

## Identification and enumeration of bacteria assimilating dimethylsulfoniopropionate (DMSP) in the North Atlantic and Gulf of Mexico

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### Abstract

The algal-derived compound dimethylsulfoniopropionate (DMSP), which is the precursor of the climatically active gas dimethylsulfide, is potentially an important source of carbon and sulfur to marine bacterioplankton. Currently, bacteria of the *Roseobacter* clade, a subgroup of  $\alpha$ -proteobacteria, are hypothesized to be the key participants in the metabolism of DMSP. To test this hypothesis, we used a combination of microautoradiography and fluorescence in situ hybridization (Micro-FISH) to identify the bacteria assimilating <sup>35</sup>S DMSP in the Gulf of Mexico, the Gulf of Maine, and the Sargasso Sea. On average, half of the bacterial community assimilated DMSP in these environments. Members of the  $\alpha$ -proteobacteria dominated DMSP assimilation, accounting for 35–40% of bacteria assimilating DMSP. *Cytophaga*-like bacteria and  $\gamma$ -proteobacteria each accounted for 15–30% of DMSP-assimilating cells. The  $\alpha$ -proteobacteria accounted for a greater fraction of the DMSP-assimilating community than expected based on their overall abundance, whereas *Cytophaga*-like bacteria were typically underrepresented in the DMSP-assimilating community. Members of the *Roseobacter* clade assimilated more DMSP on a per-cell basis than any other group, but they did not account for most of the DMSP assimilation, nor were they always present even when DMSP turnover was high. These results indicate that the biogeochemical flux of dissolved DMSP is mediated by a large and diverse group of heterotrophic bacteria.

The development of culture-independent techniques has greatly increased our knowledge of the phylogenetic composition of natural marine bacterial communities (Giovannoni et al. 1990; Amann et al. 1995), yet an understanding of the role of specific bacterial groups in biogeochemical cycles remains incomplete. Typically the phylogenetic composition of marine bacterial communities is not considered in models of marine biogeochemical cycling, implying that all bacteria have equal metabolic capacities. Previous work, however, indicates that all bacteria are not equal in their capacity to metabolize organic matter. Martinez et al. (1996) found that the capacity to hydrolyze a variety of biopolymers was not uniformly distributed among marine bacterial isolates. In addition, Cottrell and Kirchman (2000) observed differential assimilation of various organic compounds by major bacterial phylogenetic groups in the Delaware Bay. If bacterial phylogenetic groups have different metabolic capacities, then the composition of marine bacterial communities could impact biogeochemical cycling in the oceans.

Interest in the bacteria mediating the biogeochemical cycling of dimethylsulfoniopropionate (DMSP), a precursor of

dimethylsulfide (DMS), has grown since Charlson et al. (1987) proposed that oceanic DMS emissions can impact global temperatures through the formation of sulfur aerosols. DMS is produced primarily by the enzymatic hydrolysis of DMSP (Turner et al. 1988), an algal osmolyte produced in large quantities by several phytoplankton groups, including dinoflagellates, prymnesiophytes, and diatoms (Yoch 2002). Much of the DMSP produced by phytoplankton is released into the dissolved phase through senescence, viral lysis, and zooplankton grazing (Wolfe et al. 1994; Hill et al. 1998). Once released, dissolved DMSP is degraded by microbial communities. However, production of DMS is not the primary fate of dissolved DMSP. Instead, most dissolved DMSP is transformed by bacteria into nonvolatile compounds or incorporated into bacterial biomass (Kiene and Linn 2000b). Dissolved DMSP can satisfy 1–15% of the total bacterial carbon demand and virtually all of the bacterial sulfur demand (Kiene and Linn 2000a; Simó et al. 2002).

Dissolved DMSP may be metabolized primarily by bacteria of the *Roseobacter* clade, an abundant subdivision of the  $\alpha$ -proteobacteria (Giovannoni and Rappé 2000; Zubkov et al. 2002b). The capacity to degrade DMSP is common among *Roseobacter* isolates (González et al. 1999), and 80% of bacteria isolated from DMSP enrichments of coastal seawater were members of the *Roseobacter* clade (González et al. 1999). The *Roseobacter* clade is also abundant during

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### Acknowledgments

We thank Hyakubun Harada, Mary Ann Rouse, and Doris Slezak for measuring concentrations and flux rates of dissolved DMSP. This study was supported by grants from the NSF and DOE-BIOMP. An NDSEG fellowship provided support for R.M.

Table 1. Oligonucleotide probes and formamide concentrations used in fluorescence in situ hybridization.

Probe name	Probe sequence (5'→3')	Target group	Formamide concentration (%)	Reference
Eub338	GCT GCC TCC CGT AGG AGT	Bacteria	30	Amman et al. (1990)
NegControl	CCT AGT GAC GCC GTC GA	Negative control	30	Karner and Fuhrman (1997)
Alf968	GGT AAG GTT CTG CGC GIT	$\alpha$ -proteobacteria	30	Glöckner et al. (1999)
ROS537	CAA CGC TAA CCC CCT CC	<i>Roseobacter</i> clade	35	Eilers et al. (2001)
MALF1	GCC GGG GTT TCT TTA CCA	<i>Roseobacter</i> clade	20	González and Moran (1997)
RSB67	CGC TCC ACC CGA AGG TAG	<i>Roseobacter</i> ribotype	20	Zubkov et al. (2002a)
Bet42a	GCC TTC CCA CTT CGT TT	$\beta$ -proteobacteria	30	Manz et al. (1992)
Gam42a	GCC TTC CCA CAT CGT TT	$\gamma$ -proteobacteria	30	Manz et al. (1992)
Cf319a	TGG TCC GTG TCT CAG TAC	<i>Cytophaga</i> -like	35	Manz et al. (1996)

DMSP-producing algal blooms (González et al. 2000; Zubkov et al. 2002a). However, the number and identity of bacteria metabolizing DMSP, including *Roseobacter*, have not been directly examined in natural marine bacterial communities.

The goal of this study was to identify and quantify the bacteria assimilating DMSP during nonbloom conditions. To accomplish this goal we used Micro-FISH, a combination of microautoradiography and fluorescent in situ hybridization (Lee et al. 1999; Ouverney and Fuhrman 1999; Cottrell and Kirchman 2000), to identify  $^{35}\text{S}$  DMSP-assimilating bacteria. Microbial communities in the Gulf of Mexico, the Gulf of Maine, and the Sargasso Sea were investigated for DMSP assimilation. We expected members of the *Roseobacter* clade to dominate assimilation of DMSP because the capacity to assimilate DMSP is common within the *Roseobacter* clade and they are abundant in marine systems (González and Moran 1997). In contrast, we found that DMSP assimilation was spread over a variety of phylogenetic groups.

## Methods and materials

**Sample collection and incubation**—Surface seawater samples were collected in the Gulf of Mexico (November 2001, RV *Pelican*) and in the Gulf of Maine and Sargasso Sea (April 2002, RV *Oceanus*). Whole seawater (30 ml) was incubated with tracer levels of  $^{35}\text{S}$  DMSP (17–170 Bq ml $^{-1}$ ; specific activity 12–43 TBq mmol $^{-1}$ ) for 24 h in glass serum vials.  $^{35}\text{S}$  DMSP additions were <0.1 nmol L $^{-1}$  and were less than natural DMSP concentrations (1–5 nmol L $^{-1}$ ). Sample vials were placed in flow-through water baths at in situ temperature and surface light conditions (minus ultraviolet). Killed controls were treated with paraformaldehyde (final concentration of 2%) 10 min prior to addition of  $^{35}\text{S}$  DMSP. Live incubations were terminated by the addition of 20% fresh paraformaldehyde (final concentration of 2%). Samples were fixed for 24 h at 4°C before filtration onto 0.2- $\mu\text{m}$  white, polycarbonate filters backed by 0.45- $\mu\text{m}$  nitrocellulose filters. Filters were rinsed twice with 5 ml of deionized, filter-sterilized water and stored at –20°C. Identical incubations were conducted with tracer additions of  $^{35}\text{S}$  DMS to examine possible uptake of  $^{35}\text{S}$  DMS produced from  $^{35}\text{S}$  DMSP.

Bacterial production was measured by incorporation of  $^3\text{H}$

leucine into trichloroacetic acid (TCA)-insoluble material (Kirchman 1993). Triplicate 1.5-ml samples were incubated with 20 nmol L $^{-1}$   $^3\text{H}$  leucine (5.5 TBq mmol $^{-1}$ ) for 1–2 h at in situ temperatures. TCA was added to a final concentration of 5%, and the TCA-insoluble fraction was collected by centrifugation (Smith and Azam 1992). Samples were radioassayed using liquid scintillation counting. Bacterial production was calculated using a conversion factor of 3.1 kg C per mol of assimilated leucine (Kirchman 1993).

**Microautoradiography and fluorescence in situ hybridization**—The abundance of bacterial phylogenetic groups was determined using fluorescence in situ hybridization (FISH) with Cy3-labeled oligonucleotide probes (Amann et al. 1995). Sample filters were hybridized for 18 h at 42°C in hybridization solution containing 0.9 mol L $^{-1}$  NaCl, 20 mmol L $^{-1}$  Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate, 2.5 ng  $\mu\text{l}^{-1}$  of oligonucleotide probe, and 20–35% formamide depending upon the probe (Table 1). After hybridization, samples were washed for 15 min at 48°C in a solution of 20 mmol L $^{-1}$  Tris-HCl (pH 7.4), 5 mmol L $^{-1}$  ethylenediaminetetraacetic acid (EDTA), 0.01% sodium dodecyl sulfate, and the concentration of NaCl corresponding to the formamide concentration used in hybridization. Sample filter pieces were then rinsed in deionized water and air dried.

Microautoradiography preparations followed the method described by Cottrell and Kirchman (2003) with some modifications. Briefly, bacteria were treated with FISH and transferred directly to emulsion coated slides from filter pieces, while bacteria in the previous method were first transferred to a glass cover slip. In this approach, glass slides were coated with autoradiography emulsion by dipping them into LM-1 emulsion (Amersham Biosciences) diluted 50% with deionized water. FISH-treated filter pieces were placed on the coated slides with bacteria in contact with the emulsion. Slides were chilled on an ice-cold aluminum block for 10 min to solidify the emulsion. Slides were then exposed for 2–6 d in the dark at 4°C. After exposure, slides were developed with Dektol developer (Kodak) for 2 min, placed in a stop bath of deionized water for 10 s, fixed with Kodak fixer for 6 min, and washed in deionized water for 6 min. Slides were dried overnight in a vacuum desiccator then stained with 4',6-diamidino-2-phenylindole (DAPI) solution (2 ng  $\mu\text{l}^{-1}$ ) for 2 min, rinsed in 80% ethanol, dipped in 1%

Table 2. Concentration of dissolved DMSP, turnover of dissolved DMSP pool, prokaryote abundance, and percentage of the bacterial community assimilating DMSP in the Gulf of Mexico, Gulf of Maine, Sargasso Sea, and North Carolina (NC) coast. Mean  $\pm$  SE of 30 fields of view. n/d = not determined.

Location	Station	Lat. N	Lon. W	Dissolved DMSP (nmol L <sup>-1</sup> )	Turnover time (h)	Abundance (10 <sup>6</sup> cells ml <sup>-1</sup> )	% cells assimilating DMSP
Gulf of Mexico	3	29°4'	89°45'	5.5	1.7	4.0 $\pm$ 0.70	38 $\pm$ 1.7
Gulf of Mexico	4	29°27'	86°51'	5.1	16.7	1.0 $\pm$ 0.25	38 $\pm$ 2.1
Gulf of Mexico	6	27°55'	83°41'	4.5	5.2	1.1 $\pm$ 0.33	51 $\pm$ 1.7
Gulf of Mexico	7	29°70'	87°23'	2.9	n/d	1.0 $\pm$ 0.31	58 $\pm$ 3.0
Gulf of Maine	5	41°41'	68°25'	1.4	6.2	1.4 $\pm$ 0.48	56 $\pm$ 1.2
Gulf of Maine	6	38°13'	68°60'	4.4	2.3	0.9 $\pm$ 0.36	57 $\pm$ 1.5
Sargasso Sea	8	34°47'	68°59'	3.2	12.8	0.4 $\pm$ 0.24	32 $\pm$ 3.3
Sargasso Sea	12	31°30'	68°60'	1.3	28.4	0.5 $\pm$ 0.10	51 $\pm$ 1.6
Sargasso Sea	14	33°33'	74°28'	2.5	11.2	1.0 $\pm$ 0.25	49 $\pm$ 1.1
NC coast	15	34°14'	76°56'	1.9	5.1	1.8 $\pm$ 0.61	61 $\pm$ 1.9

glycerol for 1 min, and stored overnight in a vacuum desiccator. After drying, the filter was peeled away, leaving bacteria attached to the slide. The slide was mounted with a 4:1 mixture of Citifluor (Ted Pella) and Vectashield (Vector Labs) before analysis.

Sample slides were examined with a semiautomated microscopy and image analysis system described previously (Cottrell and Kirchman 2003). Briefly, images of DAPI fluorescence, Cy3 fluorescence, and of silver grain clusters formed during microautoradiography were acquired for each field of view and the images overlaid. Objects with overlapping signals in both the DAPI and Cy3 images were counted as probe positive. Silver grain clusters associated with DAPI-positive bacteria indicated cell-specific uptake of <sup>35</sup>S DMSP. Probe-positive and substrate assimilating cells were enumerated during image analysis, and the area of silver grains associated with cells was measured. Cell volumes were calculated during image analysis of DAPI fluorescence using a biovolume algorithm introduced by Sieracki et al. (1989). Cell volume data was log transformed for statistical analysis.

**DMSP concentrations and flux**—Water samples for dissolved DMSP analysis were filtered by gravity through 47-mm diameter Whatman GF/F filters. Ten milliliters of filtrate were acidified with 50  $\mu$ l of 9 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> and the sample stored for >10 h before analysis. Acidification to pH <2 preserved the DMSP and removed any DMS in the samples. DMSP was measured in 1-ml subsamples after conversion to DMS with 1 ml of 5 mol L<sup>-1</sup> NaOH. DMS analysis was by the headspace sweeping method as described previously (Kiene and Gerard 1994).

Dissolved DMSP flux was determined by incubating 30-ml water samples in the dark with tracer levels of <sup>35</sup>S DMSP. At each of four time points over 3 h, 4-ml subsamples were removed, acidified, and stored for >10 h to preserve <sup>35</sup>S DMSP and to remove volatile <sup>35</sup>S species. The <sup>35</sup>S DMSP remaining in each stored subsample was determined by treating the sample with NaOH in a sealed bottle and trapping the volatile <sup>35</sup>S DMS in 3% H<sub>2</sub>O<sub>2</sub>-soaked wicks suspended in well cups (Kiene and Linn 2000b). Rate constants were calculated from the exponential loss of <sup>35</sup>S DMSP with time.

DMSP flux was calculated as the product of the dissolved DMSP concentration and the loss rate constant (Kiene 1996).

**<sup>35</sup>S labeling of DMSP**—<sup>35</sup>S DMSP was synthesized chemically from <sup>35</sup>S L-methionine (43 TBq mmol<sup>-1</sup>; Perkin-Elmer-Amersham, NEG 009H) using L-amino acid oxidase (Sigma, A-9378) to form <sup>35</sup>S 3-methylpropionate, followed by methylation of the methyl group with methanol under acidic conditions. The resulting <sup>35</sup>S DMSP was purified by solid phase extraction (Dowex-50, H<sup>+</sup>) followed by cation exchange high-performance liquid chromatography. Radiochemical purity was >98%. Details of the synthesis and purification will be presented elsewhere. <sup>35</sup>S DMSP stock solutions were stored in 0.1 mol L<sup>-1</sup> HCl, and, if necessary to avoid significant changes in sample pH, the isotope solution was evaporated to dryness to remove HCl and reconstituted in pure water before addition to seawater.

## Results

Concentrations of dissolved DMSP ranged from 1.3 to 5.5 nmol L<sup>-1</sup> at all stations (Table 2). Although small, the dissolved DMSP pool turned over rapidly, with the turnover time averaging 10 h across all stations. Turnover times, however, varied greatly among environments, ranging from about 2 h in the Gulf of Maine to 28 h in the Sargasso Sea. The flux of DMSP through the dissolved pool was linearly correlated with bacterial production ( $r = 0.91$ ;  $p < 0.001$ ) (Fig. 1).

Large numbers of bacteria participated in the turnover of the dissolved DMSP pool (Table 2). On average, 48% of the prokaryotic community assimilated DMSP, with the fraction of the total community assimilating DMSP ranging from 32% to 61% among environments (Table 2). Unlike bacterial production, there was no significant linear relationship between the flux of dissolved DMSP and the number of cells assimilating DMSP (data not shown;  $r = 0.65$ ;  $p > 0.05$ ), implying that per-cell metabolism of DMSP varied greatly among environments.

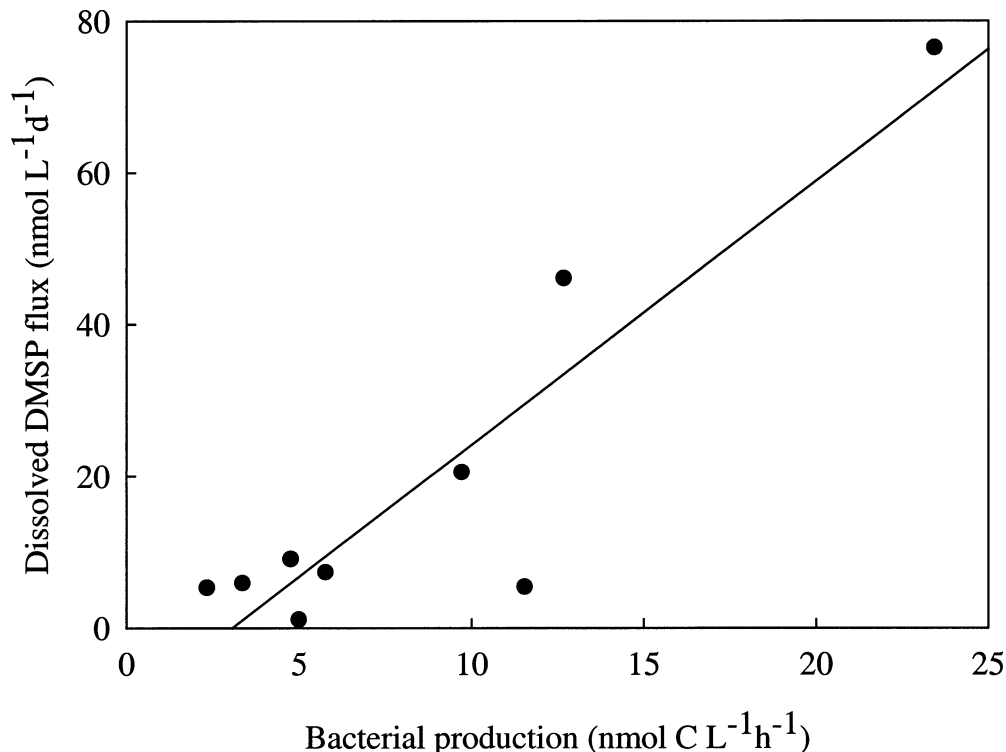


Fig. 1. Dissolved DMSP flux versus bacterial production in the Gulf of Mexico, Gulf of Maine, and Sargasso Sea ( $r = 0.90$ ;  $p < 0.01$ ).

### Composition of DMSP-assimilating community

All assayed phylogenetic groups assimilated DMSP (Fig. 2). The  $\alpha$ -proteobacteria group was the largest identified fraction of the DMSP-assimilating community in the Gulf of Maine, comprising 32% and 40% of this community at Stas. 5 and 6, respectively. Likewise,  $\alpha$ -proteobacteria accounted for approximately 40% of the DMSP-assimilating community in the Sargasso Sea, twice the percentage of the second most abundant group, the *Cytophaga*-like bacteria. In the Gulf of Maine, the  $\gamma$ -proteobacteria were the second most abundant assimilators of DMSP, comprising 27% of the DMSP-assimilating community, while about 16% of cells assimilating DMSP in the Gulf of Maine were *Cytophaga*-like. The  $\beta$ -proteobacteria were not abundant and accounted for 10% or less of the DMSP-assimilating community at all stations. Most of the cells assimilating DMSP were identified by the four major phylogenetic group probes, ranging from 73% identified at Sta. 12 in the Sargasso Sea to 91% at Sta. 6 in the Gulf of Maine.

The composition of the DMSP-assimilating community generally resembled the composition of the total bacterial community (Fig. 3). Some phylogenetic groups, however, comprised a greater fraction of the DMSP-assimilating community than predicted based on their overall abundance. The  $\alpha$ -proteobacteria were overrepresented in the DMSP-assimilating community at all stations (paired  $t$ -test;  $p < 0.05$ ). For example,  $\alpha$ -proteobacteria were approximately 24% of the total community in the Sargasso Sea but comprised approximately 40% of the DMSP-assimilating community. In contrast, *Cytophaga*-like bacteria were slightly underrepre-

sented in three of four stations (paired  $t$ -test;  $p < 0.05$ ). For example, *Cytophaga*-like bacteria were 21% of the total community in the Gulf of Maine, but only composed approximately 16% of the DMSP-assimilating community. Members of the  $\gamma$ -proteobacteria were not significantly overrepresented in the DMSP-assimilating community. Cells that did not hybridize with any phylogenetic group probe were classified as unidentified; these unidentified cells were always underrepresented in the DMSP-assimilating community.

The contribution of bacterial groups to DMSP assimilation was also examined using the surface area of silver grain clusters associated with the phylogenetic groups (Cottrell and Kirchman 2003). There were large differences in the area of silver grain clusters associated with individual DMSP-assimilating bacteria, indicating variation in per-cell assimilation (data not shown). Single-cell variation, however, did not change trends observed at the major phylogenetic group level. There was a strong linear relationship (model II geometric mean regression;  $r^2 = 0.84$ ;  $p < 0.001$ ) between the percentage of the total silver grain area associated with a group and the abundance of that group in the DMSP-assimilating community (Fig. 4). The slope of this relationship ( $1.18 \pm 0.24$ ; 95% confidence interval) was not statistically significantly different from one ( $t$ -test,  $p < 0.05$ ), indicating that the fraction of silver grain area associated with a phylogenetic group, i.e., the fraction of DMSP assimilated by the group, was approximately equal to the fraction of the DMSP-assimilating community accounted for by the group. These data support our previous conclusion that the  $\alpha$ -proteobacteria dominated DMSP assimilation, although all as-

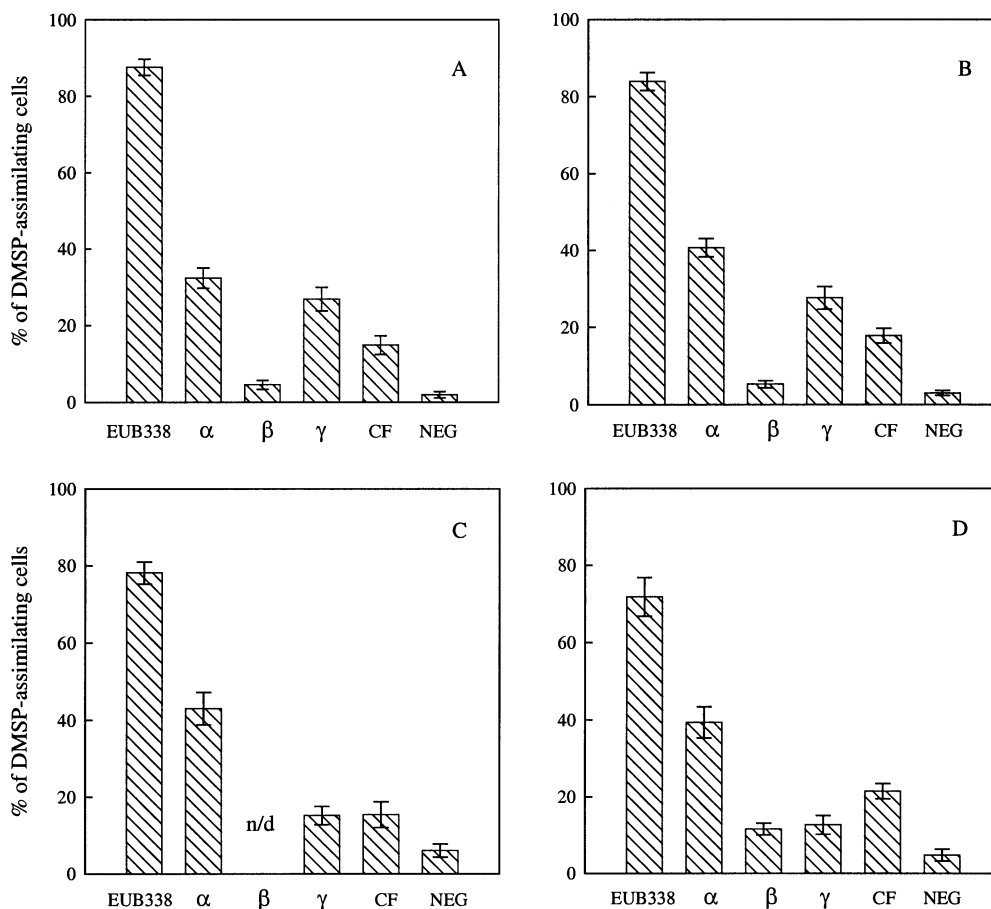


Fig. 2. Composition of the DMSP-assimilating community at the (A) Gulf of Maine Sta. 5, (B) Gulf of Maine Sta. 6, (C) Sargasso Sea Sta. 12, and (D) Sargasso Sea Sta. 14. Abundances of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -proteobacteria, *Cytophaga*-like bacteria (CF), and cells hybridizing with a general bacterial probe (EUB338) and negative control probe (NEG). Error bars represent the standard error of 30 fields of view. n/d = not determined.

sayed phylogenetic groups assimilated significant amounts of DMSP.

#### DMSP assimilation by *Roseobacter*

Members of the *Roseobacter* clade, a subgroup of the  $\alpha$ -proteobacteria, were detected at both stations in the Gulf of Maine but not in the Sargasso Sea (abundance <5% of total). *Roseobacter* cells, identified by the redundant probes MALF1 and ROS537, represented 6% to 9% of the total community in the Gulf of Maine (Fig. 5). A single ribotype of *Roseobacter*, recognized by the probe RSB67, accounted for all members of the *Roseobacter* clade at Sta. 5 and approximately half of the members at Sta. 6. *Roseobacter* also comprised 30% of the  $\alpha$ -proteobacteria in the Gulf of Mexico.

Approximately 85% of *Roseobacter* cells assimilated DMSP in the Gulf of Maine (Table 3). Since almost all *Roseobacter* cells incorporated DMSP, the *Roseobacter* clade was overrepresented in the DMSP-assimilating community. Members of the *Roseobacter* clade accounted for 9% and 12% of the cells assimilating DMSP at Stas. 5 and 6, respectively (Fig. 5). In addition, the fraction of total silver

grain area associated with the *Roseobacter* clade was large, ranging from 19% to 23% (Table 3). The fraction of DMSP assimilated by the *Roseobacter* clade, as measured by silver grain area, was more than twofold higher than expected based on their percentage of the DMSP-assimilating community.

#### Cell volumes of DMSP-assimilating bacteria

Cells incorporating DMSP (DMSP active) were significantly larger than cells that did not incorporate DMSP (DMSP inactive) at all stations (paired *t*-test;  $p < 0.001$ ) (Table 4). The average cell volume of DMSP-active cells ranged from  $0.041 \mu\text{m}^3$  at Sta. 3 in the Gulf of Mexico to  $0.060 \mu\text{m}^3$  at Sta. 8 in the Sargasso Sea. In contrast, DMSP-inactive cells ranged from  $0.032 \mu\text{m}^3$  at Sta. 3 to  $0.041 \mu\text{m}^3$  at Sta. 8. *Roseobacter* cells assimilating DMSP in the Gulf of Maine had an average volume of  $0.07\text{--}0.08 \mu\text{m}^3$  (Fig. 6). On average, DMSP-active cells were 37% larger than the DMSP-inactive cells across all stations. In addition, DMSP-active *Roseobacter* cells were 32–71% larger than the DMSP-active cells of the entire bacterial community.

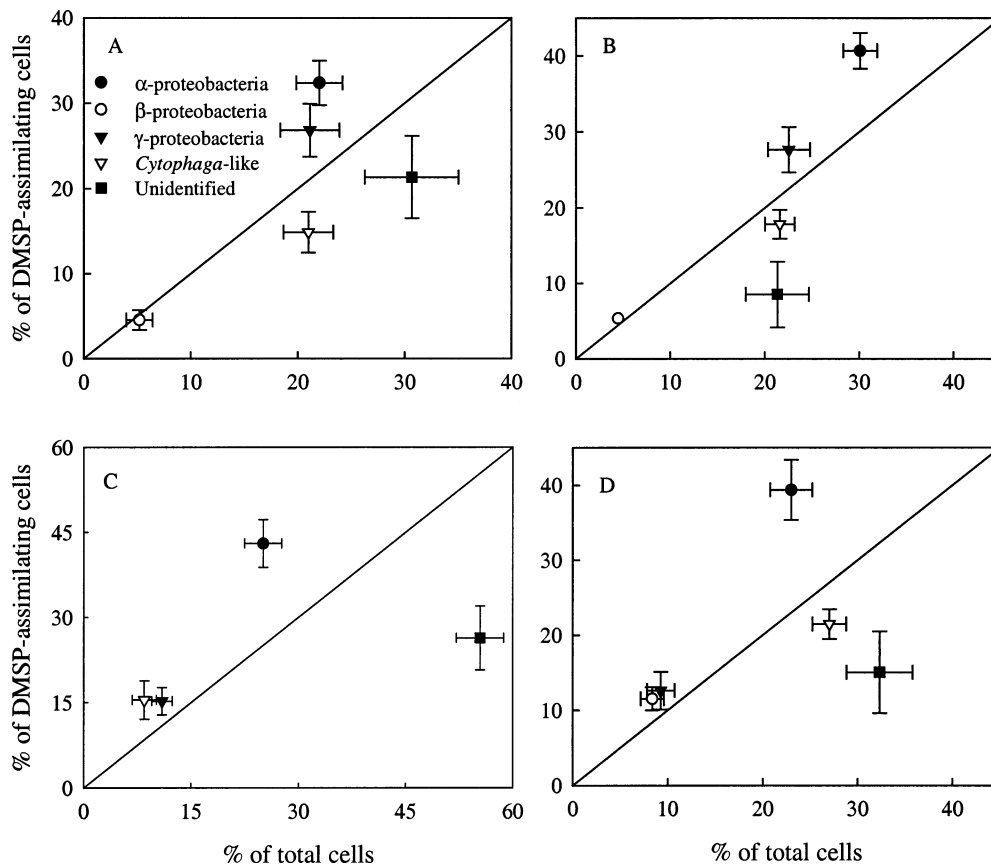


Fig. 3. Composition of the DMSP-assimilating community versus composition of the total bacterial community at the (A) Gulf of Maine Sta. 5, (B) Gulf of Maine Sta. 6, (C) Sargasso Sea Sta. 12, and (D) Sargasso Sea Sta. 14. Unidentified cells are those that did not hybridize with any phylogenetic group probe. The line represents a 1 : 1 line. Error bars are the standard error of 30 fields of view.

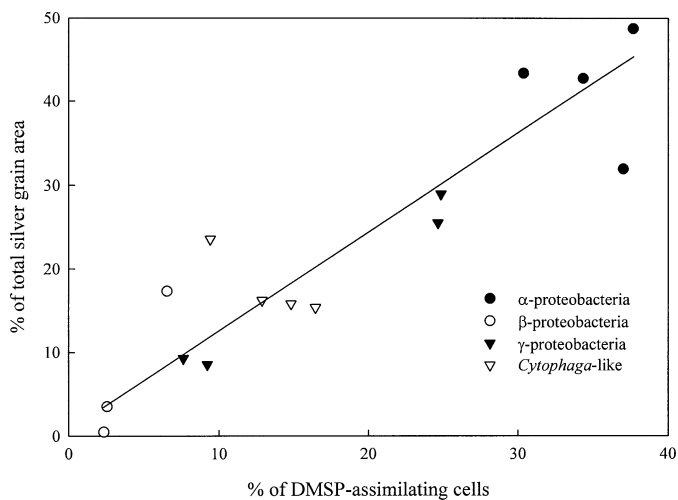


Fig. 4. Percent total silver grain area versus percent of DMSP-assimilating cells associated with  $\alpha$ -,  $\beta$ -, and  $\gamma$ -proteobacteria, *Cytophaga*-like bacteria in the Gulf of Maine and the Sargasso Sea ( $y = 1.2x + 0.7$ ;  $r^2 = 0.84$ ;  $p < 0.001$ ).

## Discussion

The fate of DMSP appears to be largely influenced by microbial metabolism, yet little is known about the bacteria involved in DMSP flux. DMSP can be a carbon and sulfur source for microbial communities or it can be cleaved into DMS, which can impact atmospheric chemistry and global climate (Charlson et al. 1987; Simó 2001). Not all bacteria, though, can cleave DMSP or assimilate it into biomass; few bacteria can do both (González et al. 1999; Yoch 2002). The capacity to assimilate DMSP is widespread among members of the *Roseobacter* clade, and the only bacteria known to both assimilate DMSP and form DMS are members of the *Roseobacter* clade (González et al. 1999). This apparent link between phylogeny and metabolic activity led to the hypothesis that the *Roseobacter* clade plays an important role in the cycling of DMSP. On a broader scale, this link implies that the phylogenetic composition of microbial communities could influence the flux of DMSP, especially if one phylogenetic group dominates the metabolism of DMSP. In this study, the *Roseobacter* clade assimilated DMSP to a greater extent than expected based on their abundance, but it did not dominate DMSP assimilation. Instead, we found that bacteria from several phylogenetic groups assimilated DMSP.

Although *Roseobacter* did not dominate assimilation of

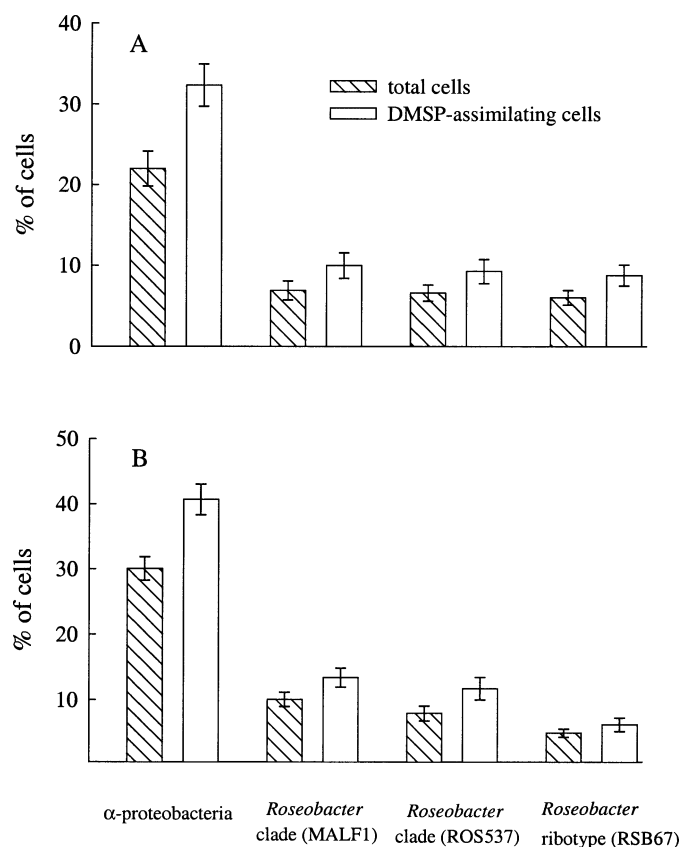


Fig. 5. Relative abundance of bacteria in both the total community and the DMSP-assimilating community at the Gulf of Maine (A) Sta. 5 and (B) Sta. 6 that hybridize with the  $\alpha$ -proteobacteria probe Alf968, redundant *Roseobacter* clade probes MALF1 and ROS537, and a *Roseobacter* ribotype recognized by probe RSB67. Error bars represent the standard error of 30 fields of view.

DMSP, our results support the hypothesis that the *Roseobacter* clade, when abundant, is important in the cycling of DMSP. Greater than 80% of *Roseobacter* cells assimilated DMSP, and approximately a third of DMSP assimilation, based on total silver grain surface area, was associated with the *Roseobacter* clade when these bacteria were present. Since the *Roseobacter* clade only accounted for roughly 10% of the DMSP-assimilating community, they appear to be able to better use DMSP on a per-cell basis than other bacteria. Their competitive advantage may be due to a high-capacity uptake system for DMSP. An analysis of the recently sequenced genome of *Silicibacter pomeroyi* DSS-3, a member of the *Roseobacter* clade, revealed five genes homologous to DMSP transporters, possibly indicating a large capacity for DMSP uptake (Moran et al. pers. comm.). The apparent affinity of the *Roseobacter* clade for DMSP may allow *Roseobacter* to out-compete other bacteria for DMSP, a potentially significant source of carbon as well as sulfur to bacteria (Simó et al. 2002). Since bacterial communities are often limited by carbon, the capacity of the *Roseobacter* clade to out-compete other bacteria for DMSP might help the *Roseobacter* clade increase its abundance when concentrations and fluxes of dissolved DMSP are high. This hypothesis is consistent with the observation that the *Roseo-*

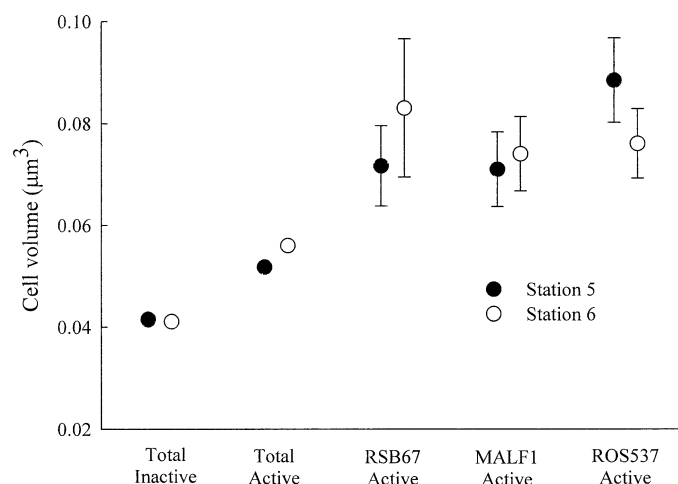


Fig. 6. Average cell volume of total DMSP-active cells (total active), total DMSP-inactive cells (total inactive), and DMSP-active cells hybridizing with the *Roseobacter* probes RSB67, MALF1, and ROS537 in the Gulf of Maine. Error bars are 95% confidence limits. Confidence limits of total active and total inactive volumes are obscured by their symbols.

bacter clade is abundant during DMSP-producing algal blooms (González et al. 2000; Zubkov et al. 2002a).

DMSP-producing algal blooms are ephemeral, however, and bacterial communities associated with blooms may not be typical of those communities mediating DMSP flux during nonbloom conditions. Our study investigated DMSP assimilation during nonbloom conditions in both productive coastal waters and the oligotrophic open ocean. Members of the *Roseobacter* clade were found in coastal waters but were not detected in the Sargasso Sea. Despite the absence of *Roseobacter*, there was still substantial turnover of the dissolved DMSP pool, and a large number of bacteria assimilated DMSP in the Sargasso Sea. Other bacteria, especially other  $\alpha$ -proteobacteria, were able to fill the niche of the missing *Roseobacter* clade. Since the capacity to assimilate DMSP is found in all the major bacterial phylogenetic groups examined, the distribution of *Roseobacter* does not appear to greatly impact the assimilation of DMSP during nonbloom conditions.

The  $\alpha$ -proteobacteria were consistently overrepresented in the DMSP-assimilating community in the Gulf of Maine and Sargasso Sea, whereas *Cytophaga*-like bacteria were typically underrepresented. It is surprising to see the same trend in DMSP assimilation in both productive coastal waters (Gulf of Maine) and oligotrophic waters (Sargasso Sea), since the bacterial communities are probably different in these environments and the  $\alpha$ -proteobacteria and *Cytophaga*-like bacteria assimilating DMSP in the Gulf of Maine are probably not the same as those assimilating DMSP in the Sargasso Sea. If the bacterial communities did differ substantially among these environments, then our data indicate that there is specialization in DMSP assimilation at the major phylogenetic group level. Alternatively, the compositions of the bacterial communities could have been similar in these environments. Rappé et al. (2000) concluded that the bacteria found in coastal waters are often closely related ( $\geq 90\%$

Table 3. Contributions of bacterial groups to the DMSP-assimilating community and to DMSP assimilation in the Gulf of Maine based on total silver grain surface area. The RSB67 ribotype is a member of the *Roseobacter* clade. Mean  $\pm$  SE of 30 fields of view.

Station	Phylogenetic group	% group assimilating DMSP	% DMSP-assimilating community	% total silver grain area
5	$\alpha$ -proteobacteria	77 $\pm$ 3.1	32 $\pm$ 2.6	46
	<i>Roseobacter</i> clade	84 $\pm$ 4.3	10 $\pm$ 1.6	25
	RSB67 ribotype	87 $\pm$ 4.5	8 $\pm$ 1.3	22
6	$\alpha$ -proteobacteria	69 $\pm$ 2.6	41 $\pm$ 2.3	57
	<i>Roseobacter</i> clade	89 $\pm$ 3.3	12 $\pm$ 1.4	36
	RSB67 ribotype	80 $\pm$ 5.6	6 $\pm$ 1.0	12

sequence similarity) to bacteria in the open ocean based on 16S rRNA sequences. If so, then these phylogenetic similarities would explain the trends in DMSP assimilation by the major phylogenetic groups in the Gulf of Maine and Sargasso Sea.

Methanethiol and DMS can be released into the water column during DMSP metabolism (Kiene and Linn 2000b) and assimilated by other bacteria. If DMS or methanethiol was released in our incubations, then some bacteria detected by microautoradiography may not have assimilated DMSP directly, but assimilated  $^{35}\text{S}$  methanethiol or  $^{35}\text{S}$  DMS produced by other bacteria. In this study, assimilation of  $^{35}\text{S}$  DMS was negligible (<1% of  $^{35}\text{S}$  DMS tracer assimilated at all stations). Additionally, of bacteria studied so far, those that assimilate methanethiol also assimilate DMSP (González et al. 1999; Kiene et al. 1999). Another possible complication is that microautoradiography could potentially identify cells that took up DMSP as an osmolyte but did not assimilate it into biomass. However, fixation with paraformaldehyde for 24 h prior to filtration onto polycarbonate filters removes unincorporated  $^{35}\text{S}$  (Kiene and Linn 1999). Therefore, autoradiography-positive results are indicative of DMSP assimilation.

The capacity to assimilate DMSP is probably common among the major phylogenetic groups because DMSP is a major source of sulfur for bacterial communities, potentially satisfying greater than 90% of the total bacterial sulfur demand (Kiene and Linn 2000a). Most DMSP-derived sulfur is incorporated into methionine and cysteine and assimilated into protein during protein synthesis (Kiene et al. 1999). If DMSP assimilation satisfies virtually all of the bacterial sulfur demand, and all bacteria synthesizing protein need sulfur, then virtually all bacteria synthesizing protein should assimilate DMSP. We found that the DMSP-assimilating community was composed of all major phylogenetic groups, indicating that bacteria from all major phylogenetic groups were actively synthesizing protein. Cottrell and Kirchman (2003) observed similar results in the Delaware estuary, where all major phylogenetic groups assimilated leucine, an indicator of protein synthesis.

DMSP-assimilating bacteria were not only diverse, but abundant as well. On average, half of all bacteria assimilated DMSP in the environments investigated. The number of DMSP-assimilating bacteria was greater than expected considering that previous investigations of natural bacterial

Table 4. Average cell volumes of DMSP assimilating (DMSP active) and nonassimilating (DMSP inactive) bacteria. Mean  $\pm$  95% confidence limits.

Location	Station	Cell volumes ( $\mu\text{m}^3$ )	
		DMSP-active cells	DMSP-inactive cells
Gulf of Mexico	3	0.040 $\pm$ 0.001	0.032 $\pm$ 0.001
Gulf of Mexico	4	0.054 $\pm$ 0.003	0.032 $\pm$ 0.002
Gulf of Mexico	6	0.053 $\pm$ 0.002	0.042 $\pm$ 0.002
Gulf of Mexico	7	0.051 $\pm$ 0.002	0.037 $\pm$ 0.003
Gulf of Maine	5	0.052 $\pm$ 0.001	0.042 $\pm$ 0.001
Gulf of Maine	6	0.056 $\pm$ 0.001	0.042 $\pm$ 0.001
Sargasso Sea	8	0.060 $\pm$ 0.002	0.041 $\pm$ 0.001
Sargasso Sea	12	0.050 $\pm$ 0.002	0.035 $\pm$ 0.002
Sargasso Sea	14	0.045 $\pm$ 0.001	0.033 $\pm$ 0.001
NC coast	15	0.047 $\pm$ 0.001	0.035 $\pm$ 0.002

communities with microautoradiography found that only 20% of marine bacteria assimilated thymidine (Smith and del Giorgio 2003). More bacteria may assimilate DMSP than thymidine because DMSP assimilation is indicative of protein synthesis, a process carried out by both dividing and nondividing-yet-active bacteria. In contrast, only dividing cells synthesize DNA, and thus incorporate thymidine. DMSP assimilation probably identified both dividing and nondividing-yet-active cells, indicating that a large number of bacteria were synthesizing protein in the Gulf of Mexico, Gulf of Maine, and Sargasso Sea.

In addition to their high abundance, DMSP-assimilating bacteria appear to be 40% larger by volume than nonassimilating bacteria on average. Similarly, leucine and thymidine-assimilating bacteria were approximately 30% larger than nonassimilating bacteria in the Delaware Bay (Cottrell and Kirchman in press). Lebaron et al. (2002) also obtained similar results in Mediterranean coastal waters, where they found a strong positive correlation between bacterial size and per-cell leucine assimilation. Data from these three studies indicate that dividing and nondividing-yet-active bacteria are significantly larger than the rest of the bacterial community, which could be comprised mainly of dormant or sluggish bacteria, i.e., cells with low levels of activity.

The large size of DMSP-assimilating cells may make them susceptible to grazing. Micrograzers preferentially graze on large and actively dividing bacteria in marine communities (Sherr et al. 1992). This selective removal process can affect the composition of the bacterial communities by depressing the abundance of the larger, more active cells (Jürgens and Matz 2002). Since all bacteria do not assimilate DMSP equally, and DMSP-assimilating bacteria are larger than nonassimilating bacteria, grazing pressure could control dissolved DMSP concentrations and flux by constraining the number and type of bacteria metabolizing DMSP.

The composition of bacterial communities has the potential to affect DMSP assimilation because not all bacteria assimilate DMSP equally (González et al. 1999; Kiene et al. 1999). Incorporation of DMSP into bacterial biomass, however, is only one possible fate for DMSP. Other fates, such as the production of DMS and nonvolatile compounds, are

also mediated by microbial communities (Kiene and Linn 2000b). As with DMSP assimilation, the capacity to produce DMS and nonvolatile compounds from DMSP is not equally distributed among bacterial isolates (González et al. 1999) and may not be equally distributed in natural communities as well. Therefore, the composition of bacterial communities could affect other aspects of DMSP processing in addition to DMSP assimilation. Further investigations into the impact of community composition on the biogeochemical flux of DMSP are warranted.

## References

- AMANN, R. I., L. KRUMHOLZ, AND D. L. STAHL. 1990. Fluorescent oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *J. Bacteriol.* **172**: 762–770.
- , W. LUDWIG, AND K. H. SCHLEIFER. 1995. Phylogenetic identification and in-situ detection of individual microbial-cells without cultivation. *Microbiol. Rev.* **59**: 143–169.
- CHARLSON, R. J., J. E. LOVELOCK, M. O. ANDREAEE, AND S. G. WARREN. 1987. Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. *Nature* **326**: 655–661.
- COTTRELL, M. T., AND D. L. KIRCHMAN. 2000. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low and high molecular weight dissolved organic matter. *Appl. Environ. Microbiol.* **66**: 1692–1697.
- AND ———. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. *Limnol. Oceanogr.* **48**: 168–178.
- AND ———. In press. Single-cell analysis of bacterial growth, cell size and community structure in the Delaware estuary. *Aquat. Microb. Ecol.*
- EILERS, H., J. PERNTHALER, J. PEPLIES, F. O. GLÖCKNER, G. GERDTS, AND R. AMANN. 2001. Isolation of novel pelagic bacteria from the German bight and their seasonal contributions to surface picoplankton. *Appl. Environ. Microbiol.* **67**: 5134–5142.
- GIOVANNONI, S. J., T. B. BRITSCHGI, C. L. MOYER, AND K. G. FIELD. 1990. Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **345**: 60–63.
- , AND M. RAPPÉ. 2000. Evolution, diversity, and molecular ecology of marine prokaryotes, pp. 47–84. *In* D. L. Kirchman [ed.], *Microbial ecology of the oceans*. Wiley-Liss.
- GLÖCKNER, F. O., B. M. FUCHS, AND R. AMANN. 1999. Bacterioplankton compositions of lakes and oceans: A first comparison based on fluorescence in situ hybridization. *Appl. Environ. Microbiol.* **65**: 3721–3726.
- GONZÁLEZ, J. M., R. P. KIENE, AND M. A. MORAN. 1999. Transformation of sulfur compounds by an abundant lineage of marine bacteria in the alpha-subclass of the class Proteobacteria. *Appl. Environ. Microbiol.* **65**: 3810–3819.
- , AND M. A. MORAN. 1997. Numerical dominance of a group of marine bacteria in the alpha-subclass of the class Proteobacteria in coastal seawater. *Appl. Environ. Microbiol.* **63**: 4237–4242.
- , AND OTHERS. 2000. Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. *Appl. Environ. Microbiol.* **66**: 4237–4246.
- HILL, R. W., B. A. WHITE, M. T. COTTRELL, AND J. W. H. DACEY. 1998. Virus-mediated total release of dimethylsulfoniopropionate from marine phytoplankton: A potential climate process. *Aquat. Microb. Ecol.* **14**: 1–6.
- JÜRGENS, K., AND C. MATZ. 2002. Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **81**: 413–434.
- KARNER, M., AND J. A. FUHRMAN. 1997. Determination of active marine bacterioplankton: A comparison of universal 16S rRNA probes, autoradiography, and nucleoid staining. *Appl. Environ. Microbiol.* **63**: 1208–1213.
- KIENE, R. P. 1996. Turnover of dissolved DMSP in estuarine and shelf water from the Northern Gulf of Mexico, pp. 337–349. *In* R. P. Kiene, M. K. Visscher, and G. Kirst [eds.], *Biological and environmental chemistry of DMSP and related sulfonium compounds*. Plenum.
- , AND G. GERARD. 1994. Determination of trace levels of dimethylsulfoxide (DMSO) in seawater and rainwater. *Mar. Chem.* **47**: 1–12.
- , AND L. J. LINN. 1999. Filter-type and handling affect determination of organic substrate uptake by bacterioplankton. *Aquat. Microb. Ecol.* **17**: 311–321.
- , AND ———. 2000a. Distribution and turnover of dissolved DMSP and its relationship with bacterial production and dimethylsulfide in the Gulf of Mexico. *Limnol. Oceanogr.* **45**: 849–861.
- , AND ———. 2000b. The fate of dissolved dimethylsulfoniopropionate (DMSP) in seawater: Tracer studies using S-35-DMSP. *Geochim. Cosmochim. Acta* **64**: 2797–2810.
- , ———, J. GONZÁLEZ, M. A. MORAN, AND J. A. BRUTON. 1999. Dimethylsulfoniopropionate and methanethiol are important precursors of methionine and protein-sulfur in marine bacterioplankton. *Appl. Environ. Microbiol.* **65**: 4549–4558.
- KIRCHMAN, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria, pp. 509–512. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], *Handbook of methods in aquatic microbial ecology*. Lewis.
- LEBARON, P., P. SERVAIS, A. C. BAUDOUX, M. BOURRAIN, C. COURTIÉS, AND N. PARTHUISOT. 2002. Variations of bacterial-specific activity with cell size and nucleic acid content assessed by flow cytometry. *Aquat. Microb. Ecol.* **28**: 131–140.
- LEE, N., AND OTHERS. 1999. Combination of fluorescent in situ hybridization and microautoradiography—a new tool for structure-function analyses in microbial ecology. *Appl. Environ. Microbiol.* **65**: 1289–1297.
- MANZ, W., R. AMANN, W. LUDWIG, M. VANCANNEYT, AND K. H. SCHLEIFER. 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum *Cytophaga-Flavobacter-Bacteroides* in the natural environment. *Microbiology (U.K.)* **142**: 1097–1106.
- , ———, ———, M. WAGNER, AND K. H. SCHLEIFER. 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria—problems and solutions. *Syst. Appl. Microbiol.* **15**: 593–600.
- MARTINEZ, J., D. C. SMITH, G. F. STEWARD, AND F. AZAM. 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquat. Microb. Ecol.* **10**: 223–230.
- OUVERNEY, C. C., AND J. A. FUHRMAN. 1999. Combined microautoradiography-16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types in situ. *Appl. Environ. Microbiol.* **65**: 1746–1752.
- RAPPÉ, M. S., K. VERGIN, AND S. J. GIOVANNONI. 2000. Phylogenetic comparisons of a coastal bacterioplankton community with its counterparts in open ocean and freshwater systems. *FEMS Microbiol. Ecol.* **33**: 219–232.
- SIERACKI, M. E., C. L. VILES, AND K. L. WEBB. 1989. Algorithm to estimate cell biovolume using image analyzed microscopy. *Cytometry* **10**: 551–557.

- SHERR, B. F., E. B. SHERR, AND J. MCDANIEL. 1992. Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. *Appl. Environ. Microbiol.* **58**: 2381–2385.
- SIMÓ, R. 2001. Production of atmospheric sulfur by oceanic plankton: Biogeochemical, ecological and evolutionary links. *Trends Ecol. Evol.* **16**: 287–294.
- , S. D. ARCHER, C. PEDRÓS-ALIÓ, L. GILPIN, AND C. E. STELFOX-WIDDICOMBE. 2002. Coupled dynamics of dimethylsulfoniopropionate and dimethylsulfide cycling and the microbial food web in surface waters of the North Atlantic. *Limnol. Oceanogr.* **47**: 53–61.
- SMITH, D. C., AND F. AZAM. 1992. A simple, economical method for measuring bacterial protein synthesis in seawater using  $^3\text{H}$ -leucine. *Mar. Microb. Food Webs* **6**: 107–114.
- SMITH, E. M., AND P. DEL GIORGIO. 2003. Low fractions of active bacteria in natural aquatic communities? *Aquat. Microb. Ecol.* **31**: 203–208.
- TURNER, S. M., G. MALIN, P. S. LISS, D. S. HARBOUR, AND P. M. HOLLIGAN. 1988. The seasonal variation of dimethyl sulfide and dimethylsulfoniopropionate concentrations in nearshore waters. *Limnol. Oceanogr.* **33**: 364–375.
- WOLFE, G. V., E. B. SHERR, AND B. F. SHERR. 1994. Release and consumption of DMSP from *Emiliania huxleyi* during grazing by *Oxyrrhis marina*. *Mar. Ecol. Prog. Ser.* **111**: 111–119.
- YOCH, D. C. 2002. Dimethylsulfoniopropionate: Its sources, role in the marine food web, and biological degradation to dimethylsulfide. *Appl. Environ. Microbiol.* **68**: 5804–5815.
- ZUBKOV, M. V., B. M. FUCHS, S. D. ARCHER, R. P. KIENE, R. AMANN, AND P. H. BURKILL. 2002a. Rapid turnover of dissolved DMS and DMSP by defined bacterioplankton communities in the stratified euphotic zone of the North Sea. *Deep-Sea Res. II* **49**: 3017–3038.
- , ———, G. A. TARRAN, P. H. BURKILL, AND R. AMANN. 2002b. Mesoscale distribution of dominant bacterioplankton groups in the northern North Sea in early summer. *Aquat. Microb. Ecol.* **29**: 135–144.

Received: 20 May 2003

Accepted: 15 September 2003

Amended: 29 September 2003