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Dimethyl Sulfide Production from Dimethylsulfoniopropionate in Coastal Seawater Samples and Bacterial Cultures†

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Dimethyl sulfide (DMS) was produced immediately after the addition of 0.1 to 2 μM β-dimethylsulfoniopropionate (DMSP) to coastal seawater samples. Azide had little effect on the initial rate of DMS production from 0.5 μM added DMSP, but decreased the rate of production after 6 h. Filtration of water samples through membrane filters (pore size, 0.2 μm) greatly reduced DMS production for approximately 10 h, after which time DMS production resumed at a high rate. Autoclaving completely eliminated the production of DMS. The antibiotics chloramphenicol, tetracycline, kanamycin, and vancomycin all had little effect on the accumulation of DMS over the first few hours of incubation, but produced significant inhibition thereafter. The effects of individual antibiotics were additive. Chloroform over a range of concentrations (0.25 to 1.25 mM) had no effects on DMS production. Similarly, organic amendments, including acrylate, glucose, protein, and starch, did not affect DMS accumulation from DMSP. Acrylate, a product of the enzymatic cleavage of DMSP, was metabolized in seawater samples, and two strains of bacteria were isolated with this compound as the growth substrate. These bacteria produced DMS from DMSP. The sensitivity to inhibitors with respect to growth and DMSP-lyase activity varied from strain to strain. These results illustrate the significant potential for microbial conversion of dissolved DMSP to DMS in coastal seawater.

Biogenic sulfur emissions from the land and seas play an important role in the global sulfur cycle. Natural emissions contribute to the acidity of precipitation (3, 22) and may play a role in marine cloud formation and climate regulation (2, 8, 34). On a global scale, the oceans contribute about 50% of the biogenic sulfur input to the atmosphere; approximately 90% of the oceanic sulfur emission is in the form of dimethyl sulfide (DMS) (1). Since the flux of DMS from the oceans is highly dependent on the concentration of DMS in surface seawater, it is critical that we understand the dynamics of the DMS pool.

DMS may be produced by degradation of a variety of organosulfur compounds (19, 29), but in the ocean it appears to be derived mainly from β-dimethylsulfoniopropionate (DMSP), an osmolyte produced by some marine plants. Dacey and Wakeham (10) have shown that disturbance of algal cells, such as through grazing by zooplankton, greatly enhances DMS production. It is likely that disruption of algal cells, either through physical means or by digestion in the guts of grazers, releases DMSP and DMS (10, 32). Dissolved DMSP concentrations of 3 to 200 nM have recently been measured in coastal and oceanic waters (15, 32, 33). Dissolved DMSP concentrations are often 2 to 10 times higher than DMS concentrations; therefore, decomposition of dissolved DMSP by microorganisms could be a major pathway for the formation of DMS in seawater.

Dacey and Blough (9) recently isolated a bacterium which produced DMS during aerobic growth on DMSP. Their isolate also grew on acrylate, which is a product of the enzymatic cleavage of DMSP (6). Thus, certain bacteria may cleave DMSP to DMS and acrylate, with the acrylate being used as a substrate for energy and growth, and the DMS released as a by-product.

The potential importance of microbial processes in DMS formation seems clear. However, very little is known about the mechanisms of DMSP degradation and DMS production in natural seawater. The present study examined the production of DMS from exogenous DMSP in coastal seawater samples. Since inhibitor experiments may be useful in elucidating the complex interactions of various microbial groups involved in biogeochemical cycling (5, 23), the effects of several chemical and physical treatments on this process were also studied. In addition, various biochemical inhibitors were tested for their effectiveness against DMSP lyase activity in two strains of acrylate-grown bacteria isolated from coastal water.

MATERIALS AND METHODS

Sampling site. Water samples were collected in summer 1989 from Marsh Landing Dock, near the mouth of the Duplin River, Sapelo Island, Ga. The Duplin River is a large tidal creek with salinities generally around 25 ‰. The Duplin River and its surrounding marshes have been described in detail elsewhere (25).

Sample processing and experimental design. Water was collected in 1-liter polycarbonate bottles and immediately returned to the laboratory, where it was dispensed in 50-ml portions to 70-ml serum bottles (Wheaton). Additions, if any, were made to the bottles by pipette or syringe, and the bottles were then sealed with Teflon-faced septa and an aluminum crimp. Samples were incubated in the dark at 30°C with gentle shaking (75 rpm, 3-cm orbit).

Each experiment was conducted on a different day; therefore, some variation in the microbial populations was expected. For this reason, experimental treatments were always evaluated relative to an uninhibited treatment. Treatments were run in duplicate or triplicate, and standard errors (for triplicates) or ranges (for duplicates) were generally less than 10%.

Controls (inhibitor or manipulation without DMSP additions) were always prepared with treatments, but in no case did these produce significant DMS when compared with samples which received DMSP additions. Autoclaved sam-

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ples were cooled to 30°C before the addition of DMSP, since heating decomposes DMSP to DMS and acrylic acid. Antibiotics were added to a final concentration of 2.5 mg · ml^{-1}, except for tetracycline, which was added at 1.25 mg · ml^{-1}. Azide was added to a final concentration of 0.25% (wt/vol) and chloroform was used over a range from 0.25 to 1.25 mM. Glutaraldehyde and formaldehyde were not used since they interfere with analysis of DMS by gas chromatography.

Bacterial isolates. Minimal medium containing 3.5 mM acrylic acid as the sole carbon substrate was used to enrich for and isolate bacteria capable of growth on acrylate. The medium contained, in grams per liter of distilled water: NaCl, 28; Na_2SO_4, 2.4; $MgSO_4$, 2.0; $CaCl_2$, 0.76; NH_4Cl, 3.0; KH_2PO_4, 2.0; and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 4.8. A trace-minerals solution (4 ml/liter of medium [35]) was also added. The pH was adjusted to 7.5 before addition of acrylic acid (250 μl). After addition of the acrylic acid, the medium pH was close to 7.1 and, if needed, was adjusted to this value.

Pure cultures were obtained by several iterations of streaking on agar plates, picking colonies, and transfer to liquid media. The purity of the isolates was checked by examination of colony morphology (on agar plates) and cell morphology (by epifluorescence microscopy). Acrylate-grown isolates were screened for their ability to produce DMS from DMSP. Two distinct strains (based on cell morphology) were used for further studies, which are described below. These bacteria were not identified taxonomically.

Cultures were incubated at 30°C in Balch tubes (150 by 20 mm; Bellco Glass, Vineland, N.J.), which were sealed with Teflon-faced septa. Each tube contained 5 ml of media and received a 0.1-ml inoculum.

Two approaches were used to study the effects of inhibitors on DMS production in these cultures. The first tested for the effects of the inhibitors on growth of cells and the expression of DMSP-lyase activity. In this case inhibitors were added at the time of inoculation, and the cultures were allowed to incubate for 24 h before DMSP (filtered through a 0.2-μm-pore-size membrane; final concentration, 20 μM) was added. The second approach tested the short-term effects of the inhibitors on DMSP-lyase activity in an existing population of growing cells. In this case, cultures were allowed to grow for 24 h, and inhibitors and DMSP were then added simultaneously. The rate of DMS production over a 6-h period following DMSP addition was used for comparisons.

Analytical determinations. DMS in the headspace of the bottles or tubes was measured by gas chromatography. A gastight syringe was used to withdraw 100 μl of headspace gas, which was then injected into a Shimadzu GC-9A equipped with a flame photometric detector. Gas chromatography conditions were as follows: oven temperature, 100°C; carrier gas, He at 60 ml/min; Teflon column (2 m long by 1/8 in. [1 in. = 2.54 cm] o.d.) filled with Carbopack BHT 100 (Supelco, Bellefonte, Pa.). Peak areas were recorded on a Shimadzu CR-6A integrator. Standards were prepared by using solutions of DMSP, which were quantitatively hydrolyzed to DMS by treatment with NaOH. Concentrations of DMS in solution were calculated from headspace concentrations by using published solubility data (11, 26). Detection limits for DMS by the headspace method were 10 to 30 nM depending on the experiment.

Acrylate was measured by ion chromatography on an Interaction ORH-801 polymeric cation-exchange column. The column was maintained at 60°C, and the eluant was 0.05 M phosphoric acid at a flow rate of 0.7 ml · min^{-1}. Acrylate was detected by A_{200}. The detection limit for acrylate in seawater was 2 μM for a 20-μl injection.

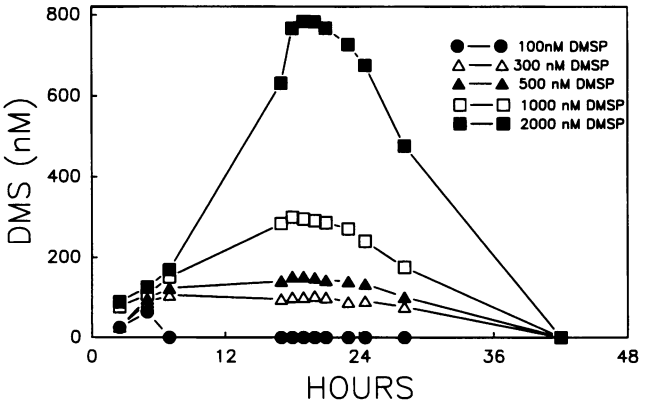

FIG. 1. Accumulation of DMS in coastal seawater samples after the addition of various concentrations of dissolved DMSP. Values below the detection limit (20 nM) are plotted as zero.

Reagents and chemicals. DMSP hydrochloride was obtained from Research Plus Inc., Bayonne, N.J. Stock solutions of DMSP were prepared in dilute HCl (pH 2.5), kept frozen during storage, and thawed only during use. DMSP hydrochloride solutions, ranging from 1 μM to 10 mM, could be kept frozen without significant deterioration for several months. Antibiotics were obtained from Sigma, and all other chemicals were of the highest purity available.

RESULTS

To choose a DMSP addition level which would be used for subsequent experiments, a preliminary experiment was conducted with a range of additions from 0.1 to 2 μM. The time course of this experiment (Fig. 1) showed that DMS was produced immediately after addition of DMSP and was easily detectable for several hours with all levels of addition. However, in the 0.1 μM DMSP addition, DMS levels quickly fell below the 30 nM detection limit. This was due to biological consumption of the DMS (R. P. Kiene, unpublished data). Higher additions of DMSP gave greater DMS accumulation rates and higher maximum accumulations. On the basis of these results, a level of 0.5 μM was chosen for further experiments, since it ensured adequate detectability of DMS during early phases (<12 h) of the incubations. This concentration of dissolved DMSP is about 3 to 50 times the concentrations observed in the Duplin River (Kiene, unpublished).

Figure 2 shows the effects of 0.25% azide, 0.2-μm-pore-size filtration, and autoclaving on the production of DMS from 0.5 μM DMSP. Azide stimulated DMS production slightly over the initial 4 h compared with the uninhibited treatment. Thereafter, DMS accumulated more slowly and did not decline as it did in the absence of the inhibitor. In water filtered through 0.2-μm membrane filters, DMS production occurred very slowly over the first 9 h but then accelerated, and DMS reached levels approximately equal to those obtained in the uninhibited and azide treatments. The DMS level did not decline in the filtered water over the period shown. However, in prolonged incubations (>30 h), DMS consumption occurred. No significant DMS production was observed in autoclaved samples, and DMS concentrations remained steady at approximately 45 nM. This level

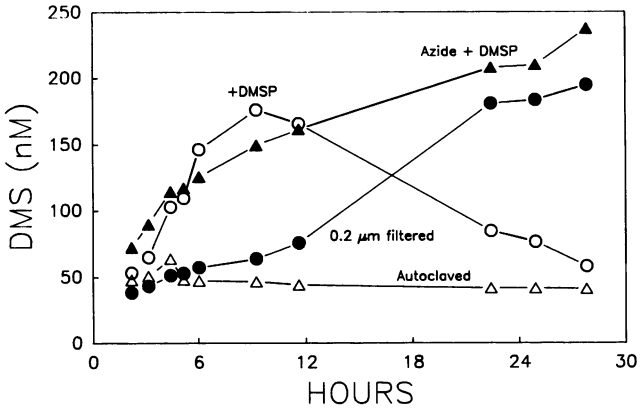

FIG. 2. Effects of azide, 0.2-μm-pore-size filtration, autoclaving, and no treatment on DMS production from 0.5 μM DMSP in coastal seawater samples.

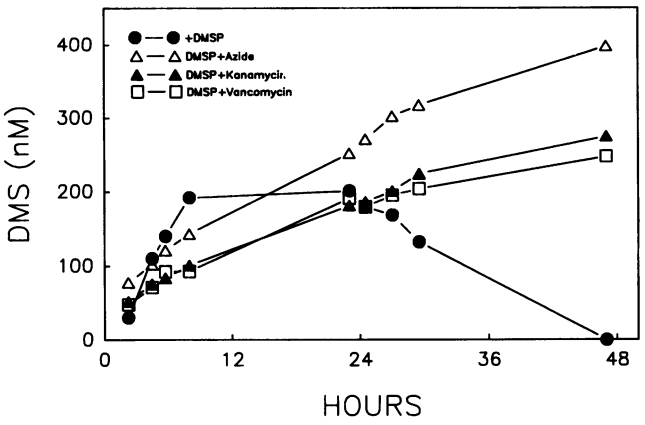

FIG. 4. Effects of azide, kanamycin, vancomycin, and no treatment on DMSP production from 0.5 μM DMSP in coastal seawater samples.

of DMS was also seen in autoclaved but unspiked samples (data not shown) and probably originated from the breakdown of endogenous DMSP during autoclaving.

Several antibiotics were tested for their effects on DMS production. Chloramphenicol and tetracycline (Fig. 3) had similar effects, causing little inhibition of DMS production over a 4-h period and a much reduced rate of production thereafter. When these antibiotics were added in combination (CAP/TET), the initial inhibition of DMS production was considerably stronger, but DMS continued to be produced at a rate equivalent to that seen with the individual antibiotics.

Kanamycin and vancomycin had little or no effect on DMS production over the first few hours of incubation (Fig. 4), but then DMS production slowed (compared with the uninhibited sample) and remained steady. Azide was included in this experiment and gave results identical to a previous experiment (Fig. 2). Again, no decrease in DMS was observed in the presence of chemical inhibitors.

Chloroform was tested at several concentrations ranging from 0.25 to 1.25 mM (Fig. 5) and showed no significant effects on the accumulation of DMS. Consumption of DMS was, however, prevented by chloroform. Additional experiments (data not shown) were carried out with additions of acrylate (0.5 to 5.0 μM), soluble starch, protein (albumin), and glucose (each at 2 mg/ml); no significant enhancement or inhibition of DMS production from added DMSP was observed.

Acrylate (90 μM addition) was consumed by biological reactions in coastal-water samples (Fig. 6), and bacteria capable of growth on acrylate were easily isolated from these enrichments. A large percentage of these isolates were capable of producing DMS from DMSP, indicating the presence of DMSP-lyase. Therefore, the effects of inhibitors were tested on two of these acrylate-grown strains (2B-2 and 6B-2) (Table 1). When inhibitors were added at the time of inoculation, all except chloroform prevented growth and expression of significant DMSP-lyase activity in strain 2B-2. Growth in the presence of chloroform was comparable to that in the uninhibited cultures, and DMSP-lyase activity was reduced by only 20%. Similar results were found with strain 6B-2, except that no inhibition of growth or DMSP-lyase activity was seen with chloroform or vancomycin.

When inhibitors and DMSP were simultaneously added to growing cultures, the results were somewhat different. In this case significant amounts of DMS were produced in all inoculated cultures (Table 1). With strain 2B-2, vancomycin had no inhibitory effects but kanamycin was strongly inhibitory (38% of control). CAP/TET, $CHCl_3$, and azide each

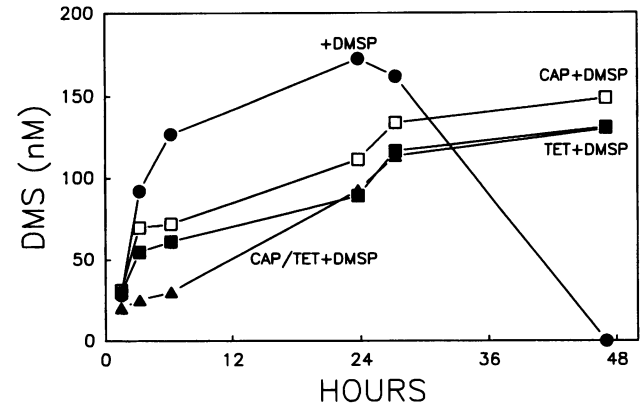

FIG. 3. Effects of chloramphenicol, tetracycline, CAP/TET, and no treatment on DMSP production from 0.5 μM DMSP in coastal seawater samples.

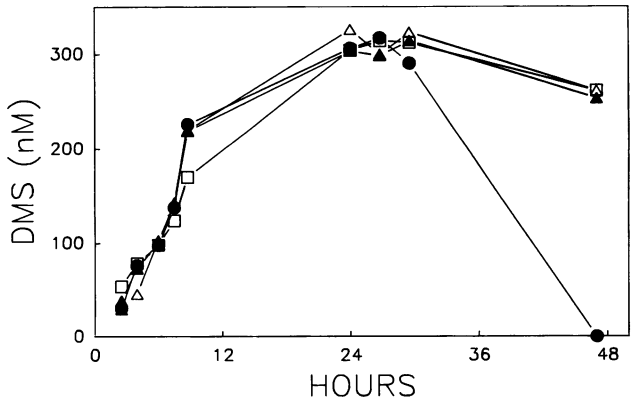

FIG. 5. Effect of several different concentrations of chloroform on DMS production from 0.5 μM DMSP in coastal seawater samples. Symbols: ●, no treatment; △, 250 μM $CHCl_3$; ▲, 500 μM $CHCl_3$; □, 1,250 μM $CHCl_3$.

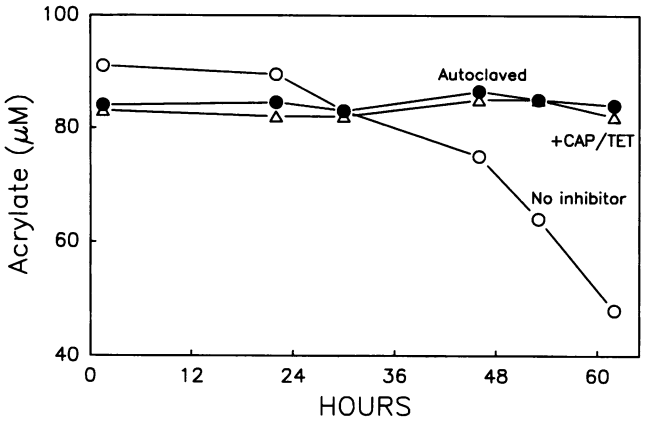

FIG. 6. Time course of acrylate concentration in seawater samples amended with acrylate. The samples were either untreated, treated with CAP/TET, or autoclaved.

gave moderate inhibition (84, 65, and 73% of control, respectively). In strain 6B-2, CAP/TET and azide proved strongly inhibitory (10.8 and 7.1% of control, respectively), whereas the other treatments had no effects.

DISCUSSION

The production of DMS from DMSP occurs via an elimination reaction which can be catalyzed by OH^- or by an enzyme according to the following equation (6, 7, 16, 31): $(CH_3)_2S^+CH_2CH_2COO^- \rightarrow CH_2{=}CHCOO^- + (CH_3)_2S + H^+$. Chemical cleavage of DMSP in seawater (pH 8.2) is very slow, yielding a half-life on the order of years (9). Therefore, production of DMS from dissolved DMSP is due primarily to enzymatic activity. An enzyme responsible for this reaction has been partially purified from the marine unicellular alga *Gyrodinium cohnii* (14, 16) and the macroalga *Polysiphonia lanosa* (6) and has the characteristics of a lyase (see reference 29 for a review). DMSP-lyase activity appears to be constitutive in coastal marine waters and in some bacteria isolated from these waters. Results from the present study indicate that DMSP-lyase activity was resilient to the addition of chemical inhibitors, antibiotics, and organic substrates. The only procedure which eliminated the activity was autoclaving (Fig. 1). Similarly, Kiene and Visscher (21) found that DMS production from DMSP in salt marsh sediments was very resistant to chemical inhibitors but was prevented by autoclaving. The production of DMS without a lag following DMSP addition and the general ineffectiveness of inhibitors suggest that there is a high potential for rapid turnover of dissolved DMSP in coastal seawater.

TABLE 1. Effects of inhibitors on the rate of DMS production from DMSP by two strains of bacteria grown on 3.5 mM acrylate in minimal media

Strain	Treatment[a]	Initial rate of DMS production (% of rate in uninhibited culture) with:	
		Inhibitor added at time of inoculation	Inhibitor added after growth and at same time as DMSP
2B-2	No inhibitor	100	100
	Uninoculated	0	0
	Vancomycin	1.3	105
	Kanamycin	0.3	38
	CAP/TET	0.9	84
	$CHCl_3$	80	65
	Azide	1	73
6B-2	No inhibitor	100	100
	Uninoculated	0	0
	Vancomycin	101	105
	Kanamycin	0.2	99.7
	CAP/TET	0.5	10.8
	$CHCl_3$	110	107
	Azide	0.1	7.1

[a] All cultures received filter-sterilized DMSP (final concentration, 20 μM) approximately 24 h after inoculation.

Removal of microorganisms by filtration greatly diminished the production of DMS (Fig. 1), but after several hours the activity resumed. Even when the 0.2-μm-filtered water was handled aseptically, similar results were observed (data not shown). This was probably due to the regrowth of bacteria in the filtered water. These microorganisms may have originated from the few cells which pass through the filter, or they could have been introduced with the gas-sampling needle. In any event, filtration significantly reduced the production of DMS for several hours, but did not eliminate it. Turner et al. (30) also found that DMS continued to be produced in seawater which was filtered through 0.2-μm filters, and this occurred without a lag as observed here. This leaves open the possibility that soluble DMSP-lyase enzymes are present in seawater, either naturally or as a result of cell rupture caused by filtration.

The antibiotics used here (chloramphenicol, tetracycline, kanamycin, and vancomycin) are considered to be relatively broad-spectrum inhibitors of microbial growth and activity. Each exhibited some inhibitory effects on DMS production (Fig. 3 and 4), although this was not always evident until several hours after addition of DMSP. It is not known whether the antibiotics had a direct effect on the enzyme responsible for DMSP degradation or whether inhibition resulted from a general slowing of microbial activity and possible cell lysis. Recently, we have found that 10 μM levels of either *p*-chloromercurobenzoate, iodoacetamide, or KCN did not inhibit DMS production from DMSP in seawater (R. P. Kiene and S. K. Service, unpublished results). These compounds were potent inhibitors of enzymatic DMS production in cell extracts of *Gyrodinium cohnii* (14, 16). Their ineffectiveness in seawater further suggests that DMSP-lyase activity in natural microbial assembleges is very resistant to chemical toxins.

When two antibiotics were added in combination (CAP/TET), the inhibition was greater than with each alone (Fig. 3). These findings could be explained by differential sensitivities of individual organisms to each of these inhibitors. This suggestion is supported by the results from experiments with the two bacterial isolates (Table 1), since the strains were affected differently by each of the inhibitors used. A single inhibitor may strongly affect one organism while leaving another unaffected, thereby resulting in partial inhibition with the entire population.

Azide, a strong inhibitor of respiration at the site of cytochrome oxidase, gave results similar to those of the antibiotics, with little effect on DMS production from DMSP over the first few hours of incubation followed by a period of slower production. This indicated that DMSP-lyase activity was not directly linked to respiration. The decrease in activity over time in the presence of azide may have been due to the death of microorganisms or to decreased ability of cells to actively transport DMSP. Preliminary results with several of the acrylate-grown bacteria indicate that DMSP is

not broken down after exhaustion of the acrylate in the media, but that the activity resumes upon addition of more acrylate. This is suggestive of an energy-dependent transport of DMSP to the site of the enzyme. It is known that in *Escherichia coli*, transport of glycine betaine, a structural analog of DMSP, requires ATP (24). DMSP could have a similar requirement.

The increasing effectiveness with time of the antibiotics and azide suggests that a period of preincubation might yield even greater inhibition. Unfortunately, the usefulness of preincubating samples with inhibitors in studies of endogenous DMS cycling may be limited. This is because significant changes in the DMSP and DMS pools occur over relatively short periods during bottle incubations (Kiene, submitted). In this regard, it is important to note that most of the inhibitors used here, when added on their own, actually do affect the endogenous DMS pool in seawater (18). These effects could not be seen in the present study because of the relatively high detection limits of the headspace analysis. Results from experiments on the effects of inhibitors on endogenous levels of DMS will be presented elsewhere (18; Kiene, submitted) and are consistent with the findings of the present study.

Chloroform had little or no effect on DMS production from DMSP, either in seawater samples (Fig. 5) or in bacterial cultures (Table 1). This finding could have some significance, since chloroform inhibits C_1 metabolism (4) and strongly inhibits DMS consumption in seawater (18) (Fig. 5). Thus, it appears that chloroform selectively inhibits DMS consumption but not its production from DMSP.

The microorganisms which degrade DMSP in seawater are not known. Bacteria such as those isolated here probably play a role, but the involvement of other heterotrophic microorganisms cannot be ruled out. Experiments with size-fractionated water samples may provide information about whether DMSP-lyase activity resides primarily in free-living bacteria or in larger size classes, which could include flagellates, ciliates, yeasts, algae, and attached bacteria.

DMSP-lyase activity may be linked to the use of acrylate as a substrate by microorganisms (9, 29, 31; see above). The consumption of acrylate in water samples and in bacterial cultures is in apparent contrast to earlier studies which reported antibiotic properties (12, 27). However, Sieburth (27) found significant inhibitory effects only at high (>125 μM) acrylate concentrations and at low pH. At submillimolar concentrations, acrylate and DMSP appear to be readily metabolized. Anaerobic fermentation of acrylate is known to occur (20, 31); however, little is known about aerobic acrylate metabolism.

The decline in the DMS level seen in all uninhibited samples was due to biological consumption. This is probably due to the activity of bacteria which are different from those producing DMS from DMSP, since the DMSP-degrading isolates did not consume DMS (Kiene, unpublished). DMS could have been consumed by methylotrophic organisms, possibly *Hyphomicrobium* spp. (13, 28), or by chemolithotrophs such as *Thiobacillus* spp. (17). In general, it appeared that DMS consumption was more sensitive to the addition of chemical inhibitors than its production was. This could explain why DMS accumulated to higher levels in the presence of some inhibitors (Fig. 2 and 4). However, the consumption of DMS was not the subject of this study, and it may be inappropriate to compare the sensitivities of these two processes. Nonetheless, microbial consumption of DMS is an important aspect of DMS biogeochemistry in seawater (18, 30).

In summary, the enzymatic production of DMS from DMSP is a constitutive property of coastal water samples. This activity is either unaffected or only partially inhibited by antibiotics and poisons during incubations of <24 h. Sterilization by autoclaving was the only procedure tested which eliminated DMS production from DMSP, although 0.2-μm filtration effectively decreased DMS production for several hours. The results presented here indicate that the potential for turnover of dissolved DMSP is high. Therefore, the decomposition of dissolved DMSP is likely to be a major mechanism for the formation of DMS in the sea. Additions of 500 nM DMSP yielded initial DMS accumulation rates of 22 to 36 nmol · liter^{-1} · h^{-1}. These rates are higher than the 8.2 nmol · liter^{-1} · h^{-1} accumulation resulting from zooplankton grazing on DMSP-containing algae (particulate DMSP concentration, ca. 340 nM) (10). Thus, if dissolved DMSP is released during grazing on phytoplankton, degradation of this pool could account for the observed increases in DMS production.

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