Bacterial Taxa That Limit Sulfur Flux from the Ocean

Erinn C. Howard, *et al.*

Science **314**, 649 (2006);
DOI: 10.1126/science.1130657

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

MS unless the timing of sample acquisition is matched to those windows. In cases where it was unlikely that the peptides would be detectable by MS, we characterized the proteins by bioinformatics approaches using the genomic data. Some of the well-known insect neuropeptide precursors that are predicted from the genome using homology-based searches include crustacean cardioactive peptide, crustacean hyperglycemic hormone, eclosion hormone, and insulin.

Because of the repetitive quality of several neuropeptide precursors, we developed a codon-scanning algorithm that searches for repetitive sequences in the genome. When multiple peptides are generated from a single precursor, they frequently share a repeating C-terminal amino acid pattern (for example, the FGLamide motif present in allatostatin peptides). Algorithms tailored to recognize repeating motifs can more successfully identify neuropeptide genes than traditional homology-based approaches. In an analysis of the *C. elegans* genome for RFamide-coding genes, two putative genes were identified by homology searches, a nominal number when compared with the 29 potential genes discovered with a tailored, pattern-matching algorithm (*15*). We used a similar approach to identify potential precursors without homologs in other species. As shown in Table 1, this method was also used to verify the positions in the genome of several of the repeating precursors, for example, allatostatin, tachykinin, and pheromone biosynthesis–activating neuropeptide.

As mentioned above, one neuropeptide gene family containing repeating C termini, the RFamides, is a well-studied family of myotropic peptides that have been identified in many species ranging from mollusks to mammals (*16*–*20*). In mammals, two of the RFamides, neuropeptides FF (NPFF) and AF (NPAF), are produced from NPFF precursors and end in a C-terminal motif, QRFamide (*21*). We probed the *Apis* genome using the codon-scanning algorithm looking for an open reading frame that encodes a motif with at least two RFamides located within 1 kilobase of each other. We discovered a putative gene that encoded a signaling protein containing three peptides terminating in QRFamide. In this particular case, the codon-scanning algorithm was the singular approach in our platform able to identify this previously unreported gene; expression of this putative *Apis* NPFF-like gene was verified by quantitative reverse transcription–polymerase chain reaction (qRT-PCR) (table S3). We also identified three additional RFamide neuropeptide genes—FLRFamide, RFamide1, and RFamide2. Because these precursors do not display significant similarity to RFamide precursors from other insects, they are not identifiable using homology searches.

Our combined approach yields many more peptides than the individual approaches used previously. As a result, in a single investigative effort, a comparable number of neuropeptides are now known in the honey bee relative to other well-studied animal models. Microarrays can be designed to include a greater number of neuropeptide gene products, thereby expanding our understanding of the expression of the neuromodulators inherent to the operation of neuronal networks. The potential of our blended technology approach to facilitate discovery of these peptides is not only significant for advancing honey bee research, it demonstrates promise for neuropeptide discovery in the large number of other new genomes currently being sequenced.

References and Notes

1. Honey Bee Genome Sequencing Consortium, *Nature* **443**, 931 (2006).
2. PubMed search for *D. melanogaster* neuropeptides pre–March 2000, yielded 305 articles; for *C. elegans* pre–December 1998, 75 articles.
3. M. A. Riehle, S. F. Garczynski, J. W. Crim, C. A. Hill, M. R. Brown, *Science* **298**, 172 (2002).
4. R. S. Hewes, P. H. Taghert, *Genome Res.* **11**, 1126 (2001).
5. J. Vanden Broeck, *Peptides* **22**, 241 (2001).
6. Multiple processing products of the genes predicted from the *D. melanogaster* genome sequence were later confirmed with MS, and sequences of interest were discovered (*22*).
7. H. Takeuchi, A. Yasuda, Y. Yasuda-Kamatani, T. Kubo, T. Nakajima, *Insect Mol. Biol.* **12**, 291 (2003).
8. P. Verleyen *et al.*, *Biochem. Biophys. Res. Commun.* **320**, 334 (2004).
9. P. Verleyen *et al.*, *Peptides* **27**, 493 (2006).
10. N. Audsley, R. J. Weaver, *Peptides* **27**, 512 (2006).
11. Materials and methods are available as supporting material on *Science* Online.
12. A. B. Hummon *et al.*, *J. Proteome Res.* **2**, 650 (2003).
13. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
14. C. W. Whitfield, A.-M. Cziko, G. E. Robinson, *Science* **302**, 296 (2003).
15. A. N. Nathoo, R. A. Moeller, B. A. Westlund, A. C. Hart, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14000 (2001).
16. M. Schaefer *et al.*, *Cell* **41**, 457 (1985).
17. R. Nichols, J. B. McCormick, I. A. Lim, *Ann. N. Y. Acad. Sci.* **897**, 264 (1999).
18. S. J. Husson, E. Clynen, G. Baggerman, A. De Loof, L. Schoofs, *Biochem. Biophys. Res. Commun.* **335**, 76 (2005).
19. G. J. Dockray, *Exp. Physiol.* **89**, 229 (2004).
20. N. Chartrel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 15247 (2003).
21. E. Bonnard *et al.*, *Peptides* **22**, 1085 (2001).
22. G. Baggerman, A. Cerstiaens, A. De Loof, L. Schoofs, *J. Biol. Chem.* **277**, 40368 (2002).

23. We dedicate this article to A. De Loof for a full career of insect research at the K. U. Leuven. We thank T. Newman for assistance with qRT-PCR, B. Southey for aiding our BLAST searches, and C. Whitfield for helpful discussions. We acknowledge M. Corona for annotating the insulin gene. Supported by the National Institutes of Health through NS31609, P30 DA01830 (J.V.S.), GM068946 (S.L.R.-Z.), and DC006395 (G.E.R.). P.V., G.B., J.H., and E.V. are postdoctoral researchers of the FWO-Flanders (Fund for Scientific Research–Flanders), supported by FWO-Grant G.0444.05.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5799/647/DC1
Materials and Methods
Tables S1 to S4
References

21 December 2005; accepted 27 July 2006
10.1126/science.1124128

Bacterial Taxa That Limit Sulfur Flux from the Ocean

Erinn C. Howard,[1] James R. Henriksen,[1] Alison Buchan,[3] Chris R. Reisch,[1] Helmut Bürgmann,[2] Rory Welsh,[2] Wenying Ye,[2] José M. González,[4] Kimberly Mace,[2] Samantha B. Joye,[2] Ronald P. Kiene,[5,6] William B. Whitman,[1] Mary Ann Moran[2]*

Flux of dimethylsulfide (DMS) from ocean surface waters is the predominant natural source of sulfur to the atmosphere and influences climate by aerosol formation. Marine bacterioplankton regulate sulfur flux by converting the precursor dimethylsulfoniopropionate (DMSP) either to DMS or to sulfur compounds that are not climatically active. Through the discovery of a glycine cleavage T-family protein with DMSP methyltransferase activity, marine bacterioplankton in the Roseobacter and SAR11 taxa were identified as primary mediators of DMSP demethylation to methylmercaptopropionate. One-third of surface ocean bacteria harbor a DMSP demethylase homolog and thereby route a substantial fraction of global marine primary production away from DMS formation and into the marine microbial food web.

Marine phytoplankton synthesize DMSP for use as an osmolyte (*1*), predator deterrent (*2*), and antioxidant (*3*). The degradation of DMSP to DMS and subsequent exchange of DMS across the ocean-atmosphere boundary is the main natural source of sulfur to the atmosphere, amounting to ~20 Tg of sulfur annually (*4*). DMS-derived atmospheric sulfur affects cloud formation and the radiative properties of Earth (*5*). Phytoplankton are known to degrade DMSP to DMS, but efforts to predict global patterns of ocean-atmosphere DMS flux based solely on phytoplankton parameters have been unsuccessful (*6*). Other members of the marine plankton must therefore influence the production and emission of DMS from the surface ocean (*7*).

[1]Department of Microbiology, University of Georgia, Athens, GA 30602, USA. [2]Department of Marine Sciences, University of Georgia, Athens, GA 30602, USA. [3]Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA. [4]Department of Microbiology, University of La Laguna, 38071 La Laguna, Tenerife, Spain. [5]Department of Marine Sciences, University of South Alabama, Mobile, AL 36688, USA. [6]Dauphin Island Sea Lab, Dauphin Island, AL 36528, USA.

*To whom correspondence should be addressed. E-mail: mmoran@uga.edu

Marine bacterioplankton are known to degrade DMSP by a pathway that first converts DMSP to methylmercaptopropionate (MMPA) in a demethylation reaction, and subsequently to methanethiol (MeSH) (*8*) or mercaptopropionate (MPA) (fig. S1) (*9*). The first step of this alternative pathway is crucial to oceanic sulfur emissions, because it removes a methyl group from DMSP and eliminates DMS as a possible degradation product. Furthermore, some of the MMPA-derived sulfur is incorporated subsequently into bacterial amino acids (*10*) and, through trophic transfers, into the marine microbial food web. Despite the estimated 50 to 90% of DMSP that is metabolized by marine bacterioplankton through this pathway (*11*, *12*), the taxa that mediate DMSP demethylation in ocean surface waters are unknown.

Bacteria in the marine Roseobacter clade have been shown to demethylate DMSP in culture (*8*). *Silicibacter pomeroyi* DSS-3 (*13*) performs both DMSP demethylation to MeSH and DMSP cleavage to DMS (*10*). A 20,000-member Tn*5*-based transposon insertion library of *S. pomeroyi* was screened for interruption of MeSH formation based on failure to produce a thiol from DMSP, and phenotypes of potential mutants were monitored by analysis of sulfur gas formation. A mutant unable to make MeSH yet able to produce DMS at wild-type levels had a transposon insertion in SPO1913 (Fig. 1), a gene encoding a protein in the glycine cleavage T-protein family (Pfam PF01571, Enzyme Commission number 2.1.2.10). DMSP degradation to MeSH was restored by complementation of the mutant in trans with an intact SPO1913 gene (fig. S2). Enzyme assays in cell-free extracts of wild-type and mutant strains showed that SPO1913 encodes the protein responsible for the first step in MeSH formation: the demethylation of DMSP to MMPA (Table 1). This DMSP demethylase gene was designated *dmdA*.

Table 1. DMSP demethylase activity in *S. pomeroyi* and *E. coli* strains with or without functional *dmdA* genes (SPO1913 or SAR11_0246) measured as MMPA formation (nmol min^{-1} mg $protein^{-1}$) in cell-free extracts. Activity in wild-type extracts was linear with both time and amount of protein, dependent on the presence of DMSP and the coenzyme tetrahydrofolate (THF), and comparable to the rate of MeSH production by whole cells. The limit of detection was 0.02 to 0.05 nmol min^{-1} mg $protein^{-1}$. Activity is shown ± SD.

Source of extract	DMSP:THF demethylase activity
S. pomeroyi DSS-3, wild-type	0.15 ± 0.02
S. pomeroyi mutant 41-H6, Tn*5* inactivation of SPO1913	0
E. coli with pABX101, recombinant SAR11_0246	0.24 ± 0.05
E. coli with pCYB1, vector alone	0

Basic Local Alignment Search Tool (BLAST) searches of genome sequences of other cultured bacteria yielded only two complete *dmdA* orthologs in any non-Roseobacter genome. Both were from marine bacteria in the SAR11 clade, *Pelagibacter ubique* HTCC1062 (*14*) and *P. ubique* HTCC1002 (*15*) (Fig. 1). One other partial *dmdA* sequence (Fig. 1) was found on a small (1.4-kb) fragment of environmental DNA contaminating the genome sequence of the sea ice bacterium *Psychroflexus torquis* (*15*); the taxonomic origin of this sequence is unknown (*16*).

We searched marine metagenomic libraries to determine whether *dmdA*-like sequences were present in natural bacterial communities. In the Sargasso Sea (*11*), *dmdA* homologs were sufficiently abundant to be harbored by about a third of bacterioplankton cells (Table 2). The Sargasso sequences formed four clades distinct from other glycine cleavage T-protein family proteins (Fig. 2). Clade A sequences clustered with DMSP demethylases from *S. pomeroyi* and other Roseobacters (table S1). Based on the number of clade A homologs relative to Roseobacter-like 16*S* rDNA sequences (*13*), at least 80% of Roseobacters captured in the Sargasso Sea metagenome possess a *dmdA* homolog. Sequences similar to clade B and clade C were not found among cultured bacteria; these sequences may be from uncultured or unsequenced marine bacterial lineages, or they may represent sequence diversity within the known *dmdA*-containing taxa. Clade D sequences clustered with the *dmdA* orthologs from *P. ubique* HTCC1062 and HTCC1002. Two sequence assemblies from the Sargasso Sea that contained clade D homologs showed similar gene organization and highest gene similarities to the *P. ubique* genomes (Fig. 1). Based on the number of clade D homologs relative to SAR11-like 16*S* rDNA sequences (*13*), only 40% of SAR11 cells demethylate DMSP; these may belong to an ecologically distinct subgroup within the taxon (*17*).

Genes adjacent to *dmdA* homologs were consistent within a clade but differed across clades (Fig. 1). Previous studies have shown that MMPA can be metabolized to MeSH or MPA in seawater (fig. S1) (*9*, *18*). Thus, whereas all DMSP demethylating taxa must have *dmdA* in common, a different, taxon-specific suite of genes may encode for the subsequent metabolism of MMPA (Fig. 1).

Although sequence coverage is small compared with that of the Sargasso Sea data set (Table 2), other marine metagenomic databases contain evidence of DMSP demethylase genes.

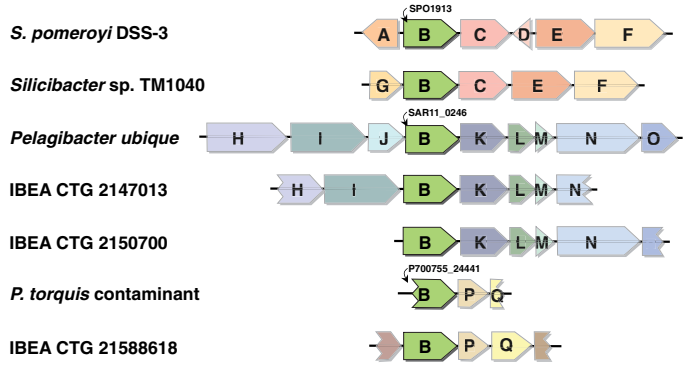

Fig. 1. Gene neighborhoods of cultured marine bacteria and selected Sargasso Sea contigs (labeled as IBEA CTG) harboring *dmdA* genes. Representative sequences that assembled into the Sargasso Sea contigs (i.e., with >97% identity) are indicated on Fig. 2. The *P. torquis* contaminant *dmdA* is a partial sequence on a small genome fragment. A, GntR family transcriptional regulator; B, glycine cleavage T-family protein (*dmdA*); C, dehydrogenase; D, glyoxalase family protein; E, aminotransferase class V; F, deoxyribodipyrimidine photolyase (*phrB*); G, protein of unknown function; H, acyl coenzyme A (CoA) dehydrogenase; I, acyl CoA synthase; J, hydrolase (*mhpC*); K, aspartate semialdehyde dehydrogenase; L, succinate dehydrogenase cytochrome b (*sdhC*); M, membrane protein; N, succinate dehydrogenase (*sdhA*); O, succinate dehydrogenase Fe-S protein; P, OsmC-like protein; Q, enoyl-CoA hydratase/isomerase.

Table 2. Abundance of *dmdA* homologs in marine bacterioplankton metagenomic surveys. Sargasso Sea data are from surface seawater samples (Stations 1 to 7) using the unassembled shotgun library (*11*). Station Aloha data are grouped into photic zone (10, 70, and 130 m) and deep water (500, 770, and 4000 m) samples according to DeLong *et al*. (*19*). Sapelo Island data are from surface seawater samples (0.5 m). *recA* homologs were determined by BLAST analysis using the *E. coli recA* sequence as the query. The percentage of cells with *dmdA* was calculated as *dmdA* × 100/*recA*. *recA* is an essential single-copy gene. Mbp, mega–base pairs.

	Library size (Mbp)	*dmdA* homologs					*recA* homologs	% of cells with *dmdA*
		Clade				Total		
		A	B	C	D			
Sargasso Sea (oceanic)	1626	29	18	83	247	377	1029	37
Station Aloha (oceanic) photic zone	24.8	1	0	0	1	2	5	40
Station Aloha (oceanic) deep water	31.1	0	0	0	0	0	17	0
Sapelo Island (coastal)	15.2	9	0	0	1	10	26	38

Two *dmdA* homologs were found in photic zone samples from the Pacific Station Aloha database (*19*); as expected, none were in the deep water samples from this site where DMSP flux is negligible. Ten *dmdA* homologs were found in a metagenome from southeastern U.S. coastal water. Similar to the Sargasso Sea metagenome, the abundance of *dmdA* homologs in both these samples indicated that about a third of bacteria in surface ocean waters may participate in DMSP demethylation (Table 2).

The marine metagenomic surveys indicated that the majority of environmental *dmdA* homologs belonged to clades for which DMSP demethylase activity has not been experimentally verified (Table 2). To address this issue, the *P. ubique* HTCC1062 *dmdA* (gene SAR11_0246) was synthesized and introduced in trans into *Escherichia coli*. Cell-free extracts of the recombinant *E. coli* formed MMPA from DMSP (Table 1), confirming demethylation by a protein in the largest environmentally occurring clade (clade D).

DMSP synthesis is estimated to account for ~1 to 10% of global marine primary production (*20*), consistent with previous evidence that a large fraction of active marine bacteria assimilate sulfur from DMSP (*21*, *22*) and the wealth of DMSP demethylation genes we found in surface water bacterioplankton communities. The evidence that oceanic *dmdA* homologs are most similar to those from cultured SAR11 bacteria (50 to 65%, Table 2), whereas coastal homologs are most similar to those from cultured Roseobacters (90%), is consistent with known differences in the ecology and distribution of these two abundant bacterioplankton groups (*13*, *14*). This evidence further suggests that SAR11 bacteria may dominate demethylation in the open ocean, where DMSP concentrations are low (10 to 15 nM) and relatively constant, whereas Roseobacters may dominate in phytoplankton blooms and coastal regions, where DMSP concentrations are high (up to 100 nM) and more variable. Knowledge of the kinetic and ecological diversity of bacterial DMSP demethylases represented by these major marine taxa is critical to understanding both the routing of reduced carbon and sulfur into the microbial food web and the bacterial controls on ocean-atmosphere sulfur flux with consequences to global climate regulation.

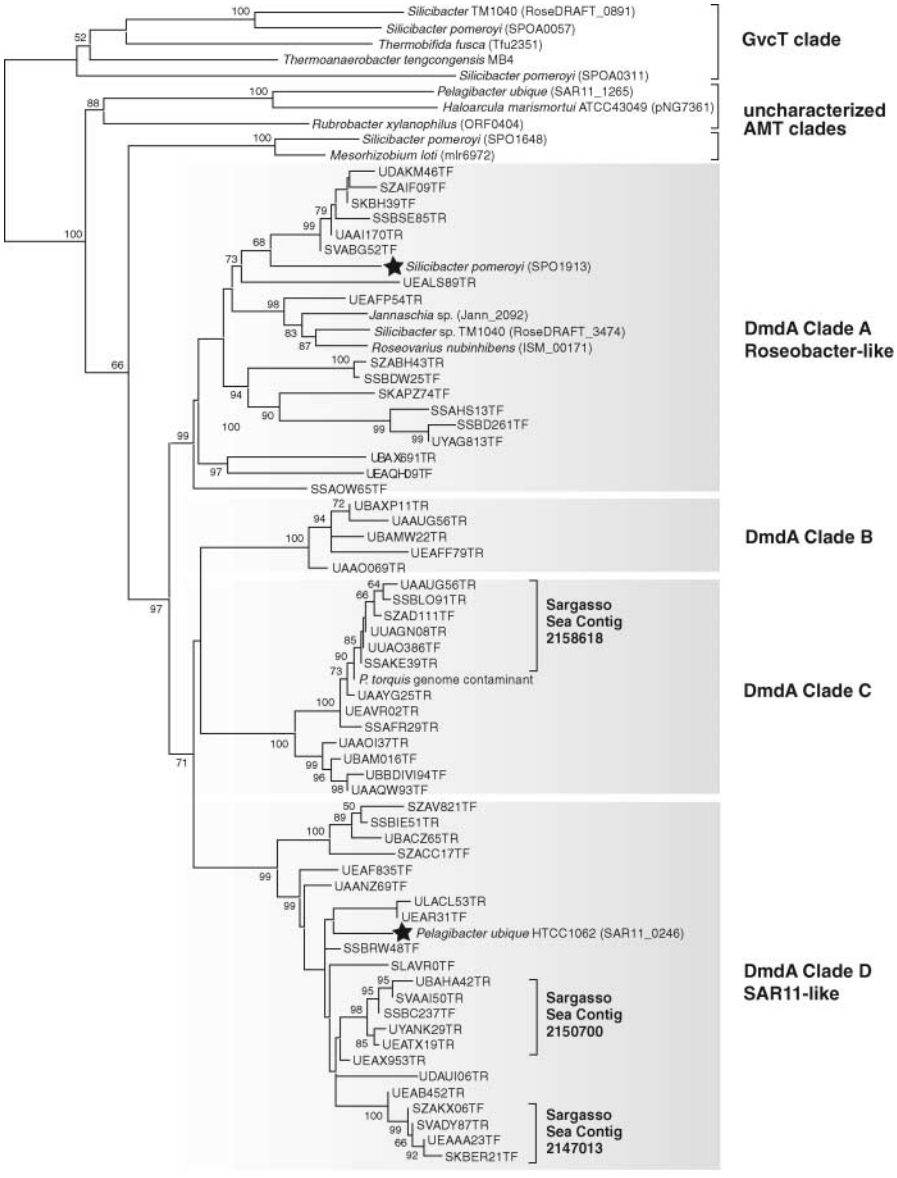

Fig. 2. Minimum evolution phylogenetic tree of amino acid sequences of glycine cleavage T-protein (GcvT) family proteins, including DmdA and related aminomethyltransferases (AMT). Sequences from cultured bacteria are labeled with organism name and gene designation. Selected Sargasso Sea metagenomic library sequences are identified by sequence identification and, if applicable, a contig designation. Proteins with confirmed DMSP demethylase activity are marked with a star. Percentage of 100 bootstrap samples supporting each node are shown if >50.

References and Notes

1. J. Stefels, *J. Sea Res.* **43**, 183 (2000).
2. G. V. Wolfe, M. Steinke, G. O. Kirst, *Nature* **387**, 894 (1997).
3. W. Sunda, J. Kieber, R. P. Kiene, S. Huntsman, *Nature* **418**, 317 (2002).
4. A. J. Kettle, M. O. Andreae, *Geophys. Res.* **105**, 26793 (2000).
5. M. O. Andreae, *Mar. Chem.* **30**, 1 (1990).
6. A. J. Kettle *et al.*, *Glob. Biogeochem. Cycles* **13**, 399 (1999).
7. R. Simó, *Trends Ecol. Evol.* **16**, 287 (2001).
8. J. M. González, R. P. Kiene, M. A. Moran, *Appl. Environ. Microbiol.* **65**, 3810 (1999).
9. P. T. Visscher, M. R. Diaz, B. F. Taylor, *Mar. Ecol. Prog. Ser.* **89**, 293 (1992).
10. R. P. Kiene, L. J. Linn, J. M. González, M. A. Moran, J. A. Bruton, *Appl. Environ. Microbiol.* **65**, 4549 (1999).
11. J. C. Venter *et al.*, *Science* **304**, 66 (2004).
12. R. P. Kiene, L. J. Linn, J. A. Bruton, *J. Sea Res.* **43**, 209 (2000).
13. M. A. Moran *et al.*, *Nature* **432**, 910 (2004).
14. S. J. Giovannoni *et al.*, *Science* **309**, 1242 (2005).
15. Gordon and Betty Moore Foundation Microbial Genome Sequencing Project (www.moore.org/microgenome/microb_list.asp).
16. The genome sequence of *P. torquis* ATCC 700755 includes contaminant sequences from several common marine taxa, as surmised from rDNA in unassembled reads. The partial *dmdA* homolog is located on a 1.4-kb fragment that does not assemble with the main *P. torquis* genome.
17. R. M. Morris *et al.*, *Limnol. Oceanogr.* **50**, 1687 (2005).
18. T. R. Miller, R. Belas, *Appl. Environ. Microbiol.* **70**, 3383 (2004).
19. E. F. DeLong *et al.*, *Science* **311**, 496 (2006).
20. On the basis of global emissions of DMS ranging from 0.47 to 1.03 Tmol per year (*4*) and the assumption that DMS emissions represent 1 to 4% of gross DMSP production, we estimated a global DMSP production rate of 11.7 to 103 Tmol of sulfur per year. Because each mole of DMSP contains 5 mol of carbon, the gross global DMSP production is 58.6 to 516 Tmol of carbon per year. With a global marine primary production of 3750 Tmol of carbon per year (*23*), 1.3 to 13.8% is in the form of DMSP. Similar contributions to marine carbon production can be estimated from DMSP:chlorophyll *a* and assumed C:chlorophyll *a* ratios (*12*).
21. M. Vila *et al.*, *Appl. Environ. Microbiol.* **70**, 4648 (2004).
22. R. R. Malmstrom, R. P. Kiene, M. T. Cottrell, D. L. Kirchman, *Appl. Environ. Microbiol.* **70**, 4129 (2004).
23. P. G. Falkowski, R. T. Barber, V. Smetacek, *Science* **281**, 200 (1998).

24. We thank P. Capes, R. Hein, and S. Napierala for technical assistance; K. Remington and S. Sun for bioinformatics assistance; R. Belas and E. Stabb for advice on genetic techniques; J. Wiegel for equipment access; J. Mou for access to Sapelo Island metagenomic data; and C. English for graphics support. This project was funded by grants from NSF (MCB-0315200 to M.A.M., W.B.W., and R.P.K.) and the Gordon and Betty Moore Foundation (to M.A.M.). Accession numbers: *dmdA*

sequences are available at NCBI under accession numbers AAV95190 (*S. pomeroyi*), ABF64177 (*Silicibacter* sp. TM1040), ABD55296 (*Jannaschia* sp. CCS1), EAP76657 (*Roseovarius nubinhibens* ISM), AAZ21068 (*P. ubique* HTCC1062), EAS85076 (*P. ubique* HTCC1002), EAS69357 (*P. torquis* genome sequence contaminant), DU750654 and DU737812 (Station Aloha metagenome), and DQ874604-DQ874613 (Sapelo Island metagenome).

Supporting Online Material
www.sciencemag.org/cgi/content/full/314/5799/649/DC1
Materials and Methods
Figs. S1 and S2
Table S1
References

31 May 2006; accepted 19 September 2006
10.1126/science.1130657

Dimethylsulfoniopropionate Uptake by Marine Phytoplankton

Maria Vila-Costa,[1]* Rafel Simó,[1]* Hyakubun Harada,[2] Josep M. Gasol,[1] Doris Slezak,[2] Ronald P. Kiene[2]

Dimethylsulfoniopropionate (DMSP) accounts for most of the organic sulfur fluxes from primary to secondary producers in marine microbial food webs. Incubations of natural communities and axenic cultures with radio-labeled DMSP showed that dominant phytoplankton groups of the ocean, the unicellular cyanobacteria *Prochlorococcus* and *Synechococcus* and diatoms, as well as heterotrophic bacteria take up and assimilate DMSP sulfur, thus diverting a proportion of plankton-produced organic sulfur from emission into the atmosphere.

Dimethylsulfoniopropionate (DMSP) is synthesized by ubiquitous phytoplankton taxa as a solute, probably for osmoregulatory and antioxidant purposes (*1*–*4*). DMSP is the precursor of the climate-active gas dimethylsulfide (DMS), the main natural source of sulfur to the global atmosphere and a major aerosol and cloud droplet precursor over the ocean (*5*–*7*). Enzymatic cleavage of DMSP into volatile DMS is the fate of only a fraction (generally <50%) of all DMSP produced (*8*). Recent research has revealed that algal DMSP plays an important role in food-web processes supplying sulfur and carbon to heterotrophic bacteria and, to a lesser extent, to microzooplankton herbivores (*9*–*12*). Thus, the biogeochemical fate and function of DMSP is largely determined by a switch between conversion into DMS and sulfur assimilation by microorganisms, which in turn depends on the composition, structure, and dynamics of the planktonic food web. The ability to assimilate DMSP sulfur seems to be widespread among taxa of heterotrophic bacterioplankton (*13*, *14*) and has also been observed in the cyanobacterium *Synechococcus* (*15*). Our work aimed to find out whether major non-DMSP–producing phytoplankton also assimilate DMSP sulfur.

To investigate the distribution of DMSP sulfur uptake and assimilation among picoplankton, we used flow cytometry cell sorting and measured assimilation by picophototrophs and heterotrophic bacteria by using radio-labeled DMSP. Surface seawater samples were collected from the coasts of the Gulf of Mexico, the northwest Mediterranean, and Gran Canaria Island and from the Sargasso Sea. After light and dark incubations with [^{35}S]DMSP, sample aliquots were passed through the flow cytometer and sorted into four major groups: heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, and autofluorescent picoeukaryotes. All groups showed some capability for assimilating DMSP sulfur (Fig. 1). The most notable DMSP sulfur assimilators were heterotrophic bacteria, followed by *Prochlorococcus*, *Synechococcus*, and picoeukaryotes. Incubation of samples in the light stimulated DMSP sulfur assimilation by picophototrophs by as much as a factor of 2.2. The phototrophs accounted for 10 to 34% of picoplanktonic DMSP consumption in the light, with the remaining 66 to 90% being carried out by heterotrophic bacteria.

Until now the only phototrophs for which DMSP use had been observed were *Synechococcus*. It seems that, in a similar way to that of heterotrophic bacteria (*10*) and *Synechococcus* (*15*), *Prochlorococcus* may also benefit from using a reduced sulfur source such as DMSP, probably by saving the energy required to reduce sulfate. Studies with cultured and natural assemblages of heterotrophic bacteria have provided evidence for a common membrane transporter for DMSP and glycine betaine (GBT) (*16*, *17*), and, interestingly, putative GBT transporter genes have been found in the genome of *P. marinus* MIT9313 (*18*).

In one of the samples (Gran Canaria Island), eukaryotic picophytoplankton also showed significant incorporation of ^{35}S from [^{35}S]DMSP (Fig. 1). It is possible, however, that some of these eukaryotes were mixotrophic bacterivores that had fed on ^{35}S-radio-labeled bacteria.

We used microautoradiography with [^{35}S]DMSP to follow DMSP sulfur assimilation by organisms larger than 5 μm collected during an annual time series in the coastal Mediterranean. Consistent with our flow-cytometric observations of picoeukaryotes, many phytoplankton cells, including dinoflagellates, cryptophytes, and diatoms, became radio-labeled (Fig. 2). Mixotrophy by bacterivory has been described for dinophytes, cryptophytes, and haptophytes (*19*), but not for diatoms, which consequently must have directly taken up ^{35}S from dissolved radio-labeled DMSP.

The DMSP-to-chlorophyll (DMSP:chl-*a*) ratio is a good indicator of how strong a DMSP producer a phytoplankton assemblage is and how much of the available carbon and sulfur are accounted for by DMSP (*9*, *11*). We found that the proportion of diatoms that had assimilated [^{35}S]DMSP sulfur followed a pattern very similar to that of independently measured DMSP:chl-*a* ratios from parallel samples (Fig. 3) through the annual course of sampling, with highest values observed in June and August. In other words, higher numbers of DMSP sulfur–assimilating diatoms did not occur when these phytoplankters were more abundant (late winter) but when DMSP was more abundant with respect to total sulfur and carbon fluxes (summer).

DMSP is a zwitterion that cannot cross cell membranes without a specific transporter (*17*). DMSP sulfur assimilation by diatoms implies, therefore, that either these algae have a DMSP transport system or they were taking up by-

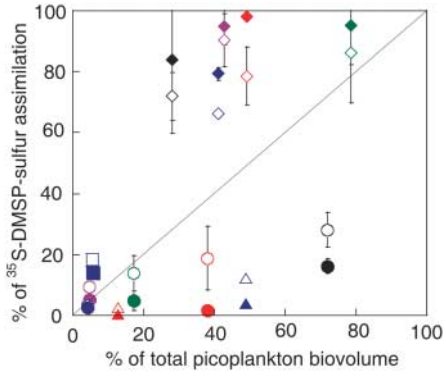

Fig. 1. Contribution of different groups of picoplankton to total picoplankton [^{35}S]DMSP assimilation versus their contribution to the total picoplankton biovolume. Solid and open symbols correspond to dark and light incubations, respectively (diamonds, heterotrophic bacteria; circles, *Synechococcus*; squares, *Prochlorococcus*; and triangles, picoeukaryotes). Green, Blanes Bay (northwest Mediterranean); black, off Dauphin Island (Gulf of Mexico); purple, Sargasso Sea; red, Pensacola beach (Gulf of Mexico); and blue, Gran Canaria Island. Error bars represent standard deviation of the mean (*n* values from 2 to 6). The 1:1 line is included as a reference.

[1]Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar (CSIC), Pg Marítim de la Barceloneta 37-49, 08003 Barcelona, Catalonia, Spain. [2]Department of Marine Sciences, University of South Alabama, Mobile, AL 36688, USA.

*To whom correspondence should be addressed. E-mail: mariavila@icm.csic.es (M.V.-C.); rsimo@icm.csic.es (R.S.)