.

Two strains of acetogenic bacteria were isolated from marine sediment to test whether they could utilize DMSP. Strain ACES was isolated from anoxic intertidal estuarine sediment of the Eems-Dollard estuary near Termuntenzijl, The Netherlands, with H2-CO2 as the substrate; strain ACM was isolated from anoxic marine intertidal sediment of the Wadden Sea.

### Table 2. Cellular fatty acid compositions of strain WN, Desulfobaculata toluolica, Desulfobacter curvatus, and Desulfobacterium autotrophicum

<table>
<thead>
<tr>
<th>ECLa</th>
<th>Fatty acid</th>
<th>Cellular fatty acid composition (%) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Strain WN</td>
</tr>
<tr>
<td>14.00</td>
<td>14:0</td>
<td>13.5</td>
</tr>
<tr>
<td>14.86</td>
<td>15:1 c9</td>
<td>1.7</td>
</tr>
<tr>
<td>15.00</td>
<td>15:0</td>
<td>5.0</td>
</tr>
<tr>
<td>15.77</td>
<td>16:1 c7</td>
<td>1.8</td>
</tr>
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<td>15.82</td>
<td>16:1 c9</td>
<td>33.1</td>
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<tr>
<td>16.00</td>
<td>16:0</td>
<td>26.2</td>
</tr>
<tr>
<td>16.43</td>
<td>16-Methyl 16:0</td>
<td>10.15</td>
</tr>
<tr>
<td>16.86</td>
<td>17:1 c11</td>
<td>4.6</td>
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<td>16.89</td>
<td>17:0 cyc</td>
<td>2.4</td>
</tr>
<tr>
<td>17.52</td>
<td>3-OH 16:0</td>
<td>1.9</td>
</tr>
<tr>
<td>17.79</td>
<td>18:1 c9</td>
<td>2.1</td>
</tr>
</tbody>
</table>

a ECL, equivalent chain length.
b Data were taken from reference 34.

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FIG. 3. Phylogenetic relationship of strain WN with other sulfate-reducing bacteria. The unrooted tree is based on a distance matrix of the 16S rRNA gene sequence (base pairs 8 to 1513) and was constructed via the neighbor-joining method as implemented in the Phylip 3.5c software package.
near Westernieland, The Netherlands, with glycine betaine as the substrate. Both strains could grow in freshwater medium for acetogenic bacteria (12) and can therefore be regarded as salt-tolerant strains. Strain ACES converted H₂-CO₂ exclusively to acetate and was also able to demethylate glycine betaine to N,N-dimethylglycine; strain ACM converted glycine betaine to acetate and an equimolar amount of N,N-dimethylglycine. Both strains had an American football shape, which is characteristic for Acetobacterium species (31). On the basis of morphology, product formation, and substrate utilization (data not shown), both strains were tentatively identified as Acetobacterium species. Strains ACM and ACES were not able to grow on DMSP.

Pure cultures of the following nonmarine acetogenic bacteria were found to be unable to grow on DMSP: A. woodii DSM 1030, S. ovata DSM 2662, S. sphaeroides DSM 2875, and B. methylophilicum Marburg DSM 3468. A salt-tolerant strain of Eubacterium limosum (strain PM31), which was isolated by Heijthuijsen (11) from anoxic intertidal sediment of the Wadden Sea with vanillic acid as the substrate, grew very poorly and converted 7.1 mM DMSP to 7.0 mM MPA, 0.4 mM acetate, and 2.0 mM butyrate in 31 days of incubation.

**DISCUSSION**

These results show that the ability to demethylate DMSP to MPA under anoxic conditions is not a property exclusive to strain PM4 but is found in several members of the Desulfobacter-Desulfobacterium cluster of the delta-Proteobacteria. None of the DMSP-degrading anaerobes demethylated DMSP to MPA; thus, pure-culture studies suggest that the observed demethylation of DMSP to MPA in anoxic sediments is the result of the combined activities of more than one type of organism. The isolation of strain WN demonstrates that it is possible to obtain anaerobic DMSP-demethylating bacteria via enrichment cultures with DMSP as a substrate, provided sufficient precautions are taken to prevent the rapid cleavage of DMSP. Strain WN differs from the DMSP-demethylating strains of the genus Desulfobacterium and is difficult to affiliate with any of the known members of the Desulfo-Desulfobacterium cluster. With respect to lipid composition, substrate utilization, and moles percent G+C, strain WN is similar to members of the genus Desulfobacterium (34, 41). Because of its autotrophic growth and hybridization with a Desulfobacterium-specific 16S rRNA probe (8), strain WN was initially identified as a strain of Desulfobacterium autotrophicum (3), although the type strain of this Desulfobacterium species does not demethylate DMSP. However, phylogenetic positioning based on the 16S rRNA gene sequence showed that strain WN is closely related to Desulfobacter hydrogenophilus and, to a lesser extent, to Desulfobacillus toluolica. Neither of these strains is able to demethylate DMSP to MPA. We are reluctant to assign strain WN to the genus Desulfobacter because it has the acetyl coenzyme A-CO dehydrogenase pathway, which is characteristic for Desulfobacterium species and is absent in Desulfobacter species (42). A similar taxonomic problem was described for a toluene-degrading sulfate-reducing bacterium (27). The new genus Desulfobacillus was created to solve that taxonomic problem. Assignment of strain WN to the genus Desulfobacillus would be improper because of the large difference in moles percent G+C and the different substrate utilization patterns.

Bacteria such as strain WN and Desulfobacterium nacini PM4 probably play a significant role in the demethylation of DMSP in anoxic intertidal sediments, since they are in relatively high abundance and have low Km values for DMSP, which are in the range of the concentrations of DMSP that have been found in marine sediments (16, 35, 38). Our Km values for strain WN, strain PM4, and a DMSP-cleaving Desulfovibrio strain (37) which had a far higher Km value for DMSP (0.4 mM) are in line with the observations made by Kiene and Taylor (17) that at higher DMSP concentrations in sediments the cleavage pathway becomes more important than the demethylation pathway.

Interestingly, the demethylation of DMSP was not restricted to sulfate-reducing bacteria but was also found to occur in a strain of Eubacterium limosum. The demethylation of DMSP by Eubacterium limosum was already suggested by Kiene and Taylor (17) on the basis of its ability to demethylate the DMSP analog glycine betaine (25). It is not clear whether organisms such as Eubacterium limosum PM31 play an important role in the conversion of DMSP in marine intertidal sediments, be-
cause this bacterium might have been a temporary guest in the intertidal sediment, originating from the rumen of sheep (see reference 10), which graze on the dikes adjacent to the intertidal flat. However, the better growth of strain PM31 at higher NaCl concentrations compared with *E. limosum* RF (10, 11) indicates that strain PM31 is better adapted to the marine environment and therefore might be active in such an environment. However, the results shown in Table 1 indicate that sulfate was required for the demethylation of DMSP, which suggests a dominant role of sulfate-reducing bacteria in this process.

The demethylation of glycine betaine by a certain strain does not imply that such a strain is able to demethylate DMSP, as was shown for two salt-tolerant strains of acetogenic bacteria of the genus *Acetobacterium* and for *Desulfo bacterium autotrophicum*. The three DMSP-demethylating species of the genus *Desulfo bacterium* and strain WN utilize the acetyl coenzyme A-CoDH dehydrogenase pathway for the oxidation of their substrate (42); the methyl group of betaine or DMSP is probably transferred by a methyltransferase system to a tetrahydrop terin, yielding methyltetrahydropterin, which is an intermediate in this pathway. Because not all glycine betaine-demethylating strains demethylate DMSP, most probably the methyltransferase system in these strains is specific for only one methyl donor, as is also the case for methanol and trimethylamine conversion by methanogens (1).

A remarkable difference between anaerobes and aerobes in the demethylation of DMSP and betaine is that the anaerobes carry out a single demethylation of the substrate to yield MMPA and dimethylglycine. For aerobic betaine-demethylating bacteria, the dimethylglycine is not an end product but is metabolized further (see, e.g., reference 9). Similarly, MMPA is usually not the end product of DMSP demethylation by aerobes. It is further demethylated to MPA (39) or metabolized in a pathway leading to the formation of methanethiol (9, 32).

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