GENETICS, DIVERSITY, AND DISTRIBUTION OF THE DIMETHYLSULFONIOPROPIONATE (DMSP) DEMETHYLASE IN MARINE BACTERIOPLANKTON

by

ERINN C. HOWARD

(Under the Direction of Mary Ann Moran)

ABSTRACT

The flux of dimethylsulfide (DMS) from ocean surface waters is the foremost natural source of sulfur to the atmosphere and influences climate via aerosol formation. Marine bacterioplankton can regulate sulfur flux by converting the precursor dimethylsulfoniopropionate (DMSP) either to DMS via a cleavage pathway or to sulfur compounds that are not climatically active via a demethylation pathway. The gene responsible for demethylating DMSP and diverting the sulfur to the climatically inactive form is identified here, and given the gene designation *dmdA*. The gene was present in cultured representatives of the Roseobacter and SAR11 taxa, suggesting these may be primary mediators of DMSP demethylation in the ocean. It was also found in the less abundant marine Rhodospiralles and in the OM60 clade of the gammaproteobacteria. Using metagenomic data from the 2007 Global Ocean Sampling (GOS) expedition, it was determined that 58% (±9%) of prokaryotic cells in marine surface waters contain a *dmdA* homolog, giving them the genetic capability to demethylate DMSP. This metagenomic data also revealed five DmdA protein clades, designated A-E. All clades except Clade B harbor a *dmdA* from a cultured marine taxon (A, Roseobacter and Rhodospiralles; C and

D, SAR11; E, gammaproteobacteria OM60). However, G+C content analysis indicates that most clades are taxonomically heterogeneous. An analysis of *dmdA* sequences over the course of an induced phytoplankton bloom in Gulf of Mexico surface waters revealed that *dmdA* diversity is high. Saturation for gene clusters at the 90% similarity level was not reached in a rarefaction analysis of ~90,000 sequences. The representation of the five recognized clades of *dmdA* did not shift substantially over the course of the phytoplankton bloom compared to non-bloom controls, and there was no evidence for "bloom" and "non-bloom" clades. These results may not be surprising in light of recent data showing similarity in the kinetic parameters of purified proteins from representatives of two different DmdA clades. The pool of *dmdA* genes was predictable based on the taxonomic composition of the bacterioplankton but not from environmental variables, including DMSP supply and concentration. Together these studies indicate that a large and diverse fraction of marine surface water bacterioplankton are capable of participating in DMSP demethylation, a critical step in the marine sulfur cycle.

INDEX WORDS: DmdA, DMSP, Dimethylsulfoniopropionate, MeSH, Methanethiol, Roseobacters, Sulfur, Marine Bacteria, Microbial ecology

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B.S., Illinois Wesleyan University, 2003

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2008

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December 2008

DEDICATION

I wish to dedicate this work to my greatest source of inspiration, support, love, and encouragement, my family, without whom I'd be lost; my wonderful mother and traveling companion, Patricia Ann (Bowling) Howard, my awesome brother, Joseph A. Howard, Jr., my Godmother, Marjorie Bowling, my grandparents Nana and Bob (Veronica and Robert Bowling), and all the rest of the clan. I love you all very much!

I also dedicate this work to my friend Bijal P. Patel, you will be forever missed.

ACKNOWLEDGEMENTS

I wish to thank my advisor, Mary Ann Moran, who has provided endless support and guidance; I couldn't have asked for a better advisor. I would also like to thank my committee members, Tim Hollibaugh, Eric Stabb and Barny Whitman for always challenging me and providing excellent feedback over the years. Thanks to Dr. Jon Dey who first sparked my interest in microbiology and encouraged me to pursue it.

I also wish to extend my gratitude to all my friends and colleagues who have supported, encouraged and assisted me, including: my entering microbiology classmates, Laura Williams, Elizabeth Burgess, Dana Cook, Theresa Rogers, Susmitha Seshadra, Noha Mesbah, Yuchen Liu, and Bijal Patel; my poker buddies, Clint Page, Andy Nuss, Ryan Aylsworth, Andrea Zbell, Ken Bradshaw, and Jess Edwards; all other marine science or microbiology friends, past and present, Chris Lasher, Wendy Ye, Ryan Brown, Jacob Shalack, Justine Lyons, Adair Johnson, Kathy (Loftis) Bowles, Vanessa Varaljay, Scott Gifford, Marshall Bowles, Cedric Fichot, Matt First, Kate Segarra, Megan Machmuller, Peter Baas, and everyone not mentioned here!

And finally I wish to thank my college roommate, Irene (Masiulis) Bowen, who always supported me, Bill Porubsky who always believes in me, and Erin Biers who is a great friend, confidant, source of encouragement, and a fellow Alias fan (seasons 1-3).

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Dimethylsulfoniopropionate (DMSP) is produced by various marine seaweeds and phytoplankton. It was first found in *Polysiphonia fastigiata* (11) and subsequently found in several marine algae, including *Polysiphonia lanosa* (10), *Enteromorpha intestinalis* (12), *Ulva lactuca* (12), as well as several non-marine plants (12). Findings suggest that DMSP is synthesized from methionine in these seaweeds and plants (2, 12, 48). Subsequent research showed DMSP production in more than just marine seaweeds, but also in marine phytoplankton (2, 68, 81). Due to the similar structure of DMSP to glycine betaine, a known osmolyte (31), combined with experimental support (17), it is hypothesized that DMSP is synthesized primarily for use as an osmolyte by marine phytoplankton (10, 68). Subsequent research showed that marine phytoplankton also can produce DMSP for use as a predator deterrent (82) and an antioxidant (63).

I. Climatic impacts of DMSP cleavage to dimethylsulfide (DMS)

Early studies concerning oceanic sulfur dynamics hypothesized hydrogen sulfide (H_2S) in the ocean surface as a significant source of sulfur to the atmosphere (29). However, the work of Lovelock, Maggs, and Rasmussen (46) found otherwise. Due to the fact that H_2S could not be detected in high concentrations in the atmosphere, and that ocean surface waters are too oxidizing to allow H_2S to be present in the concentrations necessary to support the atmospheric equilibrium necessary (28, 46, 47), Lovelock and colleagues (46) suggested that the largest source of sulfur from the ocean to the atmosphere is dimethylsulfide (DMS). Subsequently, a globally representative dataset on the distribution and emission of DMS from the oceans (6) determined that the exchange of DMS across the ocean-atmosphere boundary amounts to ~20 Tg of sulfur annually (33, 60). Due to early studies suggesting that bacteria were not likely involved in ocean nutrient dynamics, least of all DMSP cleavage (11, 85), and neglecting studies that showed marine bacteria being highly involved in the ocean food web as well as in DMSP degradation (56, 79), it was originally hypothesized that phytoplankton were the primary organisms degrading DMSP (6, 10, 13, 15, 66).

Once DMS is released from the ocean into the atmosphere, it primarily becomes oxidized to form non-sea-salt sulfate aerosol particles (SO_4^{2-}; 13, 46); intermediate oxidation products can include dimethylsulfoxide (DMSO), carbonyl sulfide (COS), methane sulfonate, and sulfide (SO_2^-; 2, 4, 13, 45). These particles are termed cloud condensation nuclei (CCN) and are the nuclei around which water droplets accumulate to form clouds (13, 80). While some evidence exists to the contrary (83), it is widely accepted that DMS production influences global climate in this fashion (3, 45).

The discovery that DMS is the primary natural source of sulfur from the ocean to the atmosphere and leads to cloud formation caused Charlson, Lovelock, Andreae, and Warren (13) to develop the CLAW hypothesis of DMSP degradation. The CLAW hypothesis suggests a negative feedback loop between phytoplankton DMSP production and DMS evolution with subsequent cloud formation. In this feedback loop, DMS released into the atmosphere leading to cloud formation acts to cover the ocean. This cloud cover allows less light to reach the ocean surface thus causing the decline of phytoplankton populations and DMSP produced. Less DMSP production means less is available for DMS production, release into the atmosphere, and subsequent cloud formation. This idea was the first DMSP degradation regulation hypothesis widely considered and is found in textbooks today (13, 18, 69).

However, initial studies attempting to correlate DMS production with chlorophyll (Chl *a*) concentrations were unsuccessful or weak, at best (6, 34, 59, 66, 67). Part of this failure is hypothesized to be from overlooking the significance of non-DMSP producing phytoplankton (60, 67, 74) and the fact that DMS released from one bloom would not lead to cloud formation directly over that same bloom (59). Clearly DMS production and Chl *a* concentration are part of an even more complex picture of DMSP degradation than was initially assumed (5, 60). To further complicate understanding, zooplankton grazing might obscure the relationship between phytoplankton concentrations and DMS release (15), and anaerobic sediment bacteria in estuaries can contribute to overall DMS (and methanethiol, see below) consumption (35) and production (70, 71). Coral reefs have also been implicated in DMS release (8). Finally, vertical mixing of the oceanic water column might play a role in DMS release from the ocean boundary (61). However these complicating factors may be minor issues for the Chl *a*:DMS ratio conundrum compared to the large number of more recent studies finding that bacterioplankton communities and individual bacterial isolates can actually degrade DMSP to DMS or MeSH (via MMPA; 44, 64, 76) and do this predominantly, in larger amounts than do phytoplankton (59, 87).

II. DMSP Demethylation: The alternative pathway

While focus had been primarily on DMS production from DMSP in marine waters, studies by Ledyard and Dacey (43) and Kiene (36), and others (50, 75, 86), found that DMSP was degraded more commonly via the demethylation pathway (~80%) as opposed to the cleavage pathway (~20%) in natural marine surface water communities. In the demethylation pathway, a methyl group is removed from the DMSP sulfur leaving 3-methyl mercaptopropionate (MMPA; 24, 36). In some bacteria, degradation proceeds further to methanethiol (MeSH) and acrylate or propionate (24, 71). The DMSP methyl group was found to be transferred to tetrahydrofolate and later to homocysteine, forming methionine (11, 32, 42, 78). This finding suggested that while DMS is the main source of sulfur from the ocean to the atmosphere, its production is rivaled by the demethylation of DMSP. DMSP demethylation effectively prohibits sulfur from becoming climatically active due to the removal of a methyl group and elimination of DMS as a possible degradation product (36, 64).

Studies of natural marine bacterioplankton communities found that, contrary to previous research suggesting that sulfate is the only sulfur source for bacterial communities (14), 15-40% of the DMSP-sulfur (via the demethylation pathway) actually becomes incorporated into sulfur-containing amino acids (24, 37, 39, 86), making DMSP an important sulfur source for marine bacterioplankton (61). It also represents an important carbon source for the marine microbial food web (30, 37, 38, 50, 84, 86). As well, DMSP is used by bacteria as a predator deterrent (82), an osmolyte (40, 55, 62), and an “anti-freeze” (40).

III. Marine bacterioplankton that degrade DMSP

Recent studies have focused on determining the groups of bacteria that are primarily responsible for DMSP consumption. The group found in abundance in DMSP-producing phytoplankton blooms and shown to be a consumer of DMSP-sulfur (via both pathways) is an alpha proteobacterial group, later determined to be the roseobacter clade (24-26, 51, 53, 54, 75). In the open ocean, 1 in 10 bacterial cells is a member of the roseobacter clade (21); this number increases to 1 in 5 cells in coastal or eutrophic waters (16, 25, 65, 77), making them dominant members of the surface ocean bacterial community (9, 16). This group of marine bacteria has been found to contain large genomes, hypothesized to allow greater flexibility in trophic strategy. They also harbor more rRNA operons than many other marine bacteria (7, 52, 53), hypothesized to give faster response times to fast-changing environmental conditions. Together, these characteristics make roseobacters a versatile group both metabolically and ecologically (1, 9, 41, 52, 53). By comparison, the ubiquitous SAR11 marine cluster of bacteria, also abundant and found to be important in DMSP degradation (26, 49, 75), contains small, highly streamlined genomes with just one rRNA operon. These characterisitics suggest SAR11s are optimized for highly oligotrophic conditions (1, 22, 52).

DMSP is degraded primarily by bacterioplankton in the surface ocean, and this degradation is primarily via the demethylation pathway (24, 36). While hypotheses exist (13, 62), studies have not yet been able to conclusively elucidate the regulation of the demethylation and cleavage degradation pathways. What causes a marine surface water bacterial community, or an individual bacterium to demethylate or cleave DMSP? The genes involved in the bacterial degradation of DMSP will provide effective tools for understanding the regulation of DMSP degradation and allow (via mRNA transcript and protein analysis) studies of the factors inducing gene expression. Because marine bacterioplankton communities demethylate ~80% of DMSP (24, 36), the first gene involved in demethylating DMSP to MMPA was selected as the focus of this dissertation. Roseobacters are valuable model organisms for this effort (23, 26, 57, 86). The first sequenced organism in the Roseobacter clade, *Silicibacter pomeroyi* DSS-3 (23), can both demethylate and cleave DMSP (24, 39), making it a prime candidate for gene-discovery research.

IV. Objectives

In the first study, a transposon-insertion mutant library using the model marine Roseobacter *Silicibacter pomeroyi* DSS-3 was employed to discover the DMSP demethylation gene. Other sequenced marine bacteria containing this gene, designated *dmdA*, were identified based on sequence identity and heterologous expression. The abundance of *dmdA* in surface water bacterioplankton from the Sargasso Sea was estimated from metagenomic sequences collected as part of the Global Ocean Sampling (73).

In the second study, the focus turned to the abundance, distribution, and diversity of *dmdA* in surface waters of the ocean. The 2007 release of the Global Ocean Sampling dataset provided an enormous resource of 7.7 million sequence reads (6.3 billion bp) from surface water bacterioplankton located along a transect from the Northwestern Atlantic to the Eastern Tropical Pacific Ocean (58). In addition to this data, newly sequenced genomes were mined to look for the presence of *dmdA*, and to characterize the neighboring genes. Finally, the %G+C content of these *dmdA*s from natural populations and cultured strains was examined to investigate the importance of horizontal gene transfer.

For the final study, a universal primer set was designed for the DMSP demethylation gene (72) and used to amplify *dmdA* throughout an induced phytoplankton bloom using Gulf of Mexico surface water. Relative changes in the diversity of this gene in conjunction with chemical data were tracked throughout the bloom. Along with the amplification of this gene, 16S rRNA genes from Bacteria and Archaea were also amplified to relate community composition changes to those of *dmdA*.

These studies set up the basis for further genetic and culture-based studies to understand the complex regulation and community interaction networks that determine the fate of DMSP in the ocean.

References

1. Alonso, C., and J. Pernthaler. 2006. *Roseobacter* and SAR11 dominate microbial glucose uptake in coastal North Sea waters. Environmental Microbiology 8:2022-2030.

2. Andreae, M. 1990. Ocean-atmosphere interactions in the global biogeochemical sulfur cycle. Marine Chemistry 30:1-29.

3. Andreae, M. O. 2007. Aerosols before pollution. Science 315:50-51.

4. Andreae, M. O., R. J. Charlson, F. Bruynseels, H. Storms, R. Van Grieken, and W. Maenhaut. 1986. Internal Mixture of Sea Salt, Silicates, and Excess Sulfate in Marine Aerosols Science 232:1620 - 1623.

5. Andreae, M. O., and P. J. Crutzen. 1997. Atmospheric aerosols: Biogeochemical sources and role in atmospheric chemistry. Science 276:1052-1058.

6. Andreae, M. O., and H. Raemdonck. 1983. Dimethyl Sulfide in the Surface Ocean and the Marine Atmosphere: A Global View. Science 221:744-747.

7. Biers, E. J., S. Sun, and E. C. Howard. Prokaryotic Genomes and Diversity in the Surface Ocean: Interrogating the Global Ocean Sampling Metagenome. Accepted *Appl Environ Microbiol*

8. Broadbent, A., and G. Jones. 2006. Seasonal and Diurnal Cycles of Dimethylsulfide, Dimethylsulfoniopropionate and Dimethylsulfoxide at One Tree Reef Lagoon. Environmental Chemistry 3:260-267.

9. Buchan, A., J. M. Gonzalez, and M. A. Moran. 2005. Overview of the Marine *Roseobacter* Lineage. Applied and Environmental Microbiology 71:5665-5677.

10. Cantoni, G. L., and D. G. Anderson. 1956. Enzymatic cleavage of dimethylpropiothetin by *Polysiphonia lanosa*. The Journal of Biological Chemistry 222:171-177.

11. Challenger, F. 1959. Aspects of the Organic Chemistry of Sulphur, vol. Butterworths Scientific Publications, London.

12. Challenger, F., R. Bywood, P. Thomas, and B. J. Hayward. 1957. Studies on Biological Methylation. XVII. The Natural Occurrence and Chemical Reactions of Some Thetins. Biochemistry and Biophysics 69:514-523.

13. Charlson, R. J., J. E. Lovelock, M. O. Andreae, and S. G. Warren. 1987. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326:655-661.

14. Cuhel, R. L., C. D. Taylor, and H. W. Jannasch. 1982. Assimilatory Sulfur Metabolism in Marine Microorganisms: Sulfur Metabolism, Protein Synthesis, and Growth of

Alteromonas luteo-violaceus and *Pseudomonas halodurans* During Perturbed Batch Growth Applied and Environmental Microbiology 43:151-159.

15. Dacey, J. W. H., and S. G. Wakeham. 1986. Ocean Dimethylsulfide: Production During Zooplankton Grazing on Phytoplankton. Science 233:1314-1316.

16. DeLong, E. F. 2005. Microbial Community Genomics in the Ocean. Nature Reviews: Microbiology 3:459-469.

17. Dickson, D. M. J., and G. O. Kirst. 1986. The role of b-dimethylsulphoniopropionate, glycine betaine and homarine in the osmoacclimation of Platymonas subcordiformis. Planta 167:536-543.

18. Duxbury, A. C., A. B. Duxbury, and K. A. Sverdrup. 2000. an introduction to the World's Oceans, 6 ed, vol. McGraw Hill, Boston.

19. Farrand, S. K., P. B. van Berkum, and P. Oger. 2003. Agrobacterium is a definable genus of the family Rhizobiaceae. International Journal of Systematic and Evolutionary Microbiology 53:1681–1687.

20. Galperin, M. Y. 2005. On the bottom of the deep blue sea. Environmental Microbiology 7:453-458.

21. Giovannoni, S., and M. Rappe. 2000. Evolution, Diversity, and Molecular Ecology of Marine Prokaryotes, p. 542. *In* D. L. Kirchman (ed.), Microbial ecology of the oceans. Wiley-Liss, New York.

22. Giovannoni, S. J., J. H. Tripp, S. Givan, M. Podar, K. L. Vergin, D. Baptista, L. Bibbs, J. Eads, T. H. Richardson, M. Noordewier, M. S. Rappe, J. M. Short, J. C. Carrington, and E. J. Mathur. 2005. Genome streamlining in a cosmopolitan oceanic bacterium. Science 309:1242-1245.

23. Gonzalez, J. M., J. S. Covert, W. B. Whitman, J. R. Henricksen, F. Mayer, B. Scharf, R. Schmitt, A. Buchan, J. A. Fuhrman, R. P. Kiene, and M. A. Moran. 2003. Silicibacter pomeroyi sp. nov. and Roseovarius nubinhibens sp. nov., dimethylsulfoniopropionate-demethylating bacteria from marine environments. International Journal of Systematic and Evolutionary Microbiology 53:1261-1269.

24. Gonzalez, 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*. Applied and Environmental Microbiology 65:3810-3819.

25. Gonzalez, J. M., and M. A. Moran. 1997. Numerical dominance of a group of marine bacteria in the alpha-subclass of the Class *Proteobacteria* in coastal seawater. Applied and Environmental Microbiology 63:4237-4242.

26. Gonzalez, J. M., R. Simo, R. Massana, J. S. Covert, E. O. Casamayor, C. Pedros-Alio, and M. A. Moran. 2000. Bacterial Community Structure Associated wtih a Dimethylsulfoniopropionate-Producing North Atlantic Algal Bloom. Applied and Environmental Microbiology 66:4237-4246.

27. Goodner, B., Gregory Hinkle, Stacie Gattung, Nancy Miller, Mary Blanchard, Barbara Qurollo, and Y. C. Barry S. Goldman, Manor Askenazi, Conrad Halling, Lori Mullin, Kathryn Houmiel, Jeffrey Gordon, Mark Vaudin, Oleg Iartchouk, Andrew Epp, Fang Liu, Clifford Wollam, Mike Allinger, Dahlia Doughty, Charlaine Scott, Courtney Lappas, Brian Markelz, Casey Flanagan, Chris Crowell, Jordan Gurson, Caroline Lomo, Carolyn Sear, Graham Strub, Chris Cielo, Steven Slater. 2001. Genome Sequence of the Plant Pathogen and Biotechnology Agent Agrobacterium tumefaciens C58. Science 294:2323-2328.

28. Gote Ostlund, H., and J. Alexander. 1963. Oxidation rate of sulfide in sea water; a preliminary study. Journal of Geophysical Research 68:3995.

29. Hartmann Hansen, M., K. Ingvorsen, and B. Barker Jpgensen. 1978. Meohanisms of hydrogen sulfide release from coastal marine sediments to the atmosphere. Limnology and Oceanography 23:68-76.

30. Howard, E. C., J. R. Henriksen, A. Buchan, C. R. Reisch, H. Bürgmann, R. Welsh, W. Ye, J. M. González, K. Mace, S. B. Joye, R. P. Kiene, W. B. Whitman, and M. A. Moran. 2006. Bacterial Taxa That Limit Sulfur Flux from the Ocean. Science 314:649-652.

31. Imhoff, J. F., and F. Rodriguez-Valera. 1984. Betaine is the main compatible solute for halophilic eubacteria. Journal of Bacteriology 160:478-479.

32. Jansen, M., and T. A. Hansen. 1998. Tetrahydrofolate serves as a methyl acceptor in the demethylation of dimethylsulfoniopropionate in cell extracts of sulfate-reducing bacteria. Archives Microbiology 169:84-87.

33. Kettle, A. J., and M. O. Andreae. 2000. Flux of dimethylsulfide from the oceans: A comparison of updated data sets and flux models. Journal of Geophysical Research 105:26793-26808.

34. Kettle, A. J. A., M. O.; Amouroux, D.; Andreae, T. W.; Bates, T. S.; Berresheim, H.; Bingemer, H.; Boniforti, R.; Curran, M. A. J.; DiTullio, G. R.; Helas, G.; Jones, G. B.; Keller, M. D.; Kiene, R. P.; Leck, C.; Levasseur, M.; Malin, G.; Maspero, M.; Matrai, P.; McTaggart, A. R.; Mihalopoulos, N.; Nguyen, B. C.; Novo, A.; Putaud, J. P.; Rapsomanikis, S.; Roberts, G.; Schebeske, G.; Sharma, S.; Simó, R.; Staubes, R.; Turner, S.; Uher, G. 1999. A global database of sea surface dimethylsulfide (DMS) measurements and a procedure to predict sea surface DMS as a function of latitude, longitude, and month. Global Biogeochemical Cycles 12:399-444.

35. Kiene, R. P. 1987. Dimethyl sulfide metabolism in salt marsh sediments. FEMS Microbiology Letters 53:71-78.

36. Kiene, R. P. 1996. Production of methanethiol from dimethylsulfoniopropionate in marine surface waters. Marine Chemistry 54:69-83.

37. Kiene, R. P., and L. J. Linn. 2000. Distribution and Turnover of Dissolved DMSP and Its Relationship with Bacterial Production and Dimethylsulfide in the Gulf of Mexico. Limnology and Oceanography 45:849-861.

38. Kiene, R. P., L. J. Linn, and J. A. Bruton. 2000. New and important roles for DMSP in marine microbial communities. Journal of SEA Research 43:209-224.

39. Kiene, R. P., L. J. Linn, J. M. Gonzalez, M. A. Moran, and J. A. Bruton. 1999. Dimethylsulfoniopropionate and methanethiol are important precursors of methionine and protein-sulfur in marine bacterioplankton. Applied and Environmental Microbiology 65:4549-4558.

40. Kirst, G. O., C. Thiel, H. Wolff, J. Nothnagel, M. Wanzek, and R. Ulmke. 1991. Dimethylsulfoniopropionate (DMSP) in ice-algae and its possible biological role. Marine Chemistry 35:381-388.

41. Konstantinidis, K. R., and J. M. Tiedje. 2004. Trends between gene content and genome size in prokaryotic species with larger genomes. Proceedings of the National Academy of Sciences 101:3160-3165.

42. Larrabee, A. R., S. Rosenthal, R. E. Cathou, and J. M. Buchanan. 1961. A Methylated Derivative of Tetrahydrofolate as an Intermediate of Methionine Biosynthesis. Journal of the American Chemical Society 83:4094-4095.

43. Ledyard, K. M., and J. W. H. Dacey. 1996. Microbial cycling of DMSP and DMS in Coastal and Oligotrophic Seawater. Limnology and Oceanography 41:33-40.

44. Ledyard, K. M., E. F. DeLong, and J. W. H. Dacey. 1993. Characterization of a DMSP-degrading bacterial isolate from the Sargasso Sea. Archives Microbiology 160:312-318.

45. Liss, P. S. 2007. Trace gas emissions from the marine biosphere. Philosophical Transactions of the Royal Society A 365:1697-1704.

46. Lovelock, J. E., R. J. Maggs, and R. A. Rasmussen. 1972. Atmospheric Dimethyl Sulphide and that Natural Sulphur Cycle. Nature 237:452-453.

47. Luther, G. W. I., and E. Tsamakis. 1989. Concentration and form of dissolved sulfide in the oxic water column of the ocean. Marine Chemistry 27:165-177.

48. Malin, G., and G. O. Kirst. 1997. Algal Production of Dimethyl Sulfide and its Atmospheric Role. Journal of Phycology 33:889-896.

49. Malmstrom, R. R., R. P. Kiene, M. T. Cottrell, and D. L. Kirchman. 2004. Contribution of SAR11 bacteria to dissolved dimethylsulfoniopropionate and amino acid uptake in the north atlantic ocean. Applied and Environmental Microbiology 70:4129-4135.

50. Merzouk, A., M. Levasseur, M. G. Scarratt, S. Michaud, R. B. Rivkin, M. S. Hale, R. P. Kiene, N. M. Price, and W. K. W. Li. 2006. DMSP and DMS dynamics during a mesoscale iron fertilization experiment in the Northeast Pacific-PartII: Biological cycling. Deep-Sea Research II 53:2370-2383.

51. Miller, T. R., and R. Belas. 2004. Dimethylsulfoniopropionate Metabolism by *Pfiesteria*-Associated *Roseobacter* spp. Applied and Environmental Microbiology 70:3383-3391.

52. Moran, M. A., R. Belas, M. A. Schell, J. M. Gonza´lez, F. Sun, S. Sun, B. J. Binder, J. Edmonds, W. Ye, B. Orcutt, E. C. Howard, C. Meile, W. Palefsky, A. Goesmann, Q. Ren, I. Paulsen, L. E. Ulrich, L. S. Thompson, E. Saunders, and A. Buchan. 2007. Ecological Genomics of Marine Roseobacters. Applied and Environmental Microbiology 73:4559-4569.

53. Moran, M. A., A. Buchan, J. M. Gonzalez, J. F. Heidelberg, W. B. Whitman, R. P. Kiene, J. R. Henricksen, G. M. King, R. Belas, C. Fuqua, L. Brinkac, M. Lewis, S. Johrl, B.

Weaver, G. Pai, J. A. Eisen, E. Rahe, W. M. Sheldon, W. Ye, T. R. Miller, J. Carlton, D. A. Rasko, I. T. Paulsen, Q. Ren, S. C. Daugherty, R. T. Deboy, R. J. Dodson, A. S. Durkin, R. Madupu, W. C. Nelson, S. A. Sullivan, M. J. Rosovitz, D. H. Haft, J. Selengut, and N. Ward. 2004. Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. Nature 432:910-913.

54. Moran, M. A., J. M. Gonzalez, and R. P. Kiene. 2002. Linking a bacterial taxon to sulfur cycling in the sea: Studies of the marine *Roseobacter* group. Geomicrobiology Journal 20:375-388.

55. Pichereau, V., J. A. Pocard, J. Hamelin, C. Blanco, and T. Bernard. 1998. Differential effects of dimethylsulfoniopropionate, dimethylsulfonioacetate, and other S-methylated compounds on the growth of Sinorhizobium meliloti at low and high osmolarities. Applied and Environmental Microbiology 64:1420-1429.

56. Pomeroy, L. R. 1974. The Ocean's Food Web, A Changing Paradigm. Bioscience 24:499-504.

57. Rappe, M. S., P. F. Kemp, and S. J. Giovannoni. 1997. Phylogenetic Diversity of Marine Coastal Picoplankton 16S rRNA Genes Cloned from the Continental Shelf Off Cape Hatteras, North Carolina. Limnology and Oceanography 42:811-826.

58. Rusch, D. B., A. L. Halpern, G. Sutton, K. B. Heidelberg, S. Williamson, S. Yooseph, D. Wu, J. A. Eisen, J. M. Hoffman, K. Remington, K. Beeson, B. Tran, H. Smith, H. Baden-Tillson, C. Stewart, J. Thorpe, J. Freeman, C. Andrews-Pfannkoch, J. E. Venter, K. Li, S. Kravitz, J. F. Heidelberg, T. Utterback, Y.-H. Rogers, L. I. Falcon, V. Souza, G. Bonilla-Rosso, L. E. Eguiarte, D. M. Karl, S. Sathyendranath, T. Platt, E. Bermingham, V. Gallardo, G. Tamayo-Castillo, M. R. Ferrari, R. L. Strausberg, K. Nealson, R. Friedman, M. Frazier, and J. C. Venter. 2007. The *Sorcerer II* Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. PLoS Biology 5:0398-0431.

59. Simo, R. 2001. Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological, evolutionary links. TRENDS in Ecology and Evolution 16:287-294.

60. Simo, R., and J. Dachs. 2002. Global ocean emission of dimethylsulfide predicted from biogeophysical data. Global Biogeochemical Cycles 16:1018.

61. Simo, R., and C. Pedros-Alio. 1999. Role of vertical mixing in controlling the oceanic production of dimethyl sulphide. Letters to Nature 402:396-399.

62. Stefels, J. 2000. Physiological aspects of the production and conversion of DMSP in marine algae and higher plants Journal of SEA Research 43:183-197.

63. Sunda, W., D. J. Kieber, R. P. Kiene, and S. Huntsman. 2002. An antioxidant function for DMSP and DMS in marine alga. Nature 418:317-320.

64. Taylor, B. F., and D. C. Gilchrist. 1991. New Routes for Aerobic Biodegradation of Dimethylsulfoniopropionate. Applied and Environmental Microbiology 57:3581-3584.

65. Teira, E., J. M. Gasol, M. Aranguren-Gassis, A. Fernandez, J. Gonzalez, I. Lekunberri, and X. A. Alvarez-Salgado. 2008. Linkages between bacterioplankton community composition, heterotrophic carbon cycling and environmental conditions in a highly dynamic coastal ecosystem. Environmental Microbiology 10:906-917.

66. Turner, S. M., and P. S. Liss. 1985. Measurements of Various Sulphur Gases in a Coastal Marine Environment. Journal of Atmospheric Chemistry 2:223-232.

67. 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. Limnology and Oceanography 33:364-375.

68. Vairavamurthy, A., M. O. Andreae, and R. L. Iverson. 1985. Biosynthesis of dimethylsulfide and dimethylpropiothetin by *Hymenomonas carterae* in relation to sulfur source and salinity variations. Limnology and Oceanography 30:59-70.

69. Vallina, S. M., and R. Simo. 2007. Strong relationship between DMS and the solar radiation dose over the global surface ocean. Science 315:506-508.

70. VAN DER Maarel, M. J. E. C., S. v. Bergeijk, A. F. v. Werkhoven, A. M. Laverman, W. G. Meijer, W. T. Stam, and T. A. Hansen. 1996. Cleavage of dimethylsulfoniopropionate and reduction of acrylate by *Desulfovibrio acrylicus* sp. nov. Archives Microbiology 166:109-115.

71. VAN DER Maarel, M. J. E. C., M. Jansen, R. Haanstra, W. G. Meijer, and T. A. Hansen. 1996. Demethylation of Dimethylsulfoniopropionate to 3-*S*-Methylmercaptopropionate by Marine Sulfate-Reducing Bacteria. Applied and Environmental Microbiology 62:3978-3984.

72. Varaljay, V. A., E. C. Howard, S. Sun, and M. A. Moran. in prep. Diversity of Dimethylsulfoniopropionate Demethylase Genes in a Marine Ecosystem Assessed by Deep Amplicon Sequencing of *dmdA* Primer Sets.

73. Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y.-H. Rogers, and H. O. Smith. 2004. Environmental Genome Shotgun Sequencing of the Sargasso Sea. Science 304:66-74.

74. Vila-Costa, M., R. P. Kiene, and R. Simo. 2008. Seasonal variability of the dynamics of dimethylated sulfur compounds in a coastal northwest Mediterranean site. Limnology and Oceanography 52:198-211.

75. Vila, M., R. Simo, R. P. Kiene, J. Pinhassi, J. M. Gonzalez, M. A. Moran, and C. Pedros-Alio. 2004. Use of Microautoradiography combined with fluorescence In Situ Hybridization to Determine Dimethylsulfoniopropionate Incorporation by Marine Bacterioplankton Taxa. Applied and Environmental Microbiology 70:4648-4657.

76. Visscher, P. T., M. R. Diaz, and B. F. Taylor. 1992. Enumeration of bacteria which cleave or demethylate dimethylsulfoniopropionate in the Caribbean Sea. Marine Ecology Progress Series 89:293-296.

77. Wagner-Dobler, I., and H. Biebl. 2006. Environmental Biology of the Marine Roseobacter Lineage. Annual Review of Microbiology 60:255-280.

78. Wagner, C., S. M. J. Lusty, H.-F. Kung, and N. L. Rogers. 1966. Trimethylsulfonium-Tetrahydrofolate Methyltransferase, a Novel Enzyme in the Utilization of 1 Carbon Units The Journal of Biological Chemistry 241:1923-1925.

79. Wagner, C., and E. R. Stadtman. 1962. Bacterial Fermentation of Dimethyl-beta-propiothetin. Archives of Biochemistry and Biophysics 98:331-336.

80. Welsh, D. T. 2000. Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate. FEMS Microbiology Reviews 24:263-290.

81. Wolfe, G. V., E. B. Sherr, and B. F. Sherr. 1994. Release and consumption of DMSP from *Emiliania huxleyi* during grazing by *Oxyrrhis marina*. Marine Ecology Progress Series 111:111-119.

82. Wolfe, G. V., M. Steinke, and G. O. Kirst. 1997. Grazing-activated chemical defence in a unicellular marine alga. Nature 387:894-897.

83. Wolff, E. W., H. Fischer, F. Fundel, U. Ruth, B. Twarloh, G. C. Littot, R. Mulvaney, R. Rathlisberger, M. de Angelis, C. F. Boutron, M. Hansson, U. Jonsell, M. A. Hutterli, F. Lambert, P. Kaufmann, B. Stauffer, T. F. Stocker, J. P. Steffensen, M. Bigler, M. L. Siggaard-Andersen, R. Udisti, S. Becagli, E. Castellano, M. Severi, D. Wagenbach, C. Barbante, P. Gabrielli, and V. Gaspari. 2006. Southern Ocean sea-ice extent, productivity and iron flux over the past eight glacial cycles. Nature 440:491-496.

84. Yoch, D. C. 2002. Dimethylsulfoniopropionate: Its sources, Role in the Marine Food Web, and Biological Degradation to Dimethylsulfide. Applied and Environmental Microbiology 68:5804-5815.

85. Zobell, C. E. 1935. Microbiological Activities at Low Temperatures with Particular Reference to Marine Bacteria. Quarterly Review of Biology 9:460-466.

86. Zubkov, M. V., B. M. Fuchs, S. D. Archer, R. P. Kiene, R. Amann, and P. H. Burkill. 2001. Linking the composition of bacterioplankton to rapid turnover of dissolved

dimethylsulfoniopropionate in an algal bloom in the North Sea. Environmental Microbiology 3:304-311.

87. Zubkov, M. V., B. M. Fuchs, S. D. Archer, R. P. Kiene, R. Amann, and P. H. Burkill. 2002. Rapid turnover of dissolved DMS and DMSP by defined bacterioplankton communities in the stratified euphotic zone of the North Sea. Deep-Sea Research II 49:3017-3038.

CHAPTER 2

BACTERIAL TAXA THAT LIMIT SULFUR FLUX FROM THE OCEAN[1]

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[1] Howard, Erinn C., James R. Henriksen, Alison Buchan, Chris R. Reisch, Helmut Burgmann, Rory Welsh, Wenying Ye, Jose M. Gonzalez, Kimberly Mace, Samantha B. Joye, Ronald P. Kiene, William B. Whitman, and Mary Ann Moran. 2006. *Science*. 314: 649-652.

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Abstract

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). 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) (Figure 2.3 in Appendix A; 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 Tn5-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 (Figure 2.1), a gene encoding a protein in the glycine cleavage T-protein family (Pfam PF01571, Enzyme Commission number 2.1.2.10 [EC]). DMSP degradation to MeSH was restored by complementation of the mutant in trans with an intact SPO1913 gene (Figure 2.4 in Appendix A). 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 2.1). This DMSP demethylase gene was designated *dmdA*.

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) (Figure 2.1). One other partial *dmdA* sequence (Figure 2.1) 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 2.2). The Sargasso sequences formed four clades distinct from other glycine cleavage T-protein family proteins (Figure 2.2). Clade A sequences clustered with DMSP demethylases from *S. pomeroyi* and other Roseobacters (Table 2.3 in Appendix A). Based on the number of clade A homologs relative to Roseobacter-like 16S 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 (Figure 2.1). Based on the number of clade D homologs relative to SAR11-like 16S 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 (Figure 2.1). Previous studies have shown that MMPA can be metabolized to MeSH or MPA in seawater (Figure 2.3 in Appendix A; 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 (Figure 2.1).

Although sequence coverage is small compared with that of the Sargasso Sea data set (Table 2.2), other marine metagenomic databases contain evidence of DMSP demethylase genes. 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 2.2).

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 2.2). 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 2.1), 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 2.2), 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.

References and Notes

1. Stefels, J. 2000. Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. J. Sea Res. 43: 183.

2. Wolfe, G. V., M. Steinke, and G. O. Kirst. 1997. Grazing-activated chemical defence in a unicellular marine alga. Nature 387: 894.

3. Sunda, W., J. Kieber, R. P. Kiene, and S. Huntsman. 2002. An antioxidant function for DMSP and DMS in marine alga. Nature 418: 317.

4. Kettle, A.J. and M. O. Andreae. Flux of dimethylsulfide from the oceans: A comparison of updated data sets and flux models. Geophys. Res. 105: 26793.

5. Andreae, M.O. Ocean-atmosphere interactions in the global biogeochemical sulfur cycle. Mar. Chem. 30: 1.

6. Kettle, A.J., D. Amouroux, T.W. Andreae, T.S. Bates, H. Berresheim, H. Bingemer, R. Boniforti, M.A.J. Curran, G.R. DiTullio, G. Helas, G. B. Jones, M.D. Keller, R.P. Kiene, C. Leck, M. Levasseur, G. Malin, M. Maspero, P. Matrai, A.R. McTaggart, N. Mihalopoulos, B.C. Nguyen, A. Novo, J.P. Putaud, S. Rapsomanikis, G. Roberts, G. Schebeske, S. Sharma, R. Simó, R. Staubes, S. Turner, and G. Uher. 1999. A global data base of sea surface dimethylsulfide (DMS) measurements and a procedure to predict sea

surface DMS as a function of latitude, longitude, and month. Glob. Biogeochem. Cycles 13: 399.

7. R. Simó. 2001. Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological, evolutionary links. Trends Ecol. Evol. 16: 287.

8. 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.

9. Visscher, P.T., M. R. Diaz, and B. F. Taylor. 1992. Enumeration of bacteria which cleave or demethylate dimethylsulfoniopropionate in the caribbean sea. Mar. Ecol. Prog. Ser. 89: 293.

10. Kiene, R.P., L. J. Linn, J. M. González, M. A. Moran, and J. A. Bruton. Dimethylsulfoniopropionate and methanethiol are important precursors of methionine and protein-sulfur in marine bacterioplankton. 1999. Appl. Environ. Microbiol. 65: 4549.

11. Venter, J. C., K. Remington, J. F. Heidelberg, A.L. Halpern, D. Rusch, J.A. Eisen, D. Wu, I. Paulsen, K.E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A.H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y.-H. Rogers, and H. O. Smith. 2004 Environmental genome shotgun sequencing of the Sargasso Sea. Science 304: 66.

12. Kiene, R.P., L. J. Linn, and J. A. Bruton. 2000. New and important roles for DMSP in marine microbial communities. J. Sea Res. 43: 209.

13. Moran, M. A., A. Buchan, J. M. Gonzalez, J. F. Heidelberg, W. B. Whitman, R. P. Kiene, J. R. Henricksen, G. M. King, R. Belas, C. Fuqua, L. Brinkac, M. Lewis, S. Johrl, B. Weaver, G. Pai, J. A. Eisen, E. Rahe, W. M. Sheldon, W. Ye, T. R. Miller, J. Carlton, D. A. Rasko, I. T. Paulsen, Q. Ren, S. C. Daugherty, R. T. Deboy, R. J. Dodson, A. S. Durkin, R. Madupu, W. C. Nelson, S. A. Sullivan, M. J. Rosovitz, D. H. Haft, J. Selengut, and N. Ward. 2004. Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. Nature 432: 910-913.

14. Giovannoni, S. J., J. H. Tripp, S. Givan, M. Podar, K. L. Vergin, D. Baptista, L. Bibbs, J. Eads, T. H. Richardson, M. Noordewier, M. S. Rappe, J. M. Short, J. C. Carrington, and E. J. Mathur. 2005. Genome streamlining in a cosmopolitan oceanic bacterium. Science 309: 1242-1245.

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. Morris, R. M., K.L. Vergin, J.-C. Cho, M.S. Rappé, C.A. Carlson, and S.J. Giovannoni. 2005. Temporal and spatial response of bacterioplankton lineages to annual convective overturn at the Bermuda Atlantic Time-series Study site. Limnol. Oceanogr. 50: 1687.

18. Miller, T.R. and R. Belas. 2004. Dimethylsulfoniopropionate Metabolism by *Pfiesteria*-Associated *Roseobacter* spp. Appl. Environ. Microbiol. 70: 3383.

19. DeLong, E.F., C.M. Preston, T. Mincer, V. Rich, S.J. Hallam, N.-U. Frigaard, A. Martinez, M.B. Sullivan, R. Edwards, B.R. Brito, S.W. Chisholm, and D.M. Karl. 2006. Science 311: 496.

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. Vila, M., R. Simo, R. P. Kiene, J. Pinhassi, J. M. Gonzalez, M. A. Moran, and C. Pedros-Alio. 2004. Use of Microautoradiography combined with fluorescence In Situ

Hybridization to Determine Dimethylsulfoniopropionate Incorporation by Marine Bacterioplankton Taxa. Applied and Environmental Microbiology 70:4648-4657.

22. Malmstrom, R. R., R. P. Kiene, M. T. Cottrell, and D. L. Kirchman. 2004. Contribution of SAR11 bacteria to dissolved dimethylsulfoniopropionate and amino acid uptake in the north atlantic ocean. Applied and Environmental Microbiology 70:4129-4135.

23. Falkowski, P.G, R. T. Barber, and V. Smetacek. Biogeochemical controls and feedbacks on ocean primary production. 1998. Science 281: 200.

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).

Table 2.1. 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 $\mathrm{protein}^{-1}$) 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 $\mathrm{protein}^{-1}$. 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, Tn5 inactivation of SPO1913	0
E. coli with pABX101, recombinant SAR11_0246	0.24 ± 0.05
E. coli with pCYB1, vector alone	0

Table 2.2. 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)	1,626	29	18	83	247	377	1029	37
Station Aloha (oceanic) photic zone	24.8	1	0	0	2	5	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

Figure 2.1. 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.

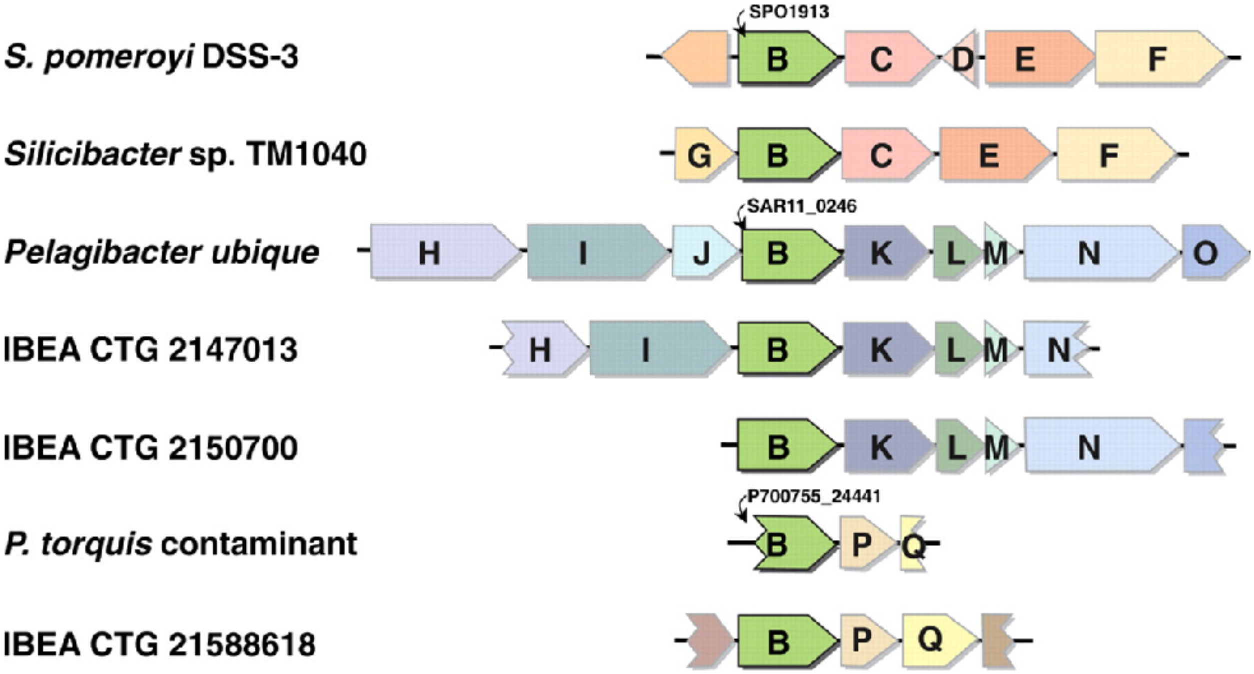
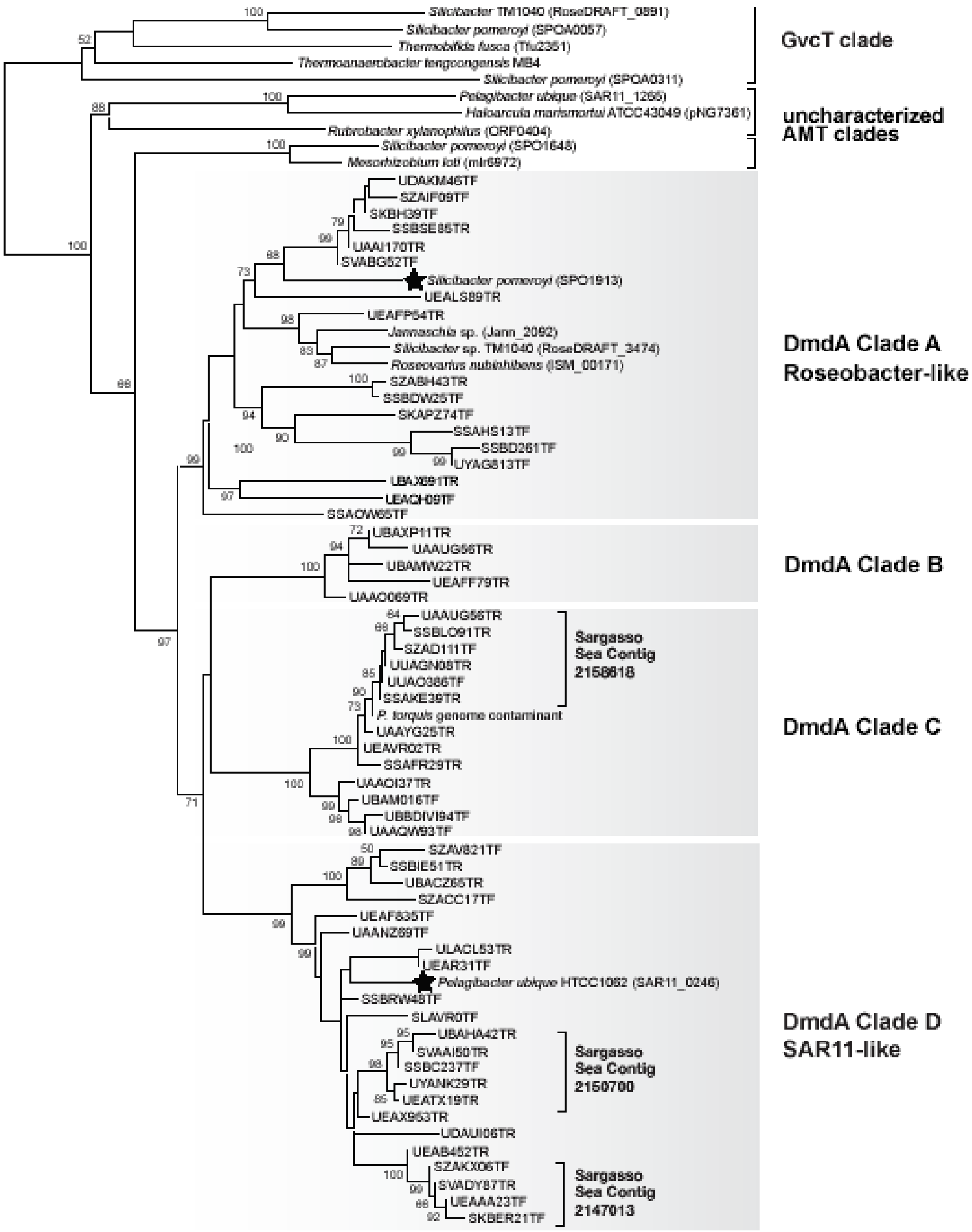

S. pomeroyi DSS-3
SPO1913
B C D E F
Silicibacter sp. TM1040
G B C E F
Pelagibacter ubique
SAR11_0246
H I J B K L M N O
IBEA CTG 2147013
H I B K L M N
IBEA CTG 2150700
B K L M N
P. torquis contaminant
P700755_24441
B P Q
IBEA CTG 21588618
B P Q

Figure 2.2. 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.


Silicibacter TM1040 (RoseDRAFT_0891)
Silicibacter pomeroyi (SPOA0057)
Thermobifida fusca (Tfu2351)
Thermoanaerobacter tengcongensis MB4
Silicibacter pomeroyi (SPOA0311)
GvcT clade
Pelagibacter ubique (SAR11_1265)
Haloarcula marismortui ATCC43049 (pNG7361)
Rubrobacter xylanophilus (ORF0404)
uncharacterized AMT clades
Silicibacter pomeroyi (SPO1648)
Mesorhizobium loti (mlr6972)
UDAKM46TF
SZAIF09TF
SKBH39TF
SSBSE85TR
UAAI170TR
SVABG52TF
Silicibacter pomeroyi (SPO1913)
UEALS89TR
UEAFP54TR
Jannaschia sp. (Jann_2092)
Silicibacter sp. TM1040 (RoseDRAFT_3474)
Roseovarius nubinhibens (ISM_00171)
SZABH43TR
SSBDW25TF
SKAPZ74TF
SSAHS13TF
SSBD261TF
UYAG813TF
UBAX691TR
UEAQH09TF
SSAOW65TF
DmdA Clade A Roseobacter-like
UBAXP11TR
UAAUG56TR
UBAMW22TR
UEAFF79TR
UAAO069TR
DmdA Clade B
UAAUG56TR
SSBLO91TR
SZAD111TF
UUAGN08TR
UUAO386TF
SSAKE39TR
P. torquis genome contaminant
UAAYG25TR
UEAVR02TR
SSAFR29TR
UAAOI37TR
UBAM016TF
UBBDIVI94TF
UAAQW93TF
Sargasso Sea Contig 2158618
DmdA Clade C
SZAV821TF
SSBIE51TR
UBACZ65TR
SZACC17TF
UEAF835TF
UAANZ69TF
ULACL53TR
UEAR31TF
Pelagibacter ubique HTCC1062 (SAR11_0246)
SSBRW48TF
SLAVR0TF
UBAHA42TR
SVAAI50TR
SSBC237TF
UYANK29TR
UEATX19TR
UEAX963TR
UDAUI06TR
UEAB452TR
SZAKX06TF
SVADY87TR
UEAAA23TF
SKBER21TF
Sargasso Sea Contig 2150700
Sargasso Sea Contig 2147013
DmdA Clade D SAR11-like

CHAPTER 3

ABUNDANT AND DIVERSE BACTERIA INVOLVED IN DMSP DEGRADATION IN MARINE SURFACE WATERS[1]

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[1] Howard, Erinn C., Shulei Sun, Erin J. Biers, and Mary Ann Moran. 2008. *Environmental Microbiology*. 10(9): 2397-2410.

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Abstract

An expanded analysis of oceanic metagenomic data indicates that the majority of prokaryotic cells in marine surface waters have the genetic capability to demethylate dimethylsulfoniopropionate (DMSP). The 1701 homologues of the DMSP demethylase gene, *dmdA*, identified in the (2007) Global Ocean Sampling (GOS) metagenome, are sufficient for 58% (±9%) of sampled cells to participate in this critical step in the marine sulfur cycle. This remarkable frequency of DMSP-demethylating cells is in accordance with biogeochemical data indicating that marine phytoplankton direct up to 10% of fixed carbon to DMSP synthesis, and that most of this DMSP is subsequently degraded by bacteria via demethylation. The GOS metagenomic data also revealed a new cluster of *dmdA* sequences (designated Clade E) that implicates marine gammaproteobacteria in DMSP demethylation, along with previously recognized alphaproteobacterial groups Roseobacter and SAR11. Analyses of G+C content and gene order indicate that lateral gene transfer is likely responsible for the wide distribution of *dmdA* among diverse taxa, contributing to the homogenization of biogeochemical roles among heterotrophic marine bacterioplankton. Candidate genes for the competing bacterial degradation process that converts DMSP to the climate-active gas dimethylsulfide (DMS) (*dddD* and *dddL*) occur infrequently in the (2007) GOS metagenome, suggesting either that the key DMS-producing bacterial genes are yet to be identified or that DMS formation by free-living bacterioplankton is insignificant relative to their demethylation activity.

Introduction

Biogeochemical studies implicate dimethylsulfoniopropionate (DMSP) as a highly labile and rapidly cycled component of the marine dissolved organic matter (DOM) pool (Kiene *et al*., 2000). Synthesized by marine phytoplankton and coastal vascular plants for use as an osmolyte and other cellular purposes (Vairavamurthy *et al*., 1985; Wolfe *et al*., 1997; Sunda *et al*., 2002), significant amounts of DMSP are released into the marine DOM pool by algal lysis and grazing. Estimates based on biogeochemical approaches (i.e. measures of DMSP concentrations and turnover in seawater) indicate that as much as 10% of primary production in marine environments is directed towards the synthesis of DMSP (Kiene *et al*., 2000; Howard *et al*., 2006). This suggests a major flow of surface ocean carbon and sulfur through a single compound. The recent discovery of key genes for bacterial DMSP degradation (Howard *et al*., 2006; Todd *et al*., 2007; Curson *et al*., 2008) provides an opportunity to address DMSP cycling from a gene-based perspective and to determine whether, as biogeochemical data would predict, DMSP degradation activities are widespread in the ocean and mediated by diverse marine bacterioplankton taxa.

There are two major fates of bacterially degraded DMSP. The first is demethylation to 3-methylmercaptopropionate (MMPA), which results in incorporation of carbon and sulfur into bacterial biomass and, through trophic transfers, the microbial food web (Kiene *et al*., 1999; Simo, 2001). The second is cleavage to dimethylsulfide (DMS) which, while quantitatively less important than demethylation (Kiene, 1996), is the largest biogenic source of sulfur to the atmosphere (Andreae and Raemdonck, 1983). As part of the atmospheric sulfur pool, DMS oxidation products form cloud condensation nuclei and influence climate on a global scale via cloud cover and solar radiation backscatter (Lovelock *et al*., 1972; Charlson *et al*., 1987; Andreae, 1990; Malin and Kirst, 1997).

Recently three bacterial genes that mediate these competing pathways of DMSP breakdown have been identified. The DMSP demethylase gene, *dmdA*, first discovered in the Roseobacter group bacterium *Silicibacter pomeroyi* DSS-3 (Howard *et al*., 2006), encodes the protein for demethylating DMSP to MMPA. Subsequent degradation to methanethiol (MeSH; Kiene, 1996; Gonzalez *et al*., 1999) or 3-mercaptopropionate (MPA; Visscher *et al.*, 1992) effectively routes DMSP-sulfur away from DMS formation and into the marine food web. *dmdA* was found in cultured roseobacters known to have DMSP-demethylating capabilities (Gonzalez *et al*., 1999; Kiene *et al*., 1999), as well as in cultured members of the abundant SAR11 clade, *Pelagibacter ubique* HTCC1062 and *P. ubique* HTCC1002 (Howard *et al*., 2006). In the Sargasso Sea (Venter *et al*., 2004), this gene was in sufficient abundance to be present in ~37% of the cells (Howard *et al*., 2006). Based on sequences from both cultured and uncultured bacteria, the *dmdA* genes fell into four distinct phylogenetic clades designated Clade A (Roseobacter-like), Clade B, Clade C and Clade D (SAR11-like). Clades B and C have no representatives among cultured bacteria (Howard *et al*., 2006).

Two bacterial genes encoding the first step in the cleavage pathway to DMS were also recently discovered (Todd *et al*., 2007; Curson *et al*., 2008). The DMSP cleavage gene *dddD* was found in marine gammaproteobacterium *Marinomonas* sp. MWYL1, and is proposed to mediate the addition of acyl coenzyme A to DMSP, leading to DMS production (Todd *et al*., 2007). The DMSP cleavage gene *dddL* was discovered in the marine Roseobacter group bacterium *Sulfitobacter* sp. EE-36, and is proposed to cleave DMSP directly to produce DMS and acrylate

(Curson *et al*., 2008). However, orthologues to *dddD* and *dddL* are not found in several of the known DMS-producing marine bacteria.

Recent augmentation of the Global Ocean Sampling (GOS) metagenomic data set (Rusch *et al*., 2007) provides an opportunity to look at the abundance, diversity and distribution of *dmdA*, *dddD* and *dddL* in a broader set of marine habitats representing a wider geographic range than the Sargasso Sea metagenome. In this article we ask three questions. First, is the abundance of DMSP degradation genes in the (2007) GOS data set consistent with biogeochemical data indicating that a major fraction of marine dissolved organic carbon and sulfur passes through DMSP? Second, is the initial identification of four *dmdA* clades supported by a considerably larger metagenomic survey of 39 additional sampling sites, and does the larger data set provide new information as to the diversity of *dmdA*-containing taxa? Third, can genes adjacent to *dmdA* in genome sequences of cultured and uncultured bacterioplankton offer insights into downstream metabolic processing?

Results

Consistent standards for identification of functional orthologues in metagenomic data sets have yet to be established. Partial gene sequences, unknown phylogenetic relatedness of source organisms, public release of assembled data sets only and limited experimental evidence for gene function pose formidable challenges to gene annotation and quantification. Our strategy was to set a relatively high expect (E) value in blast analyses ($E \leq 10^{-20}$) with multiple query sequences against unassembled translated sequences to catch all possible orthologues of the DMSP degradation genes. We then removed replicate hits, eliminated the poorer hit of paired reads, and built amino acid-based phylogenetic trees anchored with experimentally verified genes to identify evolutionarily related sequences and define clades. For environmental *dmdA* sequence analysis, previous experimental verification of DMSP demethylase activity for the protein encoded by Roseobacter and SAR11 *dmdA* sequences (Howard *et al*., 2006) provided the appropriate query sequences (SPO1913 and SAR11_0246); these cluster with environmental DmdA Clades A and D respectively. We also used a *dmdA*-like gene from the recently sequenced gammaproteobacterium HTCC2080 (MGP20802), although the function of this gene is yet to be confirmed experimentally. For the *dddD* sequence analysis, experimentally verified genes from *Marinomonas* sp. MWYL1 (Mmwyl1), *Rhizobium* NGR234 (RNGR00281) and *Burkholderia ambifaria* AMMD (basonym *Burkholderia cepacia*; Bamb_4298) were used as the query sequences. For the *dddL* analysis, experimentally verified genes from *Sulfitobacter* sp. EE36 (EE368) and *Rhodobacter sphaeroides* strain 2.4.1 (RSP_1433) were used as the query sequences.

***dmdA* abundance**

We found 1701 *dmdA* orthologues in the 39 new sites surveyed in the (2007) GOS data set (Tables 3.3 and 3.4 in Appendix B). Including the 373 orthologues previously identified from the Sargasso Sea metagenome (GOS sites 1–7; Howard *et al*., 2006), 2071 *dmdA* homologues were identified in the full GOS metagenomic data. In the (2007) GOS data, the number of *dmdA* orthologues per 100 000 sequence reads appears somewhat higher in open ocean and coastal sites (39 and 35 per 100 000 respectively; Table 1) as compared with other habitat types (estuary, 25; hypersaline lagoon, 2; all others, 22), although the differences are not statistically significant (ANOVA, $P = 0.09$).

We calculated the mean abundance of six single-copy essential genes as a metric for the number of genome equivalents captured in the metagenomic data sets (Table 3.3 in Appendix B). Estimates of genome equivalents range from 18 to 293 for the 39 sampling sites, in accordance with the different sequencing efforts at each site (Table 3.3 in Appendix B). The abundance of *dmdA* genes was compared against this value to estimate the frequency of *dmdA*-like genes in bacterioplankton genomes, assuming no more than one copy of *dmdA* per cell. Ratios suggest that 58% (±9%) of prokaryotic cells in the (2007) GOS samples carry a homologue for *dmdA* (Figure 3.1; Table 3.3 in Appendix B). By habitat type, the coastal and open ocean sites yielded the highest per-cell *dmdA* abundance (63% and 67% respectively). Average per-cell abundance was slightly lower in the estuarine sites (43%), hypersaline lagoon and freshwater lake (4% and 6%, one site each), and other environments (embayment, reef atoll, fringing reef, warm seep and mangrove; average of 58%), but not significantly so (anova, $P = 0.31$).

***dmdA* diversity**

With the addition of the (2007) GOS data, the diversity of *dmdA* expanded over the four previously identified groups (Howard *et al*., 2006) to include a new clade (designated Clade E; Figure 3.2). Environmental sequences in this new clade cluster with the putative *dmdA* in the marine gammaproteobacterium HTCC2080 (Cho and Giovannoni, 2004), but their number is relatively small (< 1% of all sequences; Figure 3.1). All but one of the (2007) GOS sites were dominated by Clade D *dmdA* homologues (Figure 3.1), and these accounted for 67% of all *dmdA* sequences identified. Clade A homologues dominated only at the hypersaline lagoon site (Figure 3.1), and overall accounted for 5% of sequences. Of the *dmdA* homologues identified in the (2007) GOS data set, 10% could not be unambiguously assigned to one of the major protein clades and are designated here as unclassified (Figures 3.1 and 3.2). As with previous analysis (Howard *et al*., 2006), DmdA sequences in Clades B, C and D form a sister group to sequences in Clade A, while Clade E groups the furthest from the other clades (Figure 3.2).

Taxa mediating DMSP demethylation

Two major groups of marine alphaproteobacteria were shown to carry out DMSP demethylation in ocean surface waters based on experimentally verified *dmdA* sequences from cultured Roseobacter and SAR11 bacteria. The identification of Clade E now suggests that a low abundance oligotrophic gammaproteobacterial taxon, the OM60 group [comprising 1.0% of the (2007) GOS 16S rRNA gene sequences], also participates in DMSP demethylation. Further, a *dmdA* in the newly sequenced *Rhodospirillales* group member alphaproteobacterium BAL199 expands the taxonomic affiliation of Clade A sequences beyond the Roseobacter group (Figure 3.2). *Rhodospirillales* account for only 0.5% of the (2007) GOS 16S rRNA gene sequences, however. As yet, there are no sequences from cultured bacteria that cluster within Clades B or C. An apparent Clade C *dmdA* sequence in the *Psychroflexus torquis* genome (NZ_AAPR00000000) was determined to be part of the metagenomic contamination of that genome sequence (Howard *et al*., 2006). We explored alternate approaches to identify other marine bacterial taxa that also harbour *dmdA* sequences.

First, a correlation analysis of clade-specific *dmdA* counts relative to 16S rRNA gene counts for the major marine taxa was carried out for the 39 sample sites. In accordance with expectations, there was a statistically significant correlation of Clade A *dmdA* sequences with Roseobacter 16S rRNA genes ($R = 0.70$, $P < 0.001$; Figure 3.5A in Appendix B) and Clade D *dmdA* sequences with SAR11 16S rRNA genes ($R = 0.91$, $P < 0.001$; Figure 3.5B in Appendix B). As for the clades of unknown taxonomic affiliation, however, Clades B and C *dmdA* counts were significantly correlated with three taxa (SAR11, SAR86 and SAR116; $R = 0.62, 0.63, 0.58$ for Clade B and $R = 0.86, 0.77, 0.70$ for Clade C, respectively, $P < 0.001$ for all; Figure 3.5C in Appendix B). These multiple statistical signals likely result from comparable ecological distributions of SAR11, SAR86 and SAR116 cells in surface ocean waters (Rappe *et al*., 1997), and limit the value of 16S rRNA gene correlations to link *dmdA* clades with phylogenetic groups. The Clade E *dmdA* count showed no significant correlation with 16S rRNA genes from any of the taxa tested, including the OM60 taxon.

In a second approach, an environmental fosmid insert containing a Clade C *dmdA* (fosmid APKI441) from the Station ALOHA metagenomic library (DeLong *et al*., 2006) was sequenced to investigate taxonomic affinities of the genes linked to *dmdA* on the fosmid. Most open reading frames (ORFs) had best blast hits to alphaproteobacterial proteins (27 of 31 ORFs); two had best hits to gammaproteobacteria, and one each had best hits to betaproteobacteria and actinobacteria (Table 3.5 in Appendix B). None of the predicted proteins encoded by APKI441 were for standard phylogenetic markers that could definitively identify the organism of origin. Further, there was no clear lower-level taxonomic signal among alphaproteobacteria-like ORFs, with hits to Roseobacter and *Rhizobiales* proteins occurring at equal frequencies and with one hit to a SAR11 protein (Table 3.5 in Appendix B). Thus while we suspect this *dmdA*-containing genome fragment derives from a marine alphaproteobacterium, the family-level taxonomic affiliation is unclear.

A third approach to identifying DMSP-demethylating bacterioplankton taxa was analysis of the G+C content in the third codon position of each *dmdA* sequence, to determine trends among clades and consistency with cultured genomes. The third codon position is under the least functional constraints in coding amino acids and has higher variance between taxonomic groups than overall G+C content, making it a useful taxonomic indicator (Majumdar *et al*., 1999; Karlin, 2001; Sullivan *et al*., 2006). Clade D *dmdA* sequences had low average third-position G+C content (19%) with minimal intra-clade variation, consistent with *dmdA* from the two sequenced

SAR11 genomes (18% and 20% respectively; Figure 3.3B). Clades A, B, C and E *dmdA* sequences had average third-position G+C contents of 41%, 41%, 26% and 49%, respectively, and quite high intra-clade variation (Figure 3.3A). The third-position G+C contents of *dmdA* sequences in cultured Roseobacter and *Rhodospiralles* BAL199 genomes are at the very high end of environmental Clade A sequences (averaging 67%), except for Roseobacter isolate HTCC2255 which is at the low end (23%; Figure 3.3A). Further, for most of the wide range of third-position G+C contents observed among the Clade A environmental sequences, there are no similar values found among the cultured Roseobacter or *Rhodospiralles* genomes. Within each clade, the average third-position G+C content was not significantly different between *dmdA* and neighbouring genes (Table 3.6 in Appendix B) or, in the case of the cultured representatives, third-position G+C content of the entire genome (data not shown), indicating that the G+C signal of *dmdA* is not anomalous relative to other genes in the genome. Thus except for Clade D, for which there is consistent evidence that these sequences are harboured by SAR11 cells, multiple taxa may be harbouring *dmdA* sequences that fall within a single evolutionary clade as defined by amino acid sequence.

dmdA **neighbourhood analysis**

Genes that encode steps in the same metabolic pathway may occur in clusters in prokaryotic genomes (Overbeek *et al*., 1999), facilitating their co-regulation within the cell. In the *S. pomeroyi* DSS-3 genome, for example, 41% of the 4283 genes are predicted to be part of operons. We therefore analysed gene neighbourhoods of *dmdA* sequences to determine whether putative function of adjacent genes provides insights into the downstream metabolism of DMSP. A total of 1061 neighbouring genes could be analysed for all five clades, including environmental sequences and regions from cultured organisms with a *dmdA* homologue.

In Clade A, a GntR family transcriptional regulator is upstream of *dmdA* in 42% of sequenced roseobacters (Figure 3.4) and may serve to regulate *dmdA*. A flanking oxidoreductase gene (directly downstream in 37% of sequenced roseobacters, and either up- or downstream in 68% of environment Clade A sequences) is co-transcribed with *dmdA* in *S. pomeroyi* DSS-3 (Bürgmann *et al.*, 2007), and may serve to oxidize the three-carbon moiety following DMSP demethylation to MMPA (Kiene, 1996). In Clade D, aspartate semi-aldehyde dehydrogenase (ASADH) is either immediately up- or downstream of 85% of *dmdA* sequences (Figure 3.4). This enzyme catalyses the second step in the aspartate biosynthetic pathway, a major route to amino acid synthesis in bacteria that yields threonine, leucine, methionine and lysine (Viola, 2001). Considering the sulfur from DMSP is readily incorporated into methionine by marine bacterioplankton carrying out demethylation (Kiene *et al.*, 1999), the carbon in DMSP may also be efficiently shunted to amino acid production by this enzyme. Components of succinate dehydrogenase are found flanking *dmdA* in a small number of Clade A sequences in the (2007) GOS data, as well as in sequenced SAR11 genomes (Howard *et al.*, 2006; Figure 3.4, genes I, J, K, L, M, N and O). Synthesis of this protein was found upregulated in the presence of DMSP in *S. pomeroyi* DSS-3 (Bürgmann *et al.*, 2007), and may indicate that succinate is an intermediate in the degradation of DMSP, possibly serving as the entry point of DMSP metabolites into the tricarboxylic acid (TCA) cycle. Overall, there was significant diversity in the function of genes adjacent to *dmdA* homologues, suggesting either a diversity of metabolic pathways following initial demethylation of DMSP (Visscher *et al.*, 1992), or that expression of this gene is not always regulated as an operon, or both. Several cases of neighbouring genes shared across clades were found, including a putative benzaldehyde lyase adjacent to *dmdA* homologues in Clades B and C, and a homologous conserved hypothetical protein in Clades A, B and C.

***dddD* and *dddL* abundance and diversity**

Homologues to the other two recently identified genes for DMSP degradation, *dddD* and *dddL*, were also identified in the (2007) GOS data set (Tables 3.7 and 3.8 in Appendix B). *dddD* and *dddL* homologues per 100 000 sequence reads averaged 0.2 for both (Table 3.1). The six *dddD* homologues were found in coastal (three sequences) and other sites (embayment, two sequences; mangrove, one sequence; Table 3.1). All the 42 *dddL* homologues were found at a single site, a hypersaline lagoon on the Galapagos Islands (Table 3.1). The estimated number of cells containing a *dddD*-like gene is quite low (coastal, 0.10%; open ocean, 0%; estuary, 0%; hypersaline lagoon, 0%; other, 0.75%), in keeping with findings from the Sargasso Sea GOS sites (Table 3.2; Todd *et al*., 2007). Likewise, the number of cells containing a *dddL*-like gene is low (hypersaline lagoon, 23%; all other site types, 0%). The environmental *dddD* and *dddL* homologues grouped with sequences having confirmed DMSP cleavage activity (Todd *et al*., 2007; Curson *et al*., 2008) and with putative *dddD* and *dddL* genes from sequenced organisms (Figures 3.6 and 3.7 in Appendix B).

Discussion

Recent identification of the genes that mediate the first step in two competing pathways for DMSP degradation (Howard *et al*., 2006; Todd *et al*., 2007; Curson *et al*., 2008), coincident with the release of large marine metagenomic data sets (Venter *et al*., 2004; Rusch *et al*., 2007), makes it possible to explore the taxonomic diversity and abundance of DMSP degraders over broad geographic regions of the ocean surface for the first time. These analyses show that DMSP demethylation via *dmdA* is mediated by diverse prokaryotic taxa in all marine surface waters sampled, with approximately three of every five bacterioplankton cells sampled in the (2007) GOS data set harbouring a putative orthologue. Although direct comparisons are complicated by different and sometimes poorly documented methods for identifying orthologues in metagenomic data, this frequency is comparable to that of proteorhodopsin-containing cells (Rusch *et al*., 2007; Table 3.2 and Figure 3.8 in Appendix B). It greatly surpasses that of several other biogeochemically important genes annotated in the GOS metagenomic data, including those encoding carbon fixation (RuBisCO; 3.6% of cells; Venter *et al*., 2004), anoxygenic phototrophy (*pufL*, *pufM*, *bchX*; 2.5%; Yutin *et al*., 2007) and methylotrophy (*mch*; 1.2%; Kalyuzhnaya *et al*., 2005) (Table 3.2 and Figure 3.8 in Appendix B). Such a high frequency of *dmdA* genes in ocean metagenomic data is a strong indicator that the ability to demethylate DMSP imparts a substantial ecological benefit to surface ocean bacterioplankton.

Previous analysis of Sargasso Sea *dmdA* genes identified four evolutionarily distinct protein clades (Howard *et al*., 2006). Most sequences from the (2007) GOS data set fell into these recognized groups (Figure 3.2), although a fifth clade of *dmdA*-like sequences linked to gammaproteobacterium HTCC2080 emerged. Clade D *dmdA* sequences dominated at the majority of sites, in accordance with the abundance of SAR11 16S rRNA gene sequences (Figure 3.1 and Figure 3.5B in Appendix B). It was hypothesized previously that Roseobacter-like *dmdA* sequences might be the majority clade in coastal oceans (Howard *et al*., 2006), but both Clade A *dmdA* sequences and Roseobacter 16S rRNA sequences were consistently poorly represented throughout the (2007) GOS metagenome (3.4% of *dmdA* homologues and 2.7% of 16S rRNA gene sequences), including at coastal sites (Figure 3.1). From an ecological viewpoint, the open ocean and coastal ocean sites (*n* = 30) show significant homogeneity in distribution and relative abundance of the *dmdA* clades (Figure 3.1; mean 63.8 ± 9% of cells across all open and coastal ocean sites), suggesting that the metabolic capability of ocean bacterioplankton to route DMSP-carbon and -sulfur away from climatically active DMS and into the marine food web is both uniform and predictable throughout marine surface waters. However, the fact that only the small, free-living bacterioplankton cells (0.1–0.8 μm size fraction) in near-surface waters were captured in the (2007) GOS data set might influence this apparent uniformity. Roseobacters and other larger or particle-associated *dmdA*-containing cells were likely systematically excluded (Moran *et al*., 2004; Yutin *et al*., 2007).

The third-position G+C content of prokaryotic genes can be a robust phylogenetic marker (Majumdar *et al*., 1999). While Clade D *dmdA* sequences have very uniform third-position G+C contents, Clades B and C *dmdA* sequences do not (Figure 3.3A), nor do their neighbouring genes (Table 3.6 in Appendix B). Clade A sequences, although originally designated as Roseobacter-like (Howard *et al*., 2006), are even less uniform in their third-position G+C contents, and the environmental sequences do not match well with those of cultured roseobacters (Figure 3.3). Further, recent sequencing of a marine bacterial isolate genome has expanded the taxonomic affiliation of Clade A *dmdA* sequences to include marine *Rhodospiralles*. These patterns suggest that members of the same DmdA protein clades may be harboured by taxonomically heterogeneous bacterioplankton that acquired similar genes through lateral transfer and recombination events (Nelson *et al*., 1999; Jain *et al*., 2002), and for which the G+C content later transitioned according to taxonomy.

Conservation of gene order is related to genome divergence time, and closely related taxa are more likely to be syntenic (Dandekar *et al*., 1998). Synteny analysis showed a high degree of consistency in gene order for Clade D *dmdA* sequences (Figure 3.4), most of which were flanked by the same two genes: ASADH and an uncharacterized hydrolase. For Clades A, B and C, however, the functional roles of the genes flanking *dmdA* were more variable (Figure 3.4). We counted the number of functionally different genes located directly adjacent to *dmdA* sequences, and normalized to the number of sequences in each clade (including both environmental sequences and those from cultured organisms; data from Figure 3.4). Clade D demethylase genes had the lowest heterogeneity in gene order (0.02), followed by Clade C (0.16), Clade A (0.21) and Clade B (0.33) (Clade E had too few sequences to analyse).

Lateral gene transfer events are most commonly identified through inconsistencies between species trees and gene trees (Ochman *et al*., 2000; Vergin *et al*., 2007). However, the limited number of *dmdA* orthologues in sequenced marine prokaryotes results in several major clades without taxonomic anchors and prevents a direct comparison of DmdA phylogeny with organism phylogeny. Nevertheless, the third-position G+C content analysis as well as gene order analysis of *dmdA* neighbourhoods suggests that one of the five *dmdA* clades (Clade D; harboured by ~40% of surface water bacteria) is taxonomically homogeneous and represents proteins from SAR11 bacterioplankton, while each of the four remaining clades (A, B, C and E; collectively harboured by ~15% of surface water bacteria) represents proteins from multiple taxonomic groups, the roseobacters, *Rhodospiralles* and oligotrophic marine gammaproteobacteria among them.

The fact that DMSP demethylation genes are poorly represented among cultured bacteria, especially given the recent major sequencing effort for marine bacterioplankton isolates (Gordon and Betty Moore Foundation, 2007), suggests that yet-to-be cultured bacterioplankton taxa are the source organisms for some DmdA clusters without phylogenetic anchor sequences. Candidate uncultured taxa include SAR86 and SAR116, both of which are ubiquitous in ocean surface waters [averaging 5.4% and 3.1%, respectively, of the 16S rRNA sequences captured in the (2007) GOS data set], and possibly marine *Archaea* (but averaging only 0.4% of 16S rRNA sequences).

Bacterial DMSP degradation is a central microbial process in the ocean mixed layer (Kiene *et al*., 2000). Biogeochemical data indicate that up to 80% of DMSP released into seawater is demethylated, supplying about 15% and 100% of the carbon and sulfur needs, respectively, of surface ocean bacteria (Kiene and Linn, 2000; Kiene *et al*., 2000; Zubkov *et al*., 2002). Metagenomic data from the (2007) GOS study confirm this fundamental role for DMSP by showing that the majority of bacterioplankton cells across several marine habitat types harbour genes for DMSP demethylation. We hypothesize that such a high gene frequency results in part from lateral transfer events of *dmdA* among major marine taxa. Evidence of lateral gene transfer has also been found for proteorhodopsin (Frigaard *et al*., 2006), another abundant and biogeochemically relevant prokaryotic gene in the ocean. Regardless of the mechanism underlying the extraordinary abundance of *dmdA*, however, widespread harbouring of this gene has the consequence of homogenizing the ecological roles of seawater bacteria (Ochman *et al*., 2000), possibly moving bacterioplankton groups away from taxon-specific specialization in biogeochemical function (Mou *et al*., 2008). Based simply on numbers of *dmdA* and proteorhodopsin homologues in marine metagenomes (Table 3.2, Figure 3.8 in Appendix B), most small bacterioplankton cells in the surface ocean are likely DMSP-demethylating photoheterotrophs.

In contrast to DMSP demethylation via *dmdA*, DMSP cleavage via *dddD* and *dddL* is not likely to be important in the ocean, unless quite rare genes can mediate major fluxes of carbon and sulfur. The low numbers of *dddD*- and *dddL*-like genes suggest either: (i) that the key genes for DMS production are yet to be identified – a third candidate gene recently found in marine Roseobacter *Roseovarius nubinhibens* ISM is reportedly also in low abundance in free-living marine bacterioplankton communities (Curson *et al*., 2008), (ii) that DMS formation is mediated by larger and particle-attached bacteria, perhaps associated with senescent phytoplankton, that were not captured in the < 0.8 μm size fraction of the GOS metagenome – metagenomic data from the only larger size fraction sequenced in the GOS (0.8–3.0 μm; Sargasso Sea Hydrostation S) had only a single sequence with high similarity to *dddD* and none with similarity to *dddL*, however, or (iii) that marine bacterioplankton are not major players in DMSP cleavage. Indeed, recent biogeochemical studies show high non-bacterial contributions to DMS production mediated directly by phytoplankton or zooplankton grazing (Slezak *et al*., 2007; Vila-Costa *et al*., 2008), and suggest that the major role for bacteria in regulating the flux of this volatile sulfur compound from the ocean surface may be in directing the sulfur into the competing demethylation pathway.

Experimental Procedures

Identification of *dmdA*, *dddD* and *dddL* orthologues in the (2007) GOS metagenome

Confirmed *dmdA* sequences SPO1913 and SAR_0246 from *S. pomeroyi* DSS-3 and *P. ubique* HTCC1062, respectively (Howard *et al*., 2006), were the query sequences for a blastp analysis of the GOS peptides at CAMERA ('GOS: all ORF peptides (p)' database; Seshadri *et al*., 2007). A maximum *E*-value of $< 10^{-20}$ was found to comprehensively capture all possible *dmdA* sequences along with a significant number of non-*dmdA* sequences; these were then manually annotated using phylogenetic tree-building (see below) to identify *dmdA* orthologues. The recently sequenced marine gammaproteobacterium isolate HTCC2080 (NZ_AAVV00000000; Cho and Giovannoni, 2004) contains a putative *dmdA* gene (MGP20802) and was similarly used as a query sequence in identifying *dmdA* orthologues. From 17 398 blastp hits, replicate sequences (i.e. those that hit more than one query sequence) were removed. The remaining 6140 unique sequences were manually annotated to remove those in incorrect COG (clusters of orthologous genes) categories, leaving 5427 potential *dmdA* homologues. These sequences were grouped according to sample site and aligned in clustalw (Thompson *et al*., 1994) with known *dmdA* sequences, while non-*dmdA* glycine cleavage T (GcvT) sequences (SPO1648), and uncharacterized aminomethyl transferase (AMT) sequences (SPOA0311, SPOA0057 and SAR11) served as out-groups. Phylogenetic trees were constructed using MEGA version 3.1, with the James–Taylor–Thornton model for distance calculation and 100 bootstrap samplings. Sequences grouping within a *dmdA* clade (A, B, C, D or E) with a bootstrap value of ≥ 70 were considered members of that clade, or were otherwise categorized as 'unclassified'. To remove duplicate sequences due to paired reads, each *dmdA* orthologue's JCVI PEP number was traced to its JCVI template number to identify sequences from the same metagenomic insert and the poorer hit was deleted. A final *dmdA* neighbour-joining tree for the (2007) GOS sampling sites (i.e. not including previously analysed Sargasso Sea sites; Howard *et al*., 2006) was constructed using the above parameters and including representatives of all clades.

Sequences of *dddD* genes from *Marinomonas* MWYL1, *B. ambifaria* AMMD (basonym *B. cepacia* AMMD) and *Rhizobium* NGR234 (Todd *et al*., 2007) were used as queries for a blastp analysis of the (2007) GOS data set as described above. After manual removal of paired reads and phylogenetic tree analysis [with *caiB* sequences from marine gammaproteobacterium HTCC2080 (HGP20804), *Roseovarius* sp. HTCC2601 (R26013), *Sagittula stellata* E-37 (SSE378) and *Escherichia coli* K12 (b0038) serving as out-groups], six putative *dddD* sequences remained.

Sequences of *dddL* genes from *Sulfitobacter* sp. EE-36 and *R. sphaeroides* 2.4.1 (Curson *et al*., 2008) were used as queries for a blastp analysis of the (2007) GOS data set as described above. After manual removal of paired reads and phylogenetic tree analysis [with related sequences from a hypothetical protein in *Rhodopsuedomonas palustris* (RPA_2344), alphaproteobacterium HTCC2255 (OM22550), *Roseobacter* sp. Azwk-3b (RAZWK3B_07579) and *Marinobacter aquaeolei* VTP (Maqu_0586) serving as out-groups], 42 putative *dddL* sequences remained. Sequences identified in these analyses are available at http://www.roseobase.org/DmdApaper.

Estimating the frequency of DMSP-degrading cells

Numbers of homologues to six essential single-copy genes (*atpD*, *rpoB*, *gyrB*, *hsp70*, *tufA* and *recA*) were determined for each site. Homologues were identified by blastp analysis at CAMERA ('GOS: all ORF peptides (p)' database) using *E. coli* K12 substrain MG1655 gene sequences as queries with a maximum *E*-value of $\leq 10^{-20}$. Manual inspection of the hits returned based on COG assignments and NCBI blast hits validated the *E*-value cut-off. Duplicate sequences due to paired reads were eliminated as described above. The homologue counts for each single-copy gene were size-normalized to the length of the *recA* gene to account for differences in length between these genes. To do this, the length of *recA* (1062 nt) was divided by the length of the single-copy gene (1383 nt for *atpD*, 4029 nt for *rpoB*, 2415 nt for *gyrB*, 1917 nt for *hsp70* and 1185 nt for *tufA*), and this value was then multiplied by the single-copy gene count. The size-normalized abundance of *dmdA* was calculated for each site as *dmdA* count × 100/(average, normalized counts of six single-copy genes).

16S rRNA gene analysis

The GOS metagenomic data were analysed by blastn with the *E. coli* K12 16S rRNA gene as the query sequence. Hits were accepted that met the following criteria: an *E*-value of $\leq 10^{-5}$, ≥ 65 bp overlapping length and $> 85\%$ similarity to the *E. coli* 16S rRNA gene sequence. Accepted hits were aligned with a taxonomic sequence library containing 195 16S rRNA gene sequences of representative cultured and uncultured marine bacteria (Moran *et al*., 2004). Sequences that overlapped for ≥ 70% of their length with a representative sequence and had ≥ 90% identity were classified to the order level, while those with poorer hits to the reference marine 16S rRNA gene library were left unclassified. *dmdA* counts were correlated against 16S rRNA gene counts for all combinations of *dmdA* clades and selected 16S rRNA clades (e.g. *dmdA* clades A, B, C, D and E versus 16S rRNA genes from Roseobacter, SAR11, SAR86, SAR116 and OM60).

Analysis of *dmdA* gene neighbourhoods

dmdA-containing GOS reads that included sequence outside the target gene were analysed further for the identity of up- and downstream genes. The neighbouring regions were analysed by blastx, with annotation criteria relaxed (an *E*-value of $\leq 10^{-5}$ or an amino acid identity of ≥ 60%) to account for the short length of most neighbouring gene fragments.

Station ALOHA fosmid sequencing

The Hawaii Ocean Time Series (HOTS) sequence library (DeLong *et al*., 2006) was queried for *dmdA* orthologues. One clone, APKI441, from the 70 m sample, was selected for full sequencing based on its outlying membership in Clade C as determined by phylogenetic analysis.

G+C content analysis

Third-codon-position G+C analysis (Majumdar *et al*., 1999; Karlin, 2001; Sullivan *et al*., 2006) was carried out on *dmdA* sequences from cultured organisms, the GOS sequences and the APKI441 fosmid. For comparison, third-position G+C analysis was also carried out on neighbouring genes and, for cultured organisms, the full genome sequence. The genome sequence of Roseobacter member HTCC2255 is contaminated with gammaproteobacterial sequence, but the *dmdA* gene is located on a large and unambiguous Roseobacter contig.

Accession Numbers

DmdA sequences are available at NCBI under Accession No. AAV95190 (*S. pomeroyi*), ABF64177 (*Silicibacter* sp. TM1040), ABD55296 (*Jannaschia* sp. CCS1), EAP76657 (*R. nubinhibens* ISM), EAQ43549 (*Roseobacter* sp. MED193), EBA11882 (*Roseobacter* sp. CCS2), EAQ26389 (*Roseovarius* sp. 217), YP_001533657 (*Dinoroseobacter shibae* DFL_12), EBA17661 (*Roseobacter* sp. SK209-2-6), EBA02880 (*Rhodobacterales* bacterium HTCC2150), ABG31871 (*Roseobacter denitrificans* OCh 114), EDM32633 (*Roseovarius* sp. TM1035), EDM71141 (*Roseobacter* sp. AzwK-3b), EAU51039 (alphaproteobacterium HTCC2255), AAZ21068 (*P. ubique*HTCC1062), EAS69357 (*P. torquis* genome sequence contaminant), EAW42451 (gammaproteobacterium HTCC2080), EDP61332 (alphaproteobacterium BAL199), DU737812 (HOT fosmid APKI441), EAD93963 (Sargasso Sea metagenome sequence UAAO069TR), EAD14694 (Sargasso Sea metagenome sequence UBAMW22TR), EAA97724 (Sargasso Sea metagenome sequence UEAVR02TR) and EAG54780 (Sargasso Sea metagenome sequence SLAVR50TF). Six single-copy and 16S rRNA gene sequences from *E. coli*K12 are available under Accession No. P0A7G6 (RecA), NP_418188 (AtpD), AAT48201 (GyrB), AAC76961 (RpoB), AAC73125 (Hsp70), NP_417798 (TufA), NC_000913 locus tag b3851 (16S rRNA). GcvT sequences are available under Accession No. AAV94935 (*S. pomeroyi*SPO1648), AAV97443 (*S. pomeroyi*SPOA0311) and AAV97197 (*S. pomeroyi*SPOA0057). DddD sequences are available under Accession No. ABR72937 (*Marinomonas*MWYL1), ABI89851[*B. ambifaria* AMMD (basonym *B. cepacia* AMMD)], AAQ87407 (*Rhizobium*NGR234), EAQ63474 (*Marinomonas* sp. MED121), EBA01716 (*Marinobacter* sp. ELB17), EAS46334 (gammaproteobacterium HTCC2207), EDQ34757 (*Hoeflea phototrophica* DFL-43), EBA08656 (*S. stellata* E-37), EAU98415 (*Burkholderia phymatum*STM815), EAV54093 (*B. ambifaria*MC40-6), ABV95365 (*D. shibae* DFL 12) and AAV94987 (*S. pomeroyi* DSS-3). CaiB sequences are available under Accession No. EAW39829 (Marine gammaproteobacterium HTCC2080), EAU45027 (*Roseovarius* sp. HTCC2601), EBA07754 (*S. stellata* E-37) and NP_414580 (*E. coli*K12). DddL sequences are available at NCBI under Accession No. ZP_00955343 (*Sulfitobacter* sp. EE-36), YP_351475 (*R. sphaeroides* 2.4.1), ZP_01439508 (*Fulvimarina pelagi*), ZP_01002705 (*Loktanella vestfoldensis*), ZP_00998135 (*Oceanicola batsensis*HTCC2597), ZP_01548404 (*Stappia aggregata* IAM 12614), YP_001534647 (*D. shibae*DFL12), ZP_01015736 (*Rhodobacterales* sp. HTCC2654), ZP_00962774 (*Sulfitobacter* sp. NAS14-1). DddL-related hypothetical protein sequences are available under Accession No. NP_947689 (*R. palustris*), ZP_01446734 (alphaproteobacterium HTCC2255), ZP_01904446 (*Roseobacter* sp. Azwk-3b) and YP_957874 (*Marinobacter aquaeolei* VTP).

Acknowledgements

We would like to thank Helmut Bürgmann, Jan Mrázek, Ron Kiene, William Whitman, Eric Stabb, Tim Hollibaugh, Scott Gifford and Vanessa Varaljay for helpful discussions, and Ed DeLong for the APKI441 fosmid. This research was supported by the National Science Foundation (OCE-0724017) and the Gordon and Betty Moore Foundation.

References

1. Andreae, M. 1990. Ocean–atmosphere interactions in the global biogeochemical sulfur cycle. Mar Chem 30: 1–29.

2. Andreae, M.O., and Raemdonck, H. 1983 Dimethyl sulfide in the surface ocean and the marine atmosphere: a global view. Science 221: 744–747.

3. Biers, E.J., Wang, K., Pennington, C., Belas, R., Chen, F., and Moran, M.A. 2008 Occurrence and expression of gene transfer agent (GTA) genes in marine bacterioplankton. Appl Environ Microbiol 74: 2933-2939.

4. Bürgmann, H., Howard, E.C., Ye, W., Sun, F., Sun, S., Napierala, S., and Moran, M.A. 2007 Transcriptional response of *Silicibacter pomeroyi* DSS-3 to dimethylsulfoniopropionate (DMSP). Environ Microbiol 9: 2742–2755.

5. Charlson, R.J., Lovelock, J.E., Andreae, M.O., and Warren, S.G. 1987 Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326: 655–661.

6. Cho, J.-C., and Giovannoni, S.J. 2004 Cultivation and growth characteristics of a diverse group of oligotrophic marine Gammaproteobacteria. Appl Environ Microbiol 70: 432–440.

7. Cottrell, M.T., Yu, L., and Kirchman, D.L. 2005 Sequence and expression analyses of cytophaga-like hydrolases in a western arctic metagenomic library and the Sargasso Sea. Appl Environ Microbiol 71: 8506–8513.

8. Curson, A.R.J., Rogers, R., Todd, J.D., Brearley, C.A., and Johnston, A.W.B. 2008 Molecular genetic analysis of a dimethylsulfoniopropionate lyase that liberates the climate-changing gas dimethylsulfide in several marine alpha-proteobacteria and Rhodobacter sphaeroides. Environ Microbiol 10: 757–767.

9. Dandekar, T., Snel, B., Huynen, M., and Bork, P. 1998 Conservation of gene order: a fingerprint of proteins that physically interact. Trends Biochem Sci 23: 324–328.

10. DeLong, E.F., C.M. Preston, T. Mincer, V. Rich, S.J. Hallam, N.-U. Frigaard, A. Martinez, M.B. Sullivan, R. Edwards, B.R. Brito, S.W. Chisholm, and D.M. Karl. 2006 Community genomics among stratified microbial assemblages in the ocean's interior. Science 311: 496–503.

11. Frigaard, N.-U., Martinez, A., Mincer, T.J., and DeLong, E.F. 2006 Proteorhodopsin lateral gene transfer between marine planktonic Bacteria and Archaea. Nature 439: 847–850.

12. Gonzalez, J.M., Kiene, R.P., and Moran, M.A. 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.

13. Gordon and Betty Moore Foundation 2007 Microbial genome sequencing project. URL http://www.moore.org/microgenome/microb_list.asp.

14. Howard, Erinn C., James R. Henriksen, Alison Buchan, Chris R. Reisch, Helmut Burgmann, Rory Welsh, Wenying Ye, Jose M. Gonzalez, Kimberly Mace, Samantha B. Joye, Ronald P. Kiene, William B. Whitman, and Mary Ann Moran. 2006 Bacterial taxa that limit sulfur flux from the ocean. Science 314: 649–652.

15. Jain, R., Rivera, M.C., Moore, J.E., and Lake, J.A. 2002 Horizonal gene transfer in microbial genome evolution. Theor Pop Biol 61: 489–495.

16. Kalyuzhnaya, M.G., Nercessian, O., Lapidus, A., and Chistoserdova, L. 2005 Fishing for biodiversity: novel methanopterin-linked C1 transfer genes deduced from the Sargasso Sea metagenome. Environ Microbiol 7: 1909–1916.

17. Karlin, S. 2001 Detecting anomalous gene clusters and pathogenicity islands in diverse bacterial genomes. Trends Microbiol 9: 335–343.

18. Kiene, R.P. 1996 Production of methanethiol from dimethylsulfoniopropionate in marine surface waters. Mar Chem 54: 69–83.

19. Kiene, R.P., and Linn, L.J. 2000 Distribution and turnover of dissolved DMSP and its relationship with bacterial production and dimethylsulfide in the Gulf of Mexico. Limnol Oceanogr 45: 849–861.

20. Kiene, R.P., Linn, L.J., Gonzalez, J.M., Moran, M.A., and Bruton, J.A. 1999 Dimethylsulfoniopropionate and methanethiol are important precursors of methionine and protein-sulfur in marine bacterioplankton. Appl Environ Microbiol 65: 4549–4558.

21. Kiene, R.P., Linn, L.J., and Bruton, J.A. 2000 New and important roles for DMSP in marine microbial communities. J Sea Res 43: 209–224.

22. Lau, W.W.Y., and Armbrust, E.V. 2006 Detection of glycolate oxidase gene glcD diversity among cultured and environmental marine bacteria. Environ Microbiol 8: 1688–1702.

23. Lovelock, J.E., Maggs, R.J., and Rasmussen, R.A. 1972 Atmospheric dimethyl sulphide and the natural sulphur cycle. Nature 237: 452–453.

24. Majumdar, S., Gupta, S.K., Sundararajan, V.S., and Ghosh, T.C. 1999 Compositional correlation studies among the three different codon positions in 12 bacterial genomes. Biochem Biophys Res Commun 266: 66–71.

25. Malin, G., and Kirst, G.O. 1997 Algal production of dimethyl sulfide and its atmospheric role. J Phycol 33: 889–896.

26. Moran, M. A., A. Buchan, J. M. Gonzalez, J. F. Heidelberg, W. B. Whitman, R. P. Kiene, J. R. Henricksen, G. M. King, R. Belas, C. Fuqua, L. Brinkac, M. Lewis, S. Johrl, B. Weaver, G. Pai, J. A. Eisen, E. Rahe, W. M. Sheldon, W. Ye, T. R. Miller, J. Carlton, D. A. Rasko, I. T. Paulsen, Q. Ren, S. C. Daugherty, R. T. Deboy, R. J. Dodson, A. S. Durkin, R. Madupu, W. C. Nelson, S. A. Sullivan, M. J. Rosovitz, D. H. Haft, J. Selengut, and N. Ward. 2004 Genome sequence of Silicibacter pomeroyi reveals adaptations to the marine environment. Nature 432: 910–913.

27. Mou, X., Sun, S., Edwards, R.A., Hodson, R.E., and Moran, M.A. 2008 Bacterial carbon processing by generalist species in the coastal ocean. Nature 451: 708–711.

28. Nelson, K.E., Clayton, R.A., Gill, S.R., Gwinn, M.L., Dodson, R.J., Haft, D.H., Hickey, E.K., Peterson, J.D., Nelson, W.C., Ketchum, K.A., McDonald, L., Utterback, T.R., Malek, J.A., Linher, K.D., Garrett, M.M., Steward, A.M., Cotton, M.D., Pratt, M.S., Phillips, C.A., Richardson, D., Heidelberg, J., Sutton, G.G., Fleischmann, R.D., Eisen, J.A., White, O., Salzberg, S.L., Smith, H.O., Venter, J.C., Fraser, CM. 1999 Evidence for

lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima*. Nature 399: 323–329.

29. Ochman, H., Lawrence, J.G., and Groisman, E.A. 2000 Lateral gene transfer and the nature of bacterial innovation. Nature 405: 299–304.

30. Overbeek, R., Fonstein, M., D'Souza, M., Pusch, G.D., and Maltsev, N. 1999 The use of gene clusters to infer functional coupling. Proc Natl Acad Sci USA 96: 2896–2901.

31. Rappe, M.S., Kemp, P.F., and Giovannoni, S.J. 1997 Phylogenetic diversity of marine coastal picoplankton 16S rRNA genes cloned from the continental shelf off Cape Hatteras, North Carolina. Limnol Oceanogr 42: 811–826.

32. Rusch, D. B., A. L. Halpern, G. Sutton, K. B. Heidelberg, S. Williamson, S. Yooseph, D. Wu, J. A. Eisen, J. M. Hoffman, K. Remington, K. Beeson, B. Tran, H. Smith, H. Baden-Tillson, C. Stewart, J. Thorpe, J. Freeman, C. Andrews-Pfannkoch, J. E. Venter, K. Li, S. Kravitz, J. F. Heidelberg, T. Utterback, Y.-H. Rogers, L. I. Falcon, V. Souza, G. Bonilla-Rosso, L. E. Eguiarte, D. M. Karl, S. Sathyendranath, T. Platt, E. Bermingham, V. Gallardo, G. Tamayo-Castillo, M. R. Ferrari, R. L. Strausberg, K. Nealson, R. Friedman, M. Frazier, and J. C. Venter. 2007 The Sorcerer II Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. PLoS Biol 5: 0398–0431.

33. Seshadri, R., Kravitz, S., Smarr, L., Gilna, P., and Frazier, M. 2007 CAMERA: a community resource for metagenomics. PLoS Biol 5: e75. doi:10.1371/journal.pbio.0050075.

34. Simo, R. 2001 Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological, evolutionary . Trends Ecol Evol 16: 287–294.

35. Slezak, D., Kiene, R.P., Toole, D.A., Simo, R., and Kieber, D.J. 2007 Effects of solar radiation on the fate of dissolved DMSP and conversion to DMS in seawater. Aquat Sci 69: 377–393.

36. Sullivan, M.B., Lindell, D., Lee, J.A., Thompson, L.R., Bielawski, J.P., and Chisholm, S.W. 2006 Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. PLoS Biol 4: 1344–1357.

37. Sunda, W., Kieber, D.J., Kiene, R.P., and Huntsman, S. 2002 An antioxidant function for DMSP and DMS in marine alga. Nature 418: 317–320.

38. Thompson, J.D., Higgins, D.G., and Gibson, T.J. 1994 clustal w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680.

39. Todd, J.D., Rogers, R., Guo Li, Y., Wexler, M., Bond, P.L., Sun, L., Curson, A.R.J., Malin, G., Steinke, M. and Johnston, A.W.B. 2007 Structural and regulatory genes required to make the gas dimethyl sulfide in bacteria. Science 315: 666–669.

40. Vairavamurthy, A., Andreae, M.O., and Iverson, R.L. 1985 Biosynthesis of dimethylsulfide and dimethylpropiothetin by Hymenomonas carterae in relation to sulfur source and salinity variations. Limnol Oceanogr 30: 59–70.

41. Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y.-H. Rogers, and H. O. Smith. 2004 Environmental genome shotgun sequencing of the Sargasso Sea. Science 304: 66–74.

42. Vergin, K.L., Tripp, J.H., Wilhelm, L.J., Denver, D.R., Rappe, M.S., and Giovannoni, S.J. 2007 High intraspecific recombination rate in a native population of Candidatus Pelagibacter ubique (SAR11). Environ Microbiol 9: 2430–2440.

43. Vila-Costa, M., Kiene, R.P., and Simo, R. 2008 Seasonal variability of the dynamics of dimethylated sulfur compounds in a coastal northwest Mediterranean site. Limnol Oceanogr 53: 198–211.

44. Viola, R.E. 2001 The central enzymes of the aspartate family of amino acid biosynthesis. Acc Chem Res 34: 339–349.

45. Visscher, P.T., Diaz, M.R., and Taylor, B.F. 1992 Enumeration of bacteria which cleave or demethylate dimethylsulfoniopropionate in the Caribbean Sea. Mar Ecol Prog Ser 89: 293–296.

46. Wolfe, G.V., Steinke, M., and Kirst, G.O. 1997 Grazing-activated chemical defence in a unicellular marine alga. Nature 387: 894–897.

47. Yutin, N., Suzuki, M.T., Teeling, H., Weber, M., Venter, J.C., Rusch, D.B., and Beja, O. 2007 Assessing diversity and biogeography of aerobic anoxygenic phototrophic bacteria in surface waters of the Atlantic and Pacific Oceans using the Global-Ocean-Sampling expedition metagenomes. Environ Microbiol 9: 1464–1475.

48. Zubkov, M.V., Fuchs, B.M., Archer, S.D., Kiene, R.P., Amann, R., and Burkill, P.H. 2002 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.

Table 3.1. *dmdA*, *dddD* and *dddL* homologues per 100 000 reads in the GOS data set by site. An asterisk indicates values > 0 but < 0.5.

Site ID number	**Sample number**	**Description**	**Name**	***dmdA* homologues per 100 000 reads**	***dddD* homologues per 100 000 reads**	***dddL* homologues per 100 000 reads**
2	8	Coastal	Gulf of Maine	21	2	0
3	9	Coastal	Brown's Bank-Gulf of Maine	39	0	0
4	10	Coastal	Outside Halifax Nova Scotia	34	0	0
5	11	Embayment	Bedford Basin Nova Scotia	26	3	0
6	12	Estuary	Bay of Fundy Nova Scotia	50	0	0
7	13	Coastal	Northern Gulf of Maine	20	0	0
8	14	Coastal	Newport Harbor Rhode Island	9	0	0
9	15	Coastal	Block Island New York	28	0	0
10	16	Coastal	Cape May New Jersey	36	0	0
11	17	Estuary	Delaware Bay New Jersey	7	0	0
12	18	Estuary	Chesapeake Bay Maryland	17	0	0
13	19	Coastal	Off Nags Head North Carolina	17	0	0
14	20	Coastal	South of Charleston South Carolina	42	0	0
15	21	Coastal	Off Key West Florida	35	0	0
16	22	Coastal	Gulf of Mexico	41	0	0
17	23	Open ocean	Yucatan Channel	32	0	0
18	24	Open ocean	Rosario Bank	43	0	0
19	25	Coastal	North-east of Colon	33	0	0
20	26	Fresh water lake	Lake Gatun	3	0	0
21	27	Coastal	Gulf of Panama	31	0	0
22	28	Open ocean	250 miles from Panama City	25	0	0
23	29	Open ocean	30 miles from Cocos Island	35	0	0
25	30	Fringing reef	Dirty Rock Cocos Island	5	0	0

26	31	Open ocean	134 miles north-east of Galapagos	41	0	0
27	32	Coastal	Devil’s Crown Floreana Island	41	0	0
28	33	Coastal	Coastal Floreana	48	0	0
29	34	Coastal	North James Bay Santigo Island	46	0	0
30	35	Warm seep (19m depth)	Warm Seep Roca Redunda	52	0	0
31	36	Coastal (12m depth)	Upwelling Fernandina Island	57	0*	0
32	37	Mangrove (0.1 m depth)	Mangrove on Isabella Island	16	1	0
33	38	Hypersaline (0.2 m depth)	Punta Cormorant Hypersaline Lagoon Floreana Island	2	0	6
34	39	Coastal	North Seamore Island	42	0	0
35	40	Coastal	Wolf Island	38	0	0
36	41	Coastal	Cabo Marshall Isabella Island	45	0	0
37	42	Open ocean	Equatorial Pacific TAO Buoy	48	0	0
47	52	Open ocean	201 miles from French Polynesia	48	0	0
51	56	Coral reef atoll	Rangirora Atoll	28	0	0
			Average homologues per 100 000 reads	**32**	**0***	**0***

Table 3.2. Frequency of homologues for biogeochemically relevant genes in the GOS metagenome.

Gene	**Gene function**	**Count**	**Abundance**	**Single-copy gene normalizer**	**Metagenome analysed**	**Study**
dmdA	DMSP demethylation	1701	58%[a]	*recA, rpoB, gyrA, dnaK, tufA, atpD*	2007 GOS	This study
dmdA	DMSP demethylation	373	37%	*recA*	Sargasso Sea pilot study	Howard, et al 2006
dddD	DMSP cleavage	6b	0.2%[a]	*recA, rpoB, gyrA, dnaK, tufA, atpD*	2007 GOS	This study
dddD	DMSP cleavage	1	0.1%[c]	*recA*[c]	Sargasso Sea pilot study	Todd, et al 2007
dddL	DMSP cleavage	42	0.6%	*recA, rpoB, gyrA, dnaK, tufA, atpD*	2007 GOS	This study
dddL	DMSP cleavage	7	0.2%[d]	*recA, rpoB, gyrA, dnaK, tufA, atpD*	Total GOS	Curson, et al 2008
15 GTA genes	Gene transfer agent	~20e	<0.7%	*recA, rpoB, gyrA, dnaK, tufA, atpD*	Total GOS	Biers, et al 2008
proteorhodopsin	Proteorhodopsin	3608	43%	*recA, rpoB, gyrA*[f]	Total GOS	Rusch, et al 2007
pufM, pufL, bchX	AanP (aerobic anoxygenic photosynthesis)	99[e,f]	2.5%	*recA, rpoB, gyrA*[f]	Total GOS	Yutin, et al 2007
glcD	Glycolate oxidase	272[f]	33%	n/a[g]	Sargasso Sea pilot study	Lau and Armbrust, 2006
celM	Cytophaga-like hydrolase	30	2.9%[c]	*recA*[c]	Sargasso Sea pilot study	Cottrell, et al 2005
mch	methenyl-H_4MPT cyclohydrolase	12	1.2%[c]	*recA*[c]	Sargasso Sea pilot study	Kalyuzhnaya, et al 2005
RuBisCO	Ribulose bis-phosphate carboxylase	37	3.6%[c]	*recA*[c]	Sargasso Sea pilot study	Venter, et al 2004

[a] Average over the 37 sampling sites in the (2007) GOS (see Fig. S3); [b] Curson and colleagues (2008) found three *dddD* homologues in the total GOS; [c] Calculated in this study based on *recA* counts from Howard and colleagues (2006); [d] Calculated in this study based on the six, single-copy gene counts; [e] Calculated as the average of multiple genes; [f] Includes paired reads; [g] Abundance generated from *glcD* OTUs relative to bacterial OTUs.

Figure 3.1. Calculated percent of bacterioplankton cells in ocean surface waters containing a *dmdA* homologue. The number of sample sites comprising each habitat type (n) is indicated.

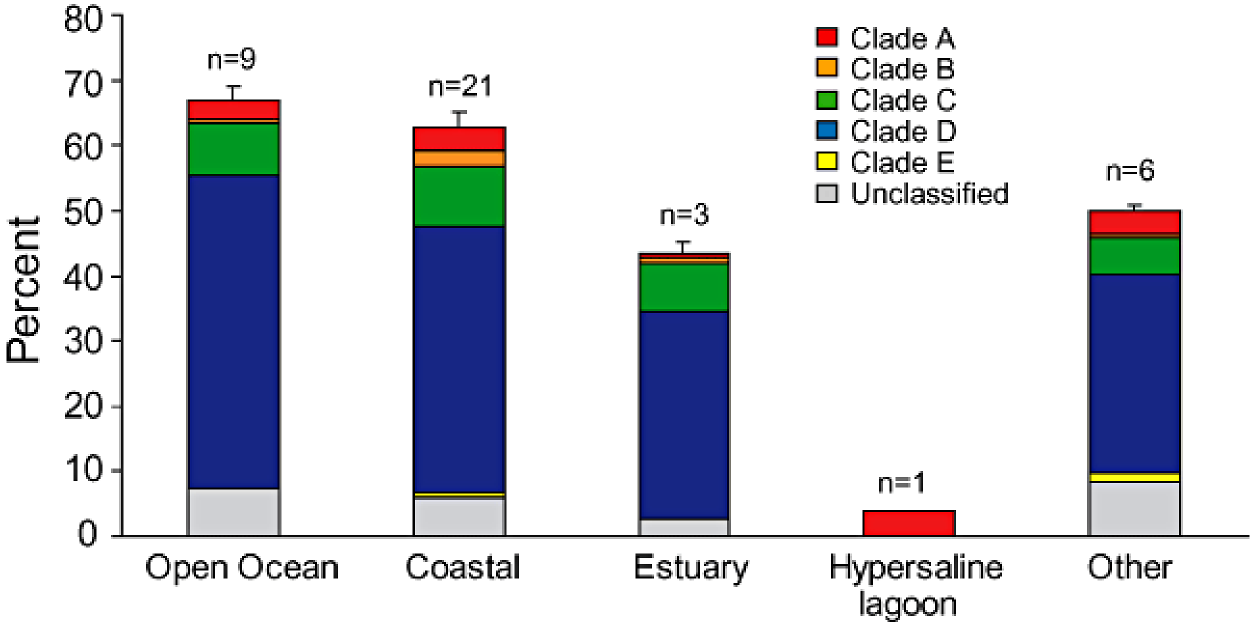

Percent
0
10
20
30
40
50
60
70
80
n=9
n=21
n=3
n=1
n=6
Clade A
Clade B
Clade C
Clade D
Clade E
Unclassified
Open Ocean
Coastal
Estuary
Hypersaline lagoon
Other

Figure 3.2. Diversity of *dmdA* homologues. The neighbour-joining tree shows *dmdA* homologues from cultured marine bacteria and selected sequences from the (2007) GOS data set, and representative sequences from the Sargasso Sea metagenome (from Howard *et al*., 2006; 'CladeB1 UAAO069TR', 'CladeB2 UBAMW22TR', 'CladeC1 UEAVR02TR' and 'CladeD SLAVR50TF'). 'APKI441 fosmid' is the *dmdA* sequence from a Station ALOHA 70-m-depth fosmid insert (DeLong *et al*., 2006).

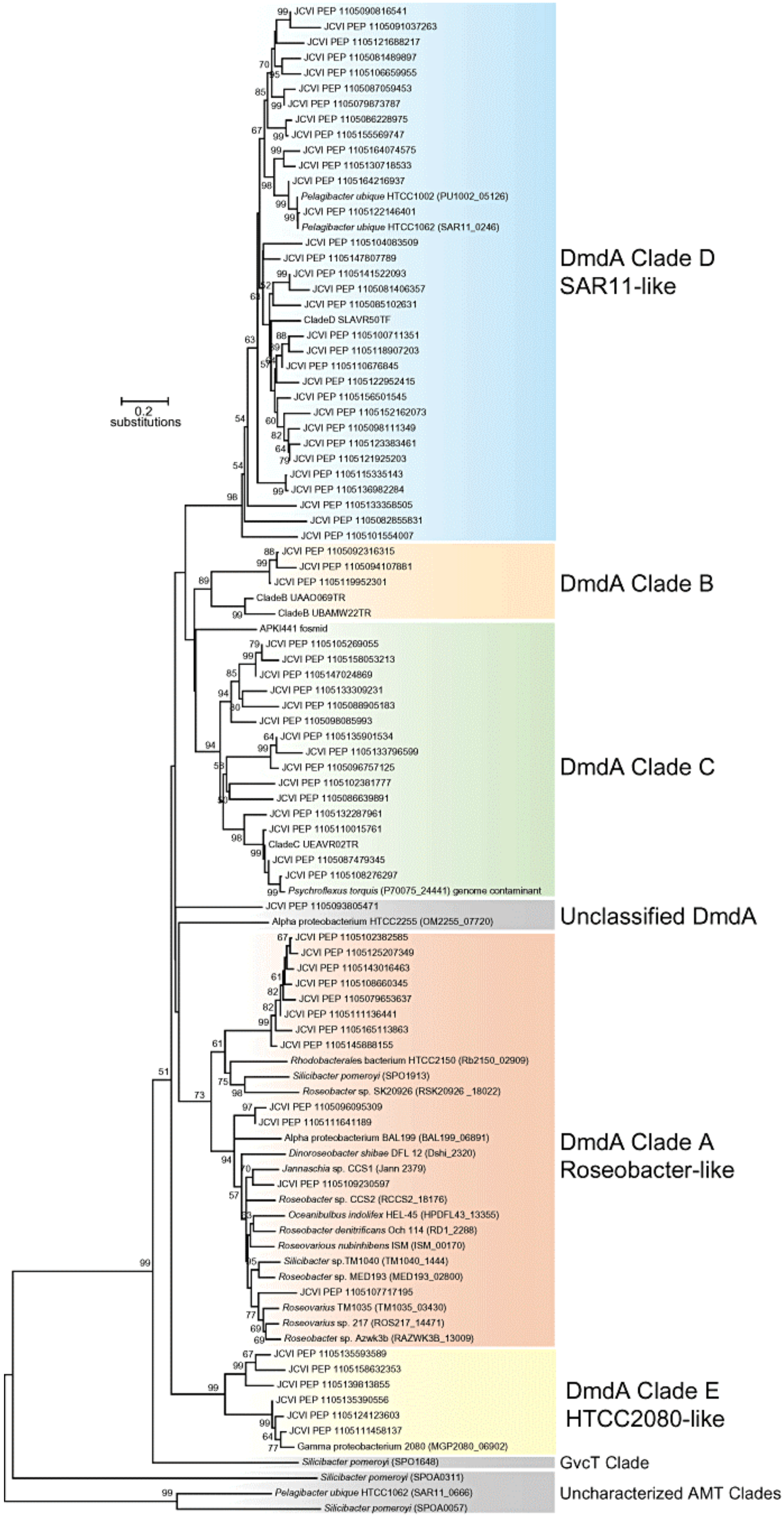

0.2
substitutions
99 JCVI PEP 1105090816541
JCVI PEP 1105091037263
JCVI PEP 1105121688217
70 JCVI PEP 1105081489897
95 JCVI PEP 1105106659955
85 JCVI PEP 1105087059453
99 JCVI PEP 1105079873787
JCVI PEP 1105086228975
67
99 JCVI PEP 1105155569747
99 JCVI PEP 1105164074575
JCVI PEP 1105130718533
98 JCVI PEP 1105164216937
Pelagibacter ubique HTCC1002 (PU1002_05126)
99 JCVI PEP 1105122146401
99 *Pelagibacter ubique* HTCC1062 (SAR11_0246)
JCVI PEP 1105104083509
JCVI PEP 1105147807789
99 JCVI PEP 1105141522093
52 JCVI PEP 1105081406357
63 JCVI PEP 1105085102631
CladeD SLAVR50TF
63 88 JCVI PEP 1105100711351
89 JCVI PEP 1105118907203
57 64 JCVI PEP 1105110676845
JCVI PEP 1105122952415
JCVI PEP 1105156501545
JCVI PEP 1105152162073
54 60 JCVI PEP 1105098111349
82 JCVI PEP 1105123383461
64 79 JCVI PEP 1105121925203
54 JCVI PEP 1105115335143
99 JCVI PEP 1105136982284
98 JCVI PEP 1105133358505
JCVI PEP 1105082855831
JCVI PEP 1105101554007
DmdA Clade D
SAR11-like
88 JCVI PEP 1105092316315
99 JCVI PEP 1105094107881
89 JCVI PEP 1105119952301
CladeB UAAO069TR
99 CladeB UBAMW22TR
DmdA Clade B
APKI441 fosmid
79 JCVI PEP 1105105269055
99 JCVI PEP 1105158053213
85 JCVI PEP 1105147024869
94 JCVI PEP 1105133309231
80 JCVI PEP 1105088905183
JCVI PEP 1105098085993
64 JCVI PEP 1105135901534
94 99 JCVI PEP 1105133796599
58 JCVI PEP 1105096757125
JCVI PEP 1105102381777
50 JCVI PEP 1105086639891
JCVI PEP 1105132287961
98 JCVI PEP 1105110015761
CladeC UEAVR02TR
99 JCVI PEP 1105087479345
JCVI PEP 1105108276297
99 *Psychroflexus torquis* (P70075_24441) genome contaminant
DmdA Clade C
JCVI PEP 1105093805471
Alpha proteobacterium HTCC2255 (OM2255_07720)
Unclassified DmdA
67 JCVI PEP 1105102382585
JCVI PEP 1105125207349
JCVI PEP 1105143016463
61 JCVI PEP 1105108660345
82 JCVI PEP 1105079653637
82 JCVI PEP 1105111136441
99 JCVI PEP 1105165113863
61 JCVI PEP 1105145888155
Rhodobacterales bacterium HTCC2150 (Rb2150_02909)
51 75 *Silicibacter pomeroyi* (SPO1913)
73 98 *Roseobacter* sp. SK20926 (RSK20926 _18022)
97 JCVI PEP 1105096095309
JCVI PEP 1105111641189
Alpha proteobacterium BAL199 (BAL199_06891)
Dinoroseobacter shibae DFL 12 (Dshi_2320)
94 70 *Jannaschia* sp. CCS1 (Jann 2379)
JCVI PEP 1105109230597
57 *Roseobacter* sp. CCS2 (RCCS2_18176)
63 *Oceanibulbus indolifex* HEL-45 (HPDFL43_13355)
Roseobacter denitrificans Och 114 (RD1_2288)
Roseovarious nubinhibens ISM (ISM_00170)
99 95 *Silicibacter* sp.TM1040 (TM1040_1444)
Roseobacter sp. MED193 (MED193_02800)
JCVI PEP 1105107717195
77 *Roseovarius* TM1035 (TM1035_03430)
69 *Roseovarius* sp. 217 (ROS217_14471)
69 *Roseobacter* sp. Azwk3b (RAZWK3B_13009)
DmdA Clade A
Roseobacter-like
67 JCVI PEP 1105135593589
99 JCVI PEP 1105158632353
99 JCVI PEP 1105139813855
JCVI PEP 1105135390556
JCVI PEP 1105124123603
99 64 JCVI PEP 1105111458137
77 Gamma proteobacterium 2080 (MGP2080_06902)
DmdA Clade E
HTCC2080-like
Silicibacter pomeroyi (SPO1648)
GvcT Clade
Silicibacter pomeroyi (SPOA0311)
99 *Pelagibacter ubique* HTCC1062 (SAR11_0666)
Silicibacter pomeroyi (SPOA0057)
Uncharacterized AMT Clades

Figure 3.3. Third-position G+C contents of *dmdA* homologues. (A) Data from environmental sequences in Clades A, B, C and E are given, along with selected cultured bacteria [the average of 13 cultured roseobacters, Roseobacter member HTCC2255 (not included in the average), *Rhodospirillales* member alphaproteobacterium BAL199 and gammaproteobacterium HTCC2080]. (B) Data from environmental sequences in Clade D are given, along with cultured bacteria in the SAR11 group (average for *P. ubique* HTCC1062 and HTCC1002).

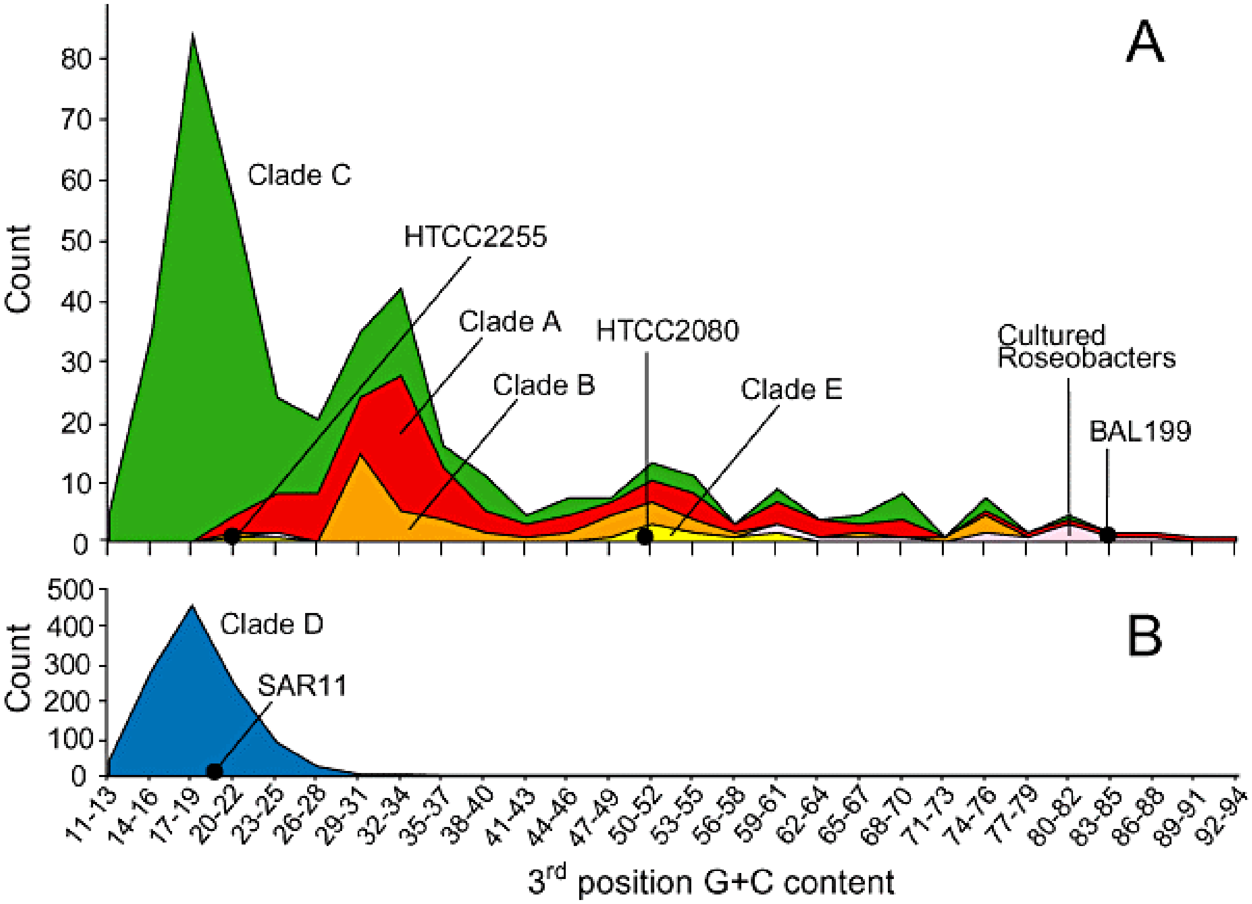
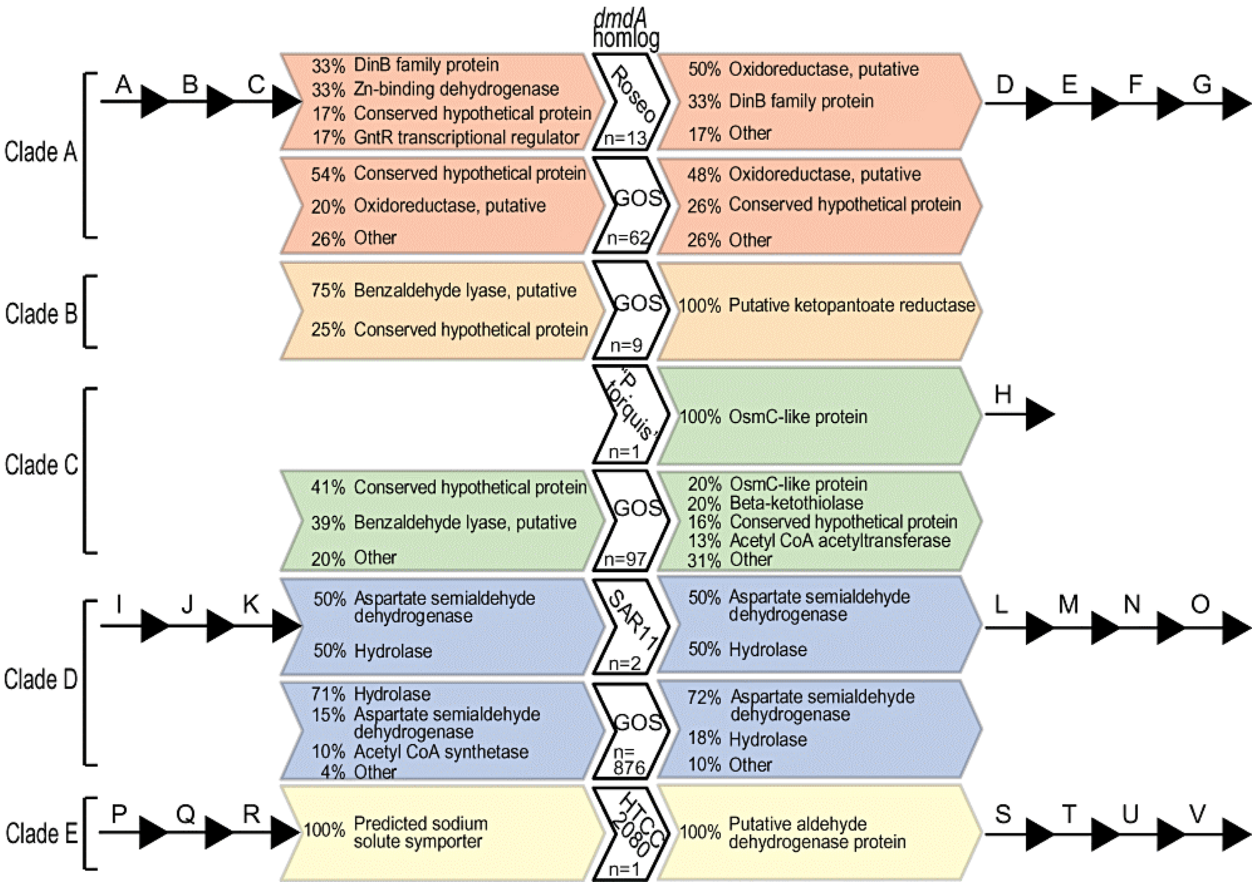

A
Count
80
70
60
50
40
30
20
10
0
Clade C
HTCC2255
Clade A
HTCC2080
Clade B
Clade E
Cultured Roseobacters
BAL199
B
Count
500
400
300
200
100
0
Clade D
SAR11
11-13
14-16
17-19
20-22
23-25
26-28
29-31
32-34
35-37
38-40
41-43
44-46
47-49
50-52
53-55
56-58
59-61
62-64
65-67
68-70
71-73
74-76
77-79
80-82
83-85
86-88
89-91
92-94
3rd position G+C content

Figure 3.4. *dmdA* neighbouring gene analysis. Numbers indicate the percent of genes found directly up- or downstream of *dmdA* homologues from cultured marine bacteria and GOS environmental sequences. The number of flanking gene regions analysed for each group is indicated (n); no neighbouring genes were retrieved for environmental Clade E sequences. Roseo = 13 Roseobacter sequences; '*P. torquis'* = the apparent contaminant sequence from the *P. torquis* genome; SAR11 = 2 SAR11 sequences; HTCC2080 = marine gammaproteobacterium HTCC2080. 'Other' refers to gene categories that constituted < 15% of the up- or downstream region. Clade A upstream 'other': RNA modification enzyme, MiaB (9%), tellurite resistance protein (3%), succinate dehydrogenase (3%), methylene THF reductase (3%), alcohol dehydrogenase (3%), DinB family protein (3%), protein of unknown function DUF1326 (3%); Clade A downstream 'other': alcohol dehydrogenase (7%), transporter (4%), RNA modification enzyme, MiaB (4%), protein of unknown function DUF1326 (7%), acyl-CoA synthase (4%), citrate lyase beta-subunit (4%), peptidyl prolyl *cis-trans* isomerase (4%), succinate dehydrogenase (4%); Clade C upstream 'other': aspartate semi-aldehyde dehydrogenase (4%), acetyl-CoA acetyltransferase (4%), hydrolase (4%), beta-ketothiolase (2%), cystathionine gamma-synthase (5%), OsmC/Ohr family protein (4%); Clade C downstream 'other': 2-dehydropantoate 2-reductase (3%), benzaldehyde lyase (8%), cystathionine gamma-synthase (5%), acyl-CoA synthase (3%), GCN5-related *N*-acetyltransferase (3%), NAD(P) transhydrogenase, beta-subunit (3%), phytanoyl-CoA dioxygenase family protein (3%), transcription regulator (5%), uracil phosphoribosyltransferase (3%); Clade D upstream 'other': GTP-binding protein (0.2%), acyl-CoA synthetase (10%), ETC complex I subunit conserved region protein (0.2%), hypothetical protein (1%), NADP oxidoreductase, coenzyme F420 dependent (1%), YbaK/prolyl-tRNA synthetase (0.6%), benzaldehyde lyase (0.2%), transporter

(0.2%), X-Pro dipeptidase (0.4%), unknown (0.2%), GTP-binding protein TypA (0.4%); Clade D downstream 'other': acyl-CoA synthetase (5%), acetyl-CoA acetyltransferase (0.3%), ETC complex I subunit (1.4%), drug/metabolite transporter permease (0.3%), hypothetical protein (2%), transporter (0.5%), X-Pro dipeptidase (0.3%), putative transporter, RarD family (0.3%), NADP oxidoreductase, coenzyme F420 dependent (0.3%). The gene neighbourhoods in the sequenced organisms are as follows (percentages indicated are those of genes that are in > 10% of each group, except HTCC2080 in which there is only one sequenced member; those that are < 10% were removed for brevity): A, GntR family transcriptional regulator (33%); B, hypothetical protein (50%), GntR family transcriptional regulator (17%); C, hypothetical protein (60%); D, BCCT transporter (17%), hypothetical protein (17%); E, aminotransferase class V (25%), hypothetical protein (17%), 3-oxoadipate enol-lactonase family protein (17%); F, hypothetical protein (50%), GntR family transcriptional regulator (17%), Putative deoxyribodipyrimidine photolyase (17%); G, LysR regulatory protein (17%), cyclopropane fatty-acyl-phospholipid synthase (17%); H, enoyl-CoA hydratase/iosmerase (100%); I, succinate dehydrogenase, flavoprotein unit *sdhA* (50%), 3-isopropyl malate dehydrogenase (50%); J, succinate dehydrogenase, putative membrane portion (50%), acyl-CoA dehydrogenase (50%); K, acyl-CoA synthetase (50%), succinate dehydrogenase *sdhC* (50%); L, succinate dehydrogenase cytochrome *b* (50%), acyl-CoA synthetase *fadD* (50%); M, succinate dehydrogenase (50%), acyl-CoA dehydrogenase *mmgC* (50%); N, succinate dehydrogenase (50%), 3-isopropylmalate dehydrogenase *leuB* (50%); O, 3-isopropylmalate dehydrogenase *leuD* (50%), succinate dehydrogenase (50%); P, dipeptidyl peptidase; Q, hypothetical protein; R, MscS mechanosensitive ion channel; S, aldehyde dehydrogenase; T, putative lignostilbene-alpha, beta-dioxygenase; U, transcriptional regulator, tetR family; V, short-chain dehydrogenase.


dmdA homolog
Clade A
A
B
C
33% DinB family protein
33% Zn-binding dehydrogenase
17% Conserved hypothetical protein
17% GntR transcriptional regulator
Roseo
n=13
50% Oxidoreductase, putative
33% DinB family protein
17% Other
D
E
F
G
54% Conserved hypothetical protein
20% Oxidoreductase, putative
26% Other
GOS
n=62
48% Oxidoreductase, putative
26% Conserved hypothetical protein
26% Other
Clade B
75% Benzaldehyde lyase, putative
25% Conserved hypothetical protein
GOS
n=9
100% Putative ketopantoate reductase
Clade C
"P. torquis"
n=1
100% OsmC-like protein
H
41% Conserved hypothetical protein
39% Benzaldehyde lyase, putative
20% Other
GOS
n=97
20% OsmC-like protein
20% Beta-ketothiolase
16% Conserved hypothetical protein
13% Acetyl CoA acetyltransferase
31% Other
Clade D
I
J
K
50% Aspartate semialdehyde dehydrogenase
50% Hydrolase
SAR11
n=2
50% Aspartate semialdehyde dehydrogenase
50% Hydrolase
L
M
N
O
71% Hydrolase
15% Aspartate semialdehyde dehydrogenase
10% Acetyl CoA synthetase
4% Other
GOS
n=876
72% Aspartate semialdehyde dehydrogenase
18% Hydrolase
10% Other
Clade E
P
Q
R
100% Predicted sodium solute symporter
HTCC 2080
n=1
100% Putative aldehyde dehydrogenase protein
S
T
U
V

CHAPTER 4

RESPONSE OF DMSP DEMETHYLATION GENES TO AN INDUCED PHYTOPLANKTON BLOOM IN GULF OF MEXICO SEAWATER[1]

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[1] Erinn C. Howard, Shulei Sun, Christopher R. Reisch, Danielle A. del Valle, Ronald P. Kiene, and Mary Ann Moran

To be submitted to *Applied and Environmental Microbiology*

Abstract

Over half of the bacterioplankton cellsin ocean surface waters are capable of routing sulfur away from the climatically-active DMS by demethylating DMSP and retaining it in the oceanic food web. In this study, >91,000 amplicons of the primary gene responsible for demethylation, *dmdA*, were sequenced over the course of an induced phytoplankton bloom in Gulf of Mexico surface waters. *dmdA* diversity was substantial, with 578 sequence clusters emerging at a conservative clustering level of $\geq$90% nucleotide sequence identity over the six-day study. The representation of the five recognized clades of DmdA did not shift substantially over the course of the phytoplankton bloom compared to controls, despite major changes in DMSP flux and concentration, and there was no evidence for "bloom" and "non-bloom" clades. 16S rRNA amplicon sequencing indicated the presence of taxa known to harbor *dmdA* genes (the Roseobacter clade, SAR11, OM60, and marine Rhodospirillales). However, the largest taxonomic change during the bloom was an increase in amplicons attributed to Flavobacteria, a taxon not known to harbor *dmdA* genes. Our results are consistent with recent data indicating that kinetic parameters of the different clades of DmdA may be quite similar. The reservoir of *dmdA* genes in this experimentally induced phytoplankton bloom was predictable from the taxonomic composition of the bacterioplankon but not from DMSP supply and concentration.

Introduction

Dimethylsulfoniopropionate (DMSP) is an ubiquitous phytoplankton product that is degraded by marine microorganisms in either of two ways. The cleavage pathway involves degradation of DMSP by phytoplankton or bacteria to produce dimethylsulfide (DMS). DMS is the largest natural source of sulfur from the ocean to the atmosphere (2), and upon oxidation, forms cloud condensation nuclei, hypothesized to affect climate on a global scale (1, 4, 21, 22). The alternative DMSP degradation pathway is carried out by bacteria alone and involves an initial demethylation to methylmercaptopropionate (MMPA), with some portion of the sulfur subsequently shunted to sulfur-containing amino acids (14, 16, 27).

The genes involved in the initial step of both DMSP degradation pathways have recently been discovered (7, 11, 32). Two different genes that each encode the first step of the DMSP cleavage pathway (*dddD* and *dddL*) have been identified (7, 32), although both are in low abundance in bacteria from the surface ocean water (an estimated 0.1 and 3.0% of cells contain the gene, respectively; 12). Conversely, the only gene found thus far that codes the first step in the DMSP demethylation pathway (*dmdA*) is highly abundant (an estimated 58% of cells contain the gene; 11, 12).

Five clades of DmdA, designated A through E, have been found in cultured marine bacteria or marine metagenomic sequences thus far. Clade A sequences include genes from various roseobacter species and a marine Rhodospirillales. Clade D sequences include genes from three SAR11 isolates (*Pelagibacter* strains HTCC1002, HTCC1062, and HTCC7211) and Clade C sequences include a second *dmdA* gene from *Pelagibacter* sp. HTCC7211. Clade E sequences include a gene from marine gamma proteobacterium HTCC2080 in the OM60 clade (11, 12, 34). To date, a Clade B *dmdA* sequence is not represented in any cultured bacterium.

The distribution of these clades can vary somewhat by environment, but their distribution across many open and coastal ocean surface water is surprisingly consistent. Generally, Clade D sequences are found in the highest abundance, and Clade E the lowest (11, 12). These *dmdA* clade distributions, however, have not been studied over time in a single environment, particularly as ecological conditions shift. Because phytoplankton blooms typically create changes in DMSP concentration and flux (9, 33, 35, 36), this study tracked the composition and diversity of the *dmdA* gene pool in Gulf of Mexico microcosms in which a phytoplankton bloom was induced by nutrient additions. Here we present relative changes in *dmdA* clade abundance based on deep sequencing of amplicons from a universal *dmdA* primer pair, in the context of simultaneous changes in DMSP concentration and fate, and bacterial and archaeal community composition.

Methods

Sample collection

Seawater was collected from oligotrophic surface waters (<1 m) in the Gulf of Mexico (30º 03.041, 87º 59.708; 27ºC, 34 PSU). Water was filtered through a 200 μm mesh into six 20L pre-rinsed cubitainers (Fold-A-Carrier; Reliance Products, Ltd.) leaving minimal headspace. Three experimental cubitainer microcosms were amended with sodium nitrate (10 μM) and potassium phosphate (0.6 μM) to induce bloom conditions; three control microcosms received no nutrient amendments. The microcosms were incubated at 27º C with a 12 h on/12 h off artificial light cycle (200 μE cm^{-2}) for the duration of the experiment.

DNA extraction

After an initial (T=0) sampling of the full contents of one control and one experimental microcosm (20 L each), samples were collected daily from each of the other four microcosms (two control and two experimental) over the next 6 days. The cubitainers collapsed as water volume was removed, resulting in relatively constant headspace volume throughout the experiment. For DNA samples, water was sequentially filtered through 8 µm (293 mm diameter) and 3 µm (47 mm diameter) prefilters, and then particles were collected on 0.2 µm (47 mm) pore-size polycarbonate filters (Poretics®) until the filters clogged (~250-500 ml volume, depending on sample). The 0.2 µm filters were flash frozen in liquid nitrogen and then stored at –20ºC until extraction. DNA was extracted using the PowerMax Soil DNA Isolation kit (MoBio Laboratories, Inc.) following manufacturer's instructions, and concentrations were estimated by absorbance on a NanoDrop Spectrophotometer (Thermo Scientific). For chemical analyses and bacterial activity measures, 500 ml of unfiltered water was collected simultaneously and immediately used in the analyses (see below).

PCR Reactions

DNA extracted from duplicate control and experimental samples (28 total samples) was used as template for PCR reactions, followed by pyrosequencing of amplicons (23). PCR reactions were carried out with Platinum High-Fidelity Taq DNA polymerase (½U; Invitrogen) in the native 10x PCR buffer with 8 ng (for *dmdA* amplifications), 5 ng (for bacterial 16S rRNA), or 10 ng (for archaeal 16S rRNA) template DNA. Universal *dmdA* primers were modified from Varaljay, et al (34) to include 454 Life Sciences A or B adaptor sequences and 14 tetranucleotide "key" sequences for sample differentiation during sequence analysis (13, 23, 30). The *dmdA* cycling conditions were as follows: 94°C for 2 min, followed by 40 cycles of 94°C for 35 s, 41°C for 35 s, and 68°C for 60 s, with a final 10 min, 68°C extension step (34). 16S rRNA bacterial and archaeal primers covering the V6 hypervariable region (developed by Sogin, et al (30) and modified by Huber, et al (13); these are a combination of 5 forward and 4 reverse bacterial 16S rRNA primers and 1 forward and 2 reverse archaeal 16S rRNA primers) were made with 14 distinct tetranucleotide keys, as described above. Bacterial or archaeal primers were mixed equally from 10 µM stock solutions, and 2 µL of this pooled solution was used in each 25 µL PCR reaction (13). The 16S rRNA gene cycling conditions were as follows: 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 58°C for 30 s, and 68°C for 30 s, with a final 10 min, 68°C extension step.

Sequencing Preparation

PCR products were run on 1% (*dmdA* amplicons) or 2% (bacterial and archaeal 16S rRNA amplicons) agarose gels, followed by purification using the QIAquick gel extraction kit (Qiagen). As a final DNA purification, products were cleaned using the Ampure® system (Agencourt Bioscience corporation), with modifications to the volume of purified PCR products (30 µL) and AMPure beads (50.4 µL). After final purification, DNA concentrations were determined as above and products (61 control and 60 experimental samples) were pooled in equal amounts, except that *dmdA* amplicons were added to the mixtures at double the amount of the 16S rRNA amplicons. Two separate pools were assembled, each of which used the distinct identification keys only once, and these were sequenced on separate halves of the PicoTiterPlate (454 Lifesciences). A total of ~1,300 ng DNA was sent for Roche GS FLX LR70 pyrosequencing at the University of South Carolina Environmental Genomics Facility (Columbia, SC).

Sequence Data Analysis

For reads of high-quality (sequences containing full and correct forward primer sequences and no uncalled bases), adaptor and key sequences were removed and sequences were clustered using the CD-HIT program (20) with ≥90% similarity for *dmdA* clusters and ≥99% similarity for 16S rRNA clusters. The average read length was 190 bp for *dmdA* amplicons, 70 for bacterial rRNA amplicons, and 72 bp for archaeal rRNA amplicons after trimming of adaptor, key, and primer sequence. For analysis of *dmdA* amplicons, reference sequences (defined as the longest read in the cluster) were analyzed via BLASTX against an in-house *dmdA* database assembled from cultured marine bacteria and the Global Ocean Sampling (26) metagenomic sequences (http://roseobase.org/DmdApaper) and augmented with a number of paralogous, non-*dmdA* sequences (12). Sequences were considered to be valid *dmdA*s if the top hit in the database was to a *dmdA* sequence with a bit score of ≥30. The sequences were assigned to the clade (either A, B, C, D, or E, or unclassified) of the top hit. For taxonomic analysis of bacterial 16S rRNA amplicons, reference sequences were aligned against a marine bacterial 16S rRNA database with representatives of all major marine taxa (3). Amplicons with ≥90% similarity and overlap of ≥70% to one of the representative marine sequences were assigned to that taxon. Otherwise they were analyzed as for the archaeal sequences. For taxonomic analysis of archaeal 16S rRNA amplicons, the SIMO RDP Agent (www.simo.marsci.uga.edu) was used to compare amplicon sequences against archaeal type species housed in the RDP database (5), using similarity cut-offs of ≥100% for species, >95% for genus, >92% for family, >91% for order, >85% for class, and >75% for phylum assignments. Because of the poor coverage of Archaea diversity among type species, most sequences were identified only to the phylum level or remained unclassified.

Statistical analysis

Clusters were analyzed via multi-dimensional scaling (MDS) using Primer 5 for Windows software (Plymouth Marine Laboratory, Plymouth, UK). A Bray-Curtis similarity matrix (4th root transformed to de-emphasize the contribution of any one particular dominant cluster) was constructed from the clusters from each replicate microcosm (two control and two nutrient-amended) over time (6 days). For *dmdA* sequences, the top 100 clusters (that contained 70% of the amplicons) were included in the statistical analysis. For bacterial 16S rRNA sequences, all clusters were analyzed (9,588 clusters representing 55 marine taxa). *dmdA* and 16S rRNA clusters were also evaluated with a SIMPER analysis (Primer 5), which determines the clusters contributing most to the differences between samples. A rarefaction curve of all *dmdA* sequences clustered at the 90% similarity level was created using EcoSim 7.0 (10) with 1,000 resamplings.

Chemical analysis and bacterial activity measurements

The ~500 mL of water collected for chemical and activity measurements was immediately subdivided as follows. Chlorophyll *a* (Chl *a*) samples were collected by filtration (50 ml) on Whatman GF/F filters. The filters were extracted in 90% acetone for 24 h at -20°C, and Chl *a* in the extracts was quantified by fluorometry (24). Samples for dissolved DMSP (DMSPd) were collected by small volume drip filtration through GF/F filters (18). Total DMSP (dissolved + particulate) was collected by acidifying whole seawater with 5 $\mu l\ ml^{-1}$ of 50% H_2SO_4, similar to the method described by Curran et al (6). Dissolved and total DMSP were quantified as DMS after alkaline hydrolysis. Bacterial production was measured by [^{3}H]leucine incorporation using the method developed by Kirchman, et al. (19) and modified by Smith and Azam (29); live and killed (5% trichloroacetic acid) controls were incubated with [^{3}H]leucine (20 nM final concentration) for 1 h in the dark. [^{35}S]DMSP was synthesized using the procedures outlined in Kiene et al. (15) and Vila et al. (35). Rate constants for DMSPd consumption were obtained with [^{35}S]DMSP as described in Slezak et al (28). Rates of DMSPd (in nM d^{-1}) were obtained by multiplying the DMSPd and DMS concentrations by their respective rate constants.

Results

Phytoplankton bloom dynamics

While control microcosms maintained low Chl *a* levels throughout the experiment (<1 µg L^{-1}), the nutrient-amended experimental microcosms had variable and higher Chl *a* concentrations that peaked at Day 4 (6 µg L^{-1}; Figure 4.1), indicating a phytoplankton bloom. Rates of bacterial secondary production indicated increased substrate availability in the experimental microcosms, consistent with bloom conditions (Figure 4.1).

The fraction of assimilated dissolved DMSP (DMSPd) degraded to DMS (maximally 15%; Figure 4.1) and the fraction of DMSPd-sulfur assimilated into macromolecules (maximally 55%; Figure 4.1) followed the same trend in both control and experimental microcosms (Figure 4.1). However, the ratio of particulate DMSP (DMSPp) to Chl *a*, a proxy for the relative amount of DMSP produced per phytoplankton cell (35), decreased over the course of the induced bloom in the experimental microcosms (Figure 4.1). This pattern suggests that the phytoplankton dominating the bloom were poor DMSP producers relative to those in the initial community and to those in the community that persisted in the control microcosms. Principal components analysis (PCA) using all environmental variables except NO_3^- and PO_4^{3-} (which were experimentally manipulated) showed that the conditions and processes in the experimental microcosms became most dissimilar to the initial samples and the control microcosms on Days 4-6 (Figure 4.2A).

DMSP demethylase gene (dmdA) dynamics

The 91,418 *dmdA* sequences obtained (28 samples representing two replicates per treatment for Days 0-6) formed 578 clusters at 90% nucleotide sequence identity (average of 158 sequences per cluster). At this similarity level, the experiment-wide richness had not yet reached a plateau (Figure 4.3). Given the error rate for FLX pyrosequencing (0.5%), this vast diversity at the 10% divergence level is real. Non-*dmdA* sequences captured by the universal *dmdA* primer set were also found (20,761 sequences); these formed 647 clusters (average of 32 sequences per cluster). Overall, 82% of the total amplicons were identified as *dmdA* sequences, while 18% were identified as encoding GcvT proteins or other related sequences.

dmdA cluster richness gradually decreased throughout the experiment in both experimental and control microcosms. The greatest cluster richness for the experimental microcosms was found on Day 1 with 240 clusters present, after which the number deceased to 185 by Day 6. Similarly, the greatest cluster richness in the control microcosms was found on Day 1 with 238 clusters present, decreasing to 164 by Day 6.

There was evidence of shifts in the relative abundance of sequences in major *dmdA* clades as the bloom in the experimental microcosms progressed, however these trends are also observed in control microcosms, albeit to a smaller extent. The percent of Clade A sequences increased in experimental microcosms from 47% to 70% of the total *dmdA* sequences between Days 3 and 4, while Clade E sequences increased from 9 to 20% of sequences at the same time.

Sequences from Clades C and D decreased in relative abundance (from 18 to 4% and from 26 to 6% of *dmdA* sequences, respectively; Figure 4.4A).

To track temporal dynamics of the community *dmdA* pool at a finer scale, the 100 largest 90% identity DmdA clusters (averaging 784 sequences per cluster and accounting for 70% of all dmdA sequences) were analyzed in more detail. A multi-dimensional scaling (MDS) plot of the percent of sequences in these clusters showed that Days 4-6 in the experimental microcosms were more similar to each other than to the other time points in both control and experimental samples (Figure 4.2B). The DMSPp:Chl *a* ratio correlated well with the observed shifts in the relative abundance of the 100 largest *dmdA* clusters (Figure 4.2B). The clusters contributing the most to differences over time in experimental vs. control microcosms were from Clades A and C (Figure 4.5).

Bacterial and archaeal communities

The 180,801 bacterial 16S rRNA sequences obtained from control and experimental microcosms formed 9,588 taxonomic clusters at 99% nucleotide sequence identity (all time points considered together). The major bacterial groups represented by these clusters were Roseobacter, SAR11, and Flavobacteria (Figure 4.4B). The relative abundance of Flavobacteria amplicons increased over time in the experimental microcosms (from 12% to 34% of total sequences), while SAR11 abundance decreased (from 31% to 12%; Figure 4.4B). These changes were larger than in the control microcosms. Roseobacters were amplified in large numbers from the microcosm bacterial communities, and numbers remained relatively stable regardless of time point or treatment (average 17% ± 7%; Figure 4.4B).

The distribution of sequences among bacterial clusters diverged over Days 4-6 in the experimental microcosms compared to the control microcosms and the initial sample (Figure

4.2C). A SIMPER analysis indicates that the increases in Flavobacteria amplicons was most responsible for this difference.

Archaeal 16S rRNA genes could be amplified from microcosm DNA only for Days 0-3. The 6,578 Archaeal 16S rRNA sequences formed 519 clusters at the 99% sequence identity level. The majority of archaeal 16S rRNA amplicons (~83%) could not be classified to phylum since they were <75% identical to any type species. These unclassified archaea likely reflect an underrepresentation of mesophilic archaea among characterized isolates. Of the sequences that could be categorized to the phylum level, the majority were Euryarchaeota (averaging 16.6% of archaeal sequences) regardless of treatment or time point (Figure 4.4C), and a small number were Crenarchaeota (averaging 0.2% of archaeal sequences). Analysis of archaeal clusters, which included the unclassified sequences, showed a shift in composition over the limited timecourse in which their 16S rRNA genes could be amplified, but this was similar in control and experimental microcosms (Figure 4.2D).

Discussion

In the context of an experimentally induced phytoplankton bloom, we asked whether shifting DMSP supply and environmental conditions influenced the relative composition of the community *dmdA* sequence pool. While at least five protein clades are found in marine bacterioplankton communities (12), little information is yet available on whether or not these *dmdA* clades represent proteins with different kinetic properties and ecological roles. Manipulation of a natural marine bacterioplankton community provided a framework in which to ask whether "bloom" and "non-bloom" clades of DmdA could be identified. Because four of the five clades are linked to specific taxa, the changes in relative DmdA abundance should likewise track changes in taxonomic composition.

Biogeochemical data confirm the initiation of a phytoplankton bloom in the experimental microcosms, peaking at Day 4 and declining by Day 6. While DMSP concentrations and flux were higher in experimental microcosms in an absolute sense due to the substantial increase in phytoplankton biomass (Figure 4.1), the ratio of DMSPp:Chl *a* showed that the blooming phytoplankton were poor producers of DMSP. Microscopic analysis (data not shown) indicated that diatoms, which are not strong DMSP producers, dominated the bloom. Thus the availability of dissolved DMSP to the bacterioplankton community in relation to other organic matter produced by the phytoplankton was likely lower in the experimental microcosms.

We found a surprising diversity of *dmdA* sequences. Overall, 578 clusters were identified at a fairly conservative definition of 90% nucleotide sequence similarity. A rarefaction curve showed amplicon nucleotide richness had not yet saturated, even at a 90% clustering level (Figure 4.3). Sequencing error (0.5%) is unlikely to be a significant source of this extensive diversity. Clusters unique to either experimental or control microcosms accounted for 29% of the total clusters. These unique clusters averaged only 1.5 sequences each, suggesting a significant amount of rare diversity that would not have been captured in shallow sequencing efforts (Figure 4.2B). There have been few exhaustive analyses of the diversity of functional genes, and even this deep sequencing approach likely underestimated diversity and richness due to mismatches with the universal primer set and PCR bias.

Despite substantial differences in the concentration and flux of DMSP between experimental and control microcosms, there were fairly limited changes in the composition of the *dmdA* genes harbored by the community. The increase in the abundance of Flavobacteria in the experimental microcosms is consistent with previous studies suggesting a role for this group during phytoplankton bloom senescence. Yet with no evidence of DMSP demethylase capabilities in cultured Flavobacteria, or indeed any member of the Bacteroidetes, this taxonomic trend alone should have had little effect on the community *dmdA* pool.

The small changes in DmdA composition might be explained by the decreasing importance of DMSPd relative to other phytoplankton-derived substrates as the bloom developed. Decreases in the Chl *a*:DMSPd ratio were highly correlated with *dmdA* composition (Figure 4.2). Other organic carbon and organic sulfur sources, including S-containing amino acids, may be supplying a larger fraction of bacterial demand in the experimental microcosms compared to the control. DmdA harboring organisms are capable of using alternative sulfur sources, including organic sulfur and (except for SAR11) sulfate. The small changes in DmdA composition might also point to ecologically insignificant differences in kinetic parameters among the protein clades. To this end, Reisch, et al (25) recently found similar K_ms, catalytic efficiencies, and pH optima for purified representative proteins from Clades A and D. Reisch et al. (25) also found evidence that bacterioplankton concentrate DMSP to high concentrations (70 mM in *S. pomeroyi*), typical for compounds used as an osmoprotectant and in agreement with field studies of natural bacterioplankton communities (31). Consistently high intracellular DMSP concentrations might decrease pressures for the protein to be optimized for external DMSP concentrations.

This study took advantage of deep amplicon sequencing made possible by pyrosequencing technology to follow the changes in the relative abundance of sequences encoding five clades of the ecologically important gene *dmdA*. While there are dynamics at the cluster level (>90% sequence similarity), *dmdA* composition at the clade level was less responsive to shifting environmental DMSP availability or other environmental variables than hypothesized, at least among the genes that can be amplified by the universal *dmdA* primer set. Relative constancy in *dmdA* clade composition was also found spatially in the GOS metagenomic data (12). In this bloom of low DMSP-producing phytoplankton, the relative importance of major *dmdA* clades was predictable from the taxonomic composition of the bacterioplankon but not from the environmental dynamics, including DMSPd flux and concentrations and relative importance of DMSP-derived carbon and sulfur.

Acknowledgements

We thank the captain of the R/V E.O. Wilson (Dauphin Island, AL) for help in sample collection, and Bradley Tolar, Erin J. Biers, Helmut Bürgmann, Montserrat Coll-Lladó, Allison Rellinger, Casey Smith, Scott Gifford, Xiaozhen Mou, and Adam Spaulding for their assistance in sample collecting and processing. This research was supported by NSF grant OCE070724017 (to M.A.M. and R.P.K.) and by a grant from the Gordon and Betty Moore Foundation (to M.A.M.).

References

1. Andreae, M. 1990. Ocean-atmosphere interactions in the global biogeochemical sulfur cycle. Marine Chemistry 30:1-29.

2. Andreae, M. O., and H. Raemdonck. 1983. Dimethyl Sulfide in the Surface Ocean and the Marine Atmosphere: A Global View. Science 221:744-747.

3. Biers, E. J., S. Sun, and E. C. Howard. accepted Applied and Environmental Microbiology. Prokaryotic Genomes and Diversity in the Surface Ocean: Interrogating the Global Ocean Sampling Metagenome.

4. Charlson, R. J., J. E. Lovelock, M. O. Andreae, and S. G. Warren. 1987. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326:655-661.

5. Cole, J. R., B. Chai, R. J. Farris, Q. Wang, A. S. Kulam-Syed-Mohideen, D. M. McGarrell, A. M. Bandela, E. Cardenas, G. M. Garrity, and J. M. Tiedje. 2007. The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. Nucleic Acids Research 35:D169-D172.

6. Curran, M. A. J., G. B. Jones, and H. Burton. 1998. Spatial distribution of dimethylsulfide and dimethylsulfoniopropiate in the Australasian sector of the Southern Ocean. Journal of Geophysical Research 103:16667-16689.

7. Curson, A. R. J., R. Rogers, J. D. Todd, C. A. Brearley, and A. W. B. Johnston. 2008. Molecular genetic analysis of a dimethylsulfoniopropionate lyase that liberates the climate-changing gas dimethylsulfide in several marine alpha-proteobacteria and *Rhodobacter sphaeroides*. Environmental Microbiology 10:757-767.

8. Del Valle, D. A., D. J. Kieber, and R. P. Kiene. 2006. Depth-dependent fate of biologically-consumed dimethylsulfide in the Sargasso Sea. Marine Chemistry 103:197-208.

9. Gonzalez, J. M., R. Simo, R. Massana, J. S. Covert, E. O. Casamayor, C. Pedros-Alio, and M. A. Moran. 2000. Bacterial Community Structure Associated wtih a Dimethylsulfoniopropionate-Producing North Atlantic Algal Bloom. Applied and Environmental Microbiology 66:4237-4246.

10. Gotelli, N. J., and G. L. Entsminger. 2008. EcoSim: Null models software for ecology. Version 7. Acquired Intelligence Inc. & Kesey-Bear, Jericho.

11. Howard, E. C., J. R. Henriksen, A. Buchan, C. R. Reisch, H. Bürgmann, R. Welsh, W. Ye, J. M. González, K. Mace, S. B. Joye, R. P. Kiene, W. B. Whitman, and M. A. Moran. 2006. Bacterial Taxa That Limit Sulfur Flux from the Ocean. Science 314:649-652.

12. Howard, E. C., S. Sun, E. J. Biers, and M. A. Moran. 2008. Abundant and diverse bacteria involved in DMSP degradation in marine surface waters. Environmental Microbiology 10:2397-2410.

13. Huber, J. A., D. B. M. Welch, H. G. Morrison, S. M. Huse, P. R. Neal, D. A. Butterfield, and M. L. Sogin. 2007. Microbial Population Structure in the Deep Marine Biosphere. Science 318:97-100.

14. Kiene, R. P. 1996. Production of methanethiol from dimethylsulfoniopropionate in marine surface waters. Marine Chemistry 54:69-83.

15. Kiene, R. P., L. P. Hoffman Williams, and J. E. Walker. 1998. Seawater microorganisms have a high affinity glycine betaine uptake system which also recognizes dimethylsulfoniopropionate. Aquatic Microbial Ecology 15:39-51.

16. Kiene, R. P., L. J. Linn, J. M. Gonzalez, M. A. Moran, and J. A. Bruton. 1999. Dimethylsulfoniopropionate and methanethiol are important precursors of methionine and protein-sulfur in marine bacterioplankton. Applied and Environmental Microbiology 65:4549-4558.

17. Kiene, R. P., and S. K. Service. 1991. Decomposition of dissolved DMSP and DMS in estuarine waters: dependence on temperature and substrate concentration. Marine Ecology Progress Series 76:1-11.

18. Kiene, R. P., and D. Slezak. 2006. Low dissolved DMSP concentrations in seawater revealed by small volume gravity filtration and dialysis sampling. Limnology and Oceanography 4:80-95.

19. Kirchman, D. L., E. K'nees, and R. E. Hodson. 1985. Leucine incorporation and its potential as a measure of protein synthesis by baceria in natural aquatic systems. Applied and Environmental Microbiology 49:599-607.

20. Li, W., and A. Godzik. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658-1659.

21. Lovelock, J. E., R. J. Maggs, and R. A. Rasmussen. 1972. Atmospheric Dimethyl Sulphide and that Natural Sulphur Cycle. Nature 237:452-453.

22. Malin, G., and G. O. Kirst. 1997. Algal Production of Dimethyl Sulfide and its Atmospheric Role. Journal of Phycology 33:889-896.

23. Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bemben, J. Berka, M. S. Braverman, Y.-J. Chen, Z. Chen, S. B. Dewell, L. Du, J. M. Fierro, X. V. Gomes, B. C. Godwin, W. He, S. Helgesen, C. Heen Ho, G. P. Irzyk, S. C. Jando, M. L. I. Alenquer, T. P. Jarvie, K. B. Jirage, J.-B. Kim, J. R. Knight, J. R. Lanza, J. H. Leamon, S. M. Lefkowitz, M. Lei, J. Li, K. L. Lohman, H. Lu, V. B. Makhijani, K. E. McDade, M. P. McKenna, E. W. Myers, E. Nickerson, J. R. Nobile, R. Plant, B. P. Puc, M. T. Ronan, G.

T. Roth, G. J. Sarkis, J. F. Simons, J. W. Simpson, M. Srinivasan, K. R. Tartaro, A. Tomasz, K. A. Vogt, G. A. Volkmer, S. H. Wang, Y. Wang, M. P. Weiner, P. Yu, R. F. Begley, and J. M. Rothberg. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376-380.

24. Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A manual for chemical and biological methods for seawater analysis, vol. Pergamon, New York, NY.

25. Reisch, C. R., M. A. Moran, and W. B. Whitman. accepted Journal of Bacteriology. Purification and Characterization of DMSP Dependent Demethylase (DmdA) from Marine Bacteria.

26. Rusch, D. B., A. L. Halpern, G. Sutton, K. B. Heidelberg, S. Williamson, S. Yooseph, D. Wu, J. A. Eisen, J. M. Hoffman, K. Remington, K. Beeson, B. Tran, H. Smith, H. Baden-Tillson, C. Stewart, J. Thorpe, J. Freeman, C. Andrews-Pfannkoch, J. E. Venter, K. Li, S. Kravitz, J. F. Heidelberg, T. Utterback, Y.-H. Rogers, L. I. Falcon, V. Souza, G. Bonilla-Rosso, L. E. Eguiarte, D. M. Karl, S. Sathyendranath, T. Platt, E. Bermingham, V. Gallardo, G. Tamayo-Castillo, M. R. Ferrari, R. L. Strausberg, K. Nealson, R. Friedman, M. Frazier, and J. C. Venter. 2007. The *Sorcerer II* Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. PLoS Biology 5:0398-0431.

27. Simo, R. 2001. Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological, evolutionary links. TRENDS in Ecology and Evolution 16:287-294.

28. Slezak, D., R. P. Kiene, D. A. Toole, R. Simo, and D. J. Kieber. 2007. Effects of solar radiation on the fate of dissolved DMSP and conversion to DMS in seawater. Aquatic Science 69:377-393.

29. Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. Marine Microbial Food Webs 6:107-114.

30. Sogin, M. L., H. G. Morrison, J. A. Huber, D. M. Welch, S. M. Huse, P. R. Neal, J. M. Arrieta, and G. J. Herndl. 2006. Microbial diversity in the deep sea and the unexplored "rare biosphere". Proceedings of the National Academy of Sciences 103:12115-12120.

31. Sunda, W., D. J. Kieber, R. P. Kiene, and S. Huntsman. 2002. An antioxidant function for DMSP and DMS in marine alga. Nature 418:317-320.

32. Todd, J. D., R. Rogers, Y. Guo Li, m. Wexler, P. L. Bond, L. Sun, A. R. J. Curson, G. Malin, M. Steinke, and A. W. B. Johnston. 2007. Structural and Regulatory Genes Required to Make the Gas Dimethyl Sulfide in Bacteria. Science 315:666-669.

33. 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. Limnology and Oceanography 33:364-375.

34. Varaljay, V. A., E. C. Howard, S. Sun, and M. A. Moran. in prep. Diversity of Dimethylsulfoniopropionate Demethylase Genes in a Marine Ecosystem Assessed by Deep Amplicon Sequencing of *dmdA* Primer Sets.

35. Vila, M., R. Simo, R. P. Kiene, J. Pinhassi, J. M. Gonzalez, M. A. Moran, and C. Pedros-Alio. 2004. Use of Microautoradiography combined with fluorescence In Situ Hybridization to Determine Dimethylsulfoniopropionate Incorporation by Marine Bacterioplankton Taxa. Applied and Environmental Microbiology 70:4648-4657.

36. Zubkov, M. V., B. M. Fuchs, S. D. Archer, R. P. Kiene, R. Amann, and P. H. Burkill. 2001. Linking the composition of bacterioplankton to rapid turnover of dissolved dimethylsulfoniopropionate in an algal bloom in the North Sea. Environmental Microbiology 3:304-311.

Figure 4.1. Control and experimental microcosm chemical measurements.

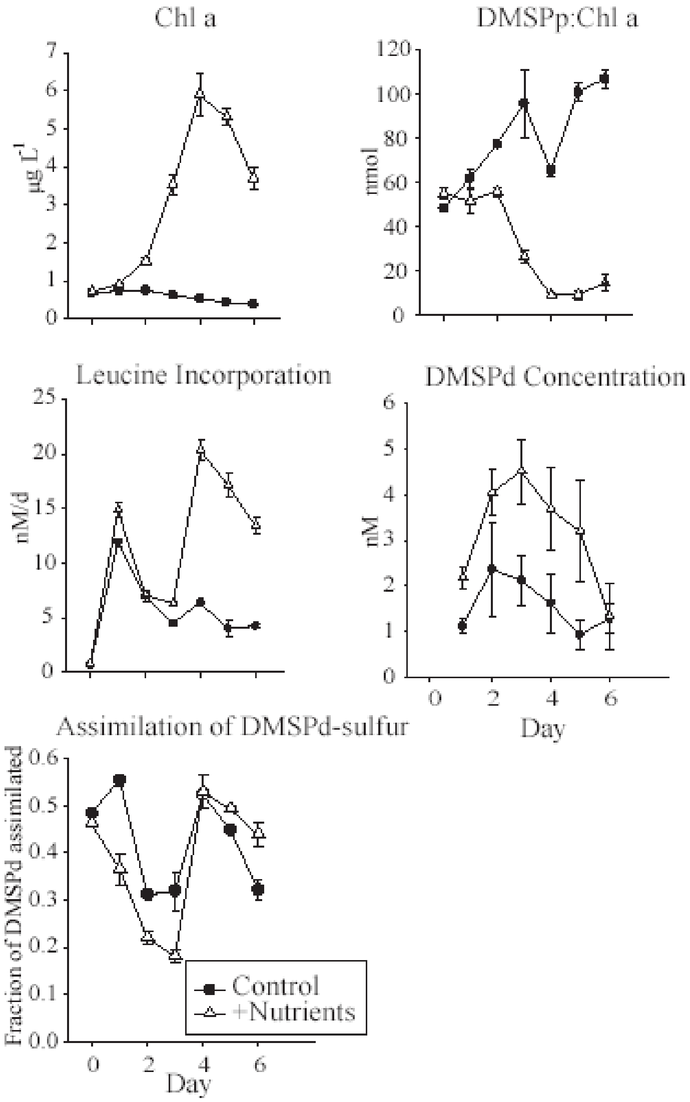
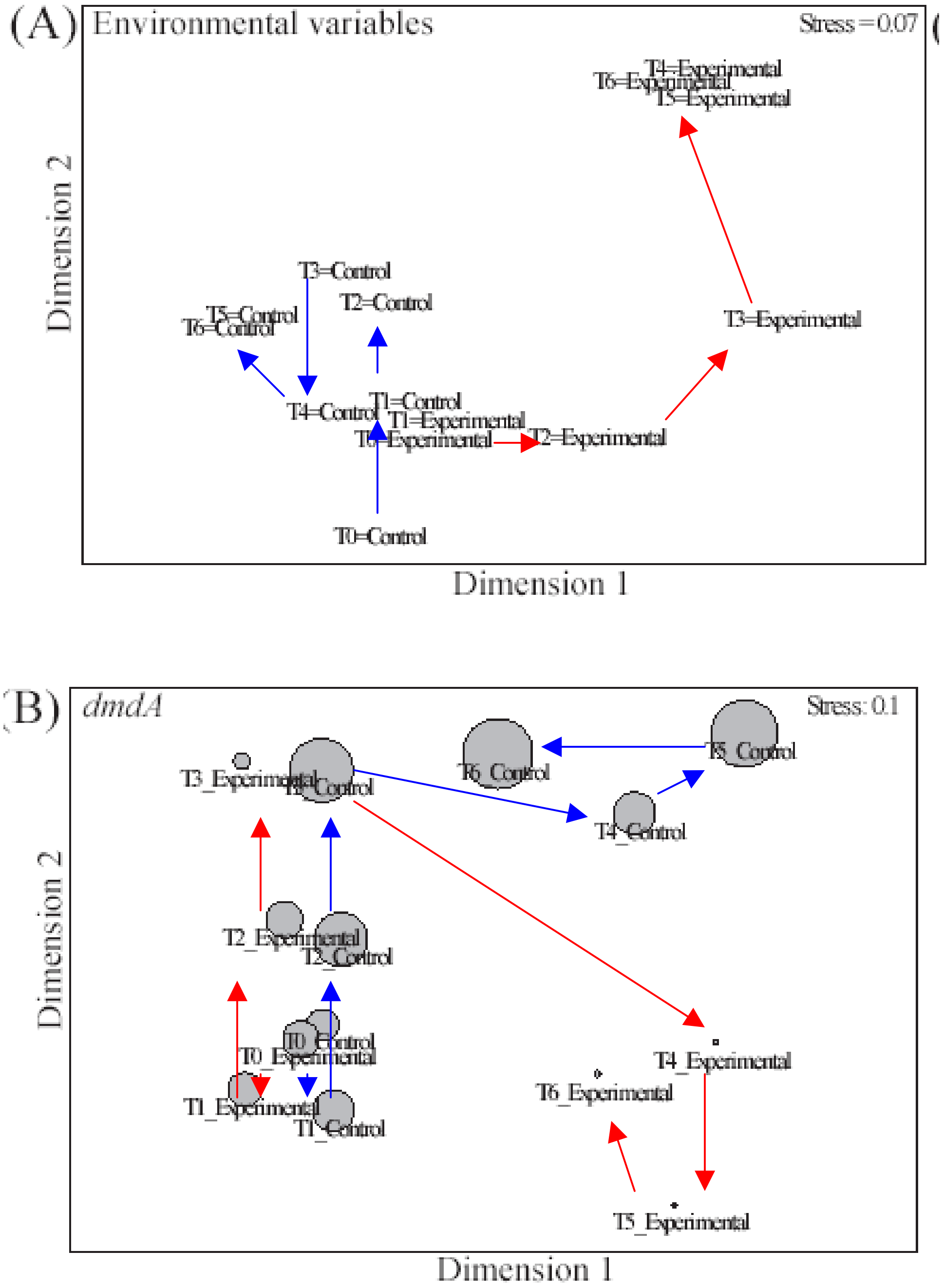
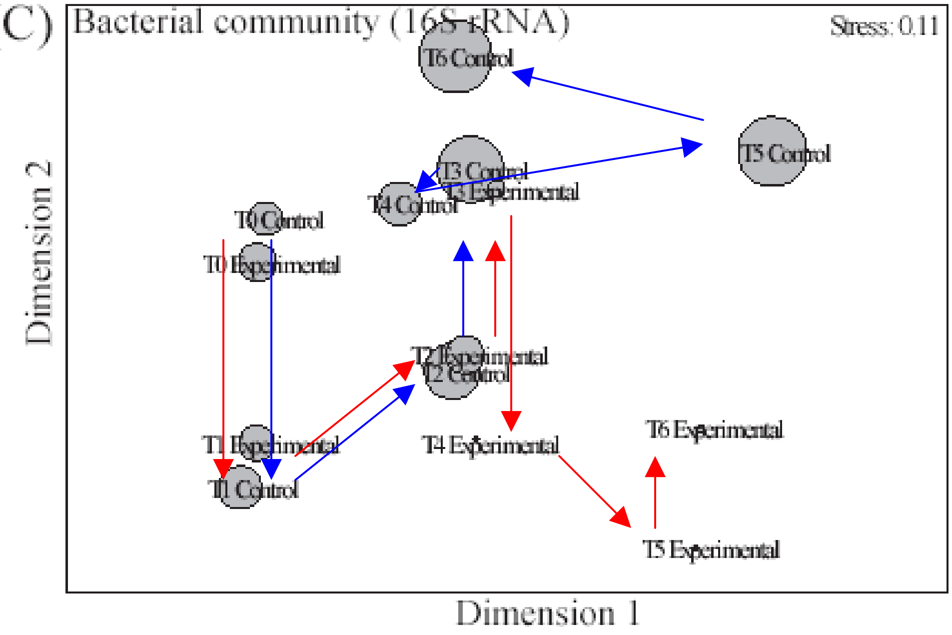

Chl a
μg L-1
0
1
2
3
4
5
6
7
DMSPp:Chl a
nmol
0
20
40
60
80
100
120
Leucine Incorporation
nM/d
0
5
10
15
20
25
DMSPd Concentration
nM
0
1
2
3
4
5
6
0
2
4
6
Day
Assimilation of DMSPd-sulfur
Fraction of DMSPd assimilated
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
2
4
6
Day
Control
+Nutrients

Figure 4.2. Multidimensional scaling of the Bray-Curtis similarity matrix generated using the relative concentrations of environmental variables (A) and the relative abundance of clusters of *dmdA* sequences (B), clusters of bacterial 16S rRNA sequences (C), and clusters of archaeal 16S rRNA sequences (D) over the time course in experimental and control samples. Bubbles (plots B-D) indicate the ratio of DMSPp:Chl a at the indicated time point and sample. Arrows follow the sample time progression through the control (blue) and experimental (red) time courses.


(A)
Environmental variables
Stress=0.07
T4=Experimental
T6=Experimental
T5=Experimental
T3=Control
T2=Control
T5=Control
T6=Control
T3=Experimental
T4=Control
T1=Control
T1=Experimental
T6=Experimental
T2=Experimental
T0=Control
Dimension 2
Dimension 1
(B)
dmdA
Stress: 0.1
T3_Experimental
T3_Control
T6_Control
T5_Control
T4_Control
T2_Experimental
T2_Control
T0_Control
T0_Experimental
T1_Experimental
T1_Control
T4_Experimental
T6_Experimental
T5_Experimental
Dimension 2
Dimension 1

(C)


Bacterial community (16S rRNA)
Stress: 0.11
T6 Control
T5 Control
T3 Control
T3 Experimental
T4 Control
T0 Control
T0 Experimental
T2 Experimental
T2 Control
T1 Experimental
T1 Control
T4 Experimental
T6 Experimental
T5 Experimental
Dimension 1
Dimension 2

(D)

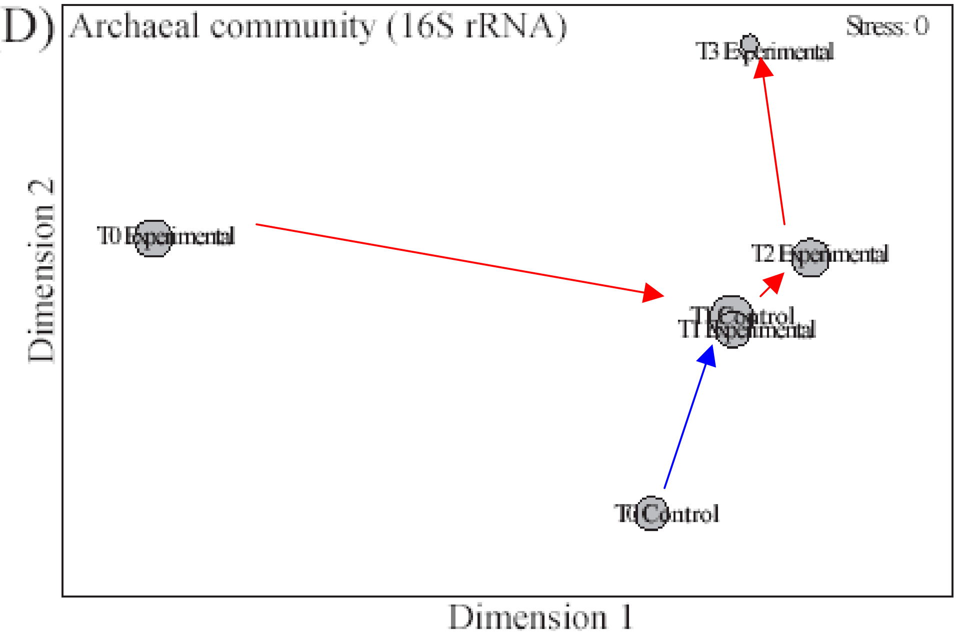
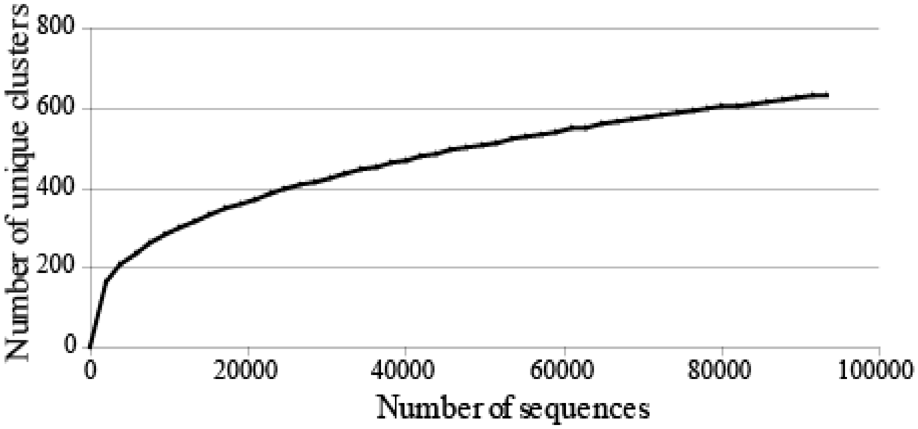

Archaeal community (16S rRNA)
Stress: 0
T3 Experimental
T0 Experimental
T2 Experimental
T1 Control
T1 Experimental
T0 Control
Dimension 1
Dimension 2

Figure 4.3. Rarefaction curve of *dmdA* sequences clustered at the 90% similarity level.



Number of unique clusters
800
600
400
200
0
0
20000
40000
60000
80000
100000
Number of sequences

Figure 4.4. Relative abundance of DmdA clades (A), bacterial taxa (B), and archaeal taxa (C) over the time course between control and experimental samples. The y-axis represents percent of sequences in each sample in the indicated classification.

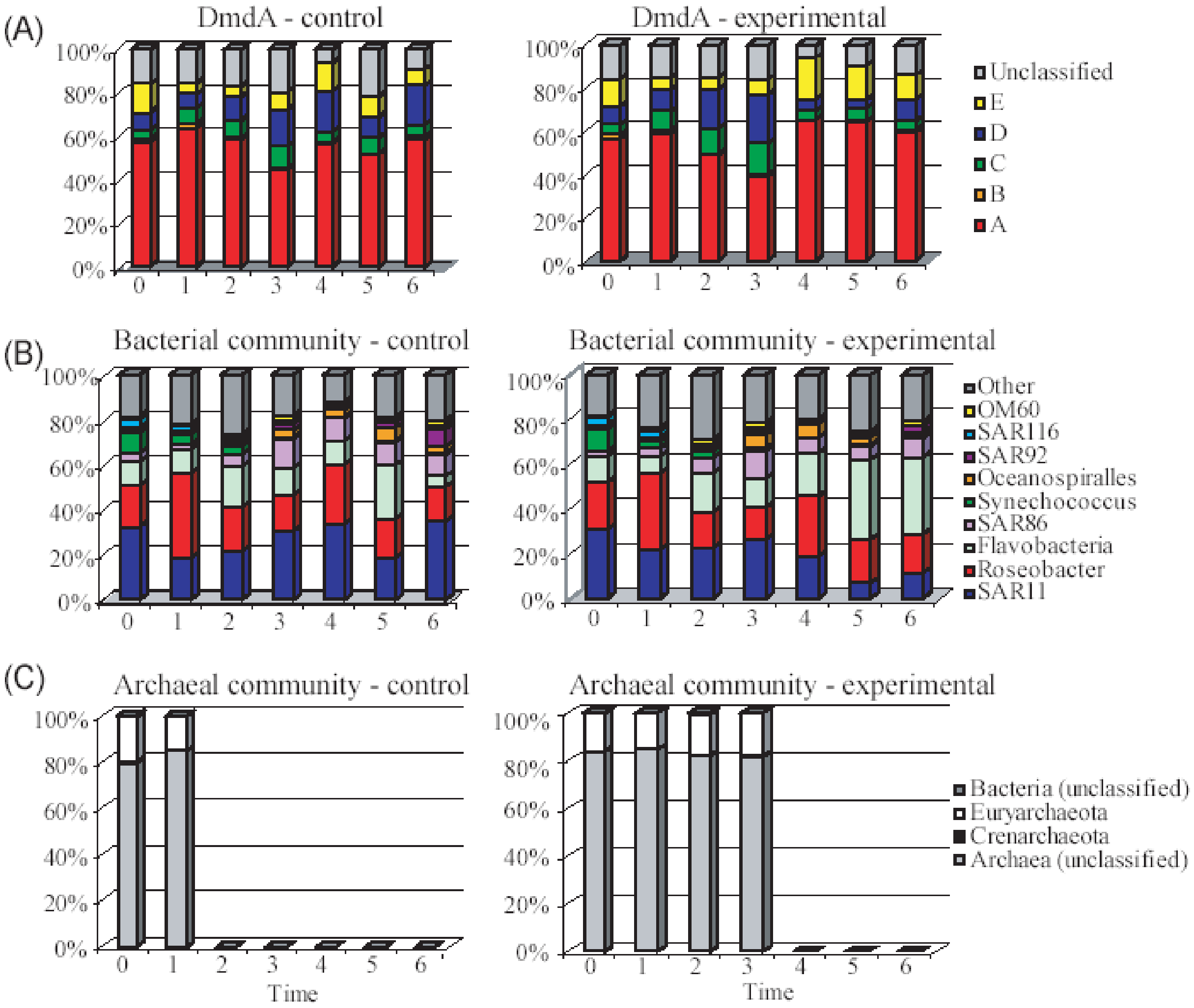
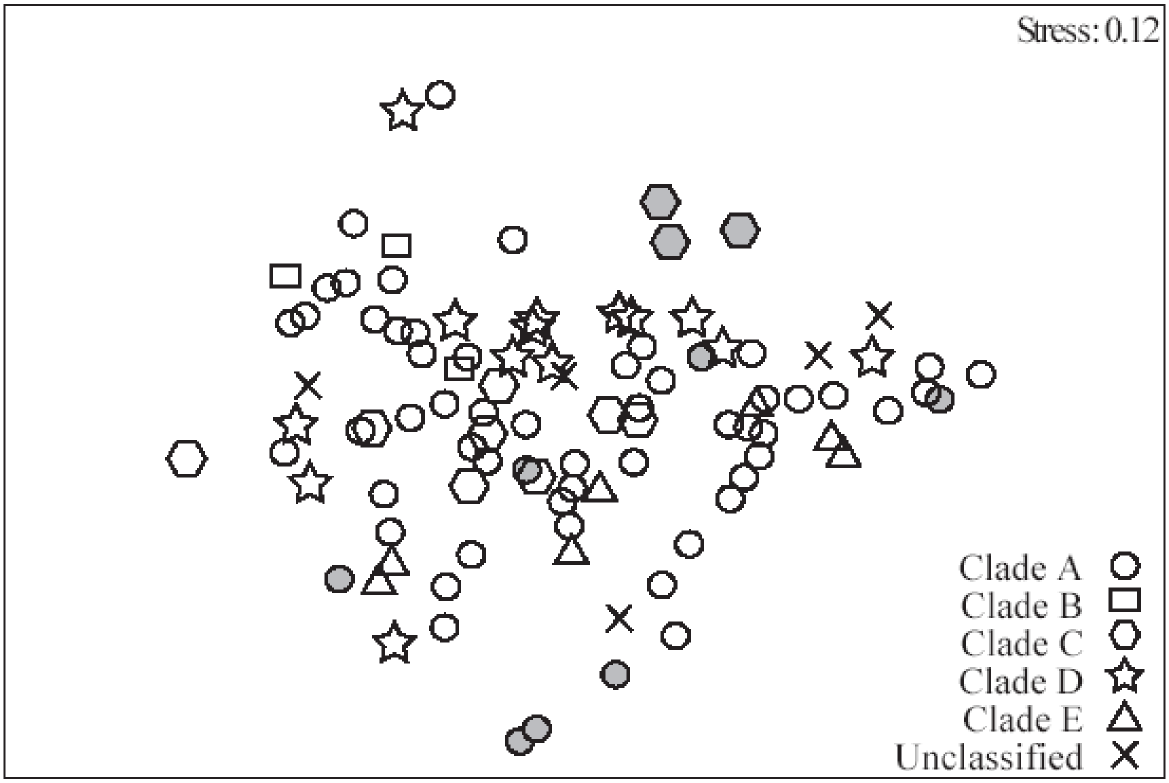

(A)
DmdA - control
DmdA - experimental
100%
80%
60%
40%
20%
0%
0 1 2 3 4 5 6
Unclassified
E
D
C
B
A
(B)
Bacterial community - control
Bacterial community - experimental
Other
OM60
SAR116
SAR92
Oceanospiralles
Synechococcus
SAR86
Flavobacteria
Roseobacter
SAR11
(C)
Archaeal community - control
Archaeal community - experimental
Bacteria (unclassified)
Euryarchaeota
Crenarchaeota
Archaea (unclassified)
Time

Figure 4.5. Multidimensional scaling of the Bray-Curtis similarity matrix generated using the relative abundance of each top 100 DmdA cluster (observes the relative similarity between each indicated cluster among samples/days). Filled symbols indicate the top 10 clusters contributing to the dissimilarity between control and experimental samples as indicated by the SIMPER analysis.


Stress: 0.12
Clade A
Clade B
Clade C
Clade D
Clade E
Unclassified

CHAPTER 5

CONCLUSIONS

The intent of this study was to understand how the capability for demethylating dimethylsulfoniopropionate (DMSP) is temporally and spatially distributed among surface ocean marine bacteria. This metabolic process is responsible for diverting DMSP-sulfur away from the climatically-active gas dimethylsulfide (DMS), and for providing substantial amounts of reduced carbon and sulfur to the marine microbial food web.

In Chapter 2, the roseobacter clade bacterium *Silicibacter pomeroyi* DSS-3, which can both demethylate and cleave DMSP, was used to identify the gene responsible for bacterial demethylation. A mutant library was created by random transposon mutagenesis. A mutant was found that was unable to demethylate DMSP to methylmercaptopropionate (MMPA) but retained DMSP cleavage function. In this mutant, the transposon had inserted into gene SPO1913, originally annotated as an “amino methyltransferase” (Figure 2.1; 1). Demethylation function was restored upon complementation with a fully functional SPO1913 gene copy (Figure 2.4; 1). This gene, conclusively demonstrated to be responsible for demethylating DMSP, was named *dmdA*. DmdA was also found in the ubiquitous marine SAR11 group, represented by strains *Pelagibacter ubique* HTCC1002 and *P. ubique* HTCC1062, as well as several other roseobacter species (Figure 2.2). DMSP demethylation function was demonstrated experimentally for the *dmdA* from *P. ubique*, by synthesizing the gene with *Escherichia coli* codon usage and testing for function in trans (Figure 2.1; 1). Finding *dmdA* in the roseobacter taxon (highly abundant coastal bacteria) as well as in the SAR11 taxon (highly abundant open ocean bacteria) suggests the capability for DMSP demethylation throughout much of the surface ocean.

Using the Sargasso Sea pilot project portion of the Global Ocean Sampling (GOS) expedition (4), four clades of *dmdA* (designated A, B, C, and D) were found. Roseobacter organisms were found to harbor Clade A *dmdA* sequences and SAR11 organisms to harbor Clade D (Figure 2.2; 1). In Chapter 3, a bioinformatic analysis that included more of the GOS samples (Northwest Atlantic through the Eastern tropical Pacific; 3) provided evidence of another *dmdA* clade, designated Clade E. Clustering with the environmental sequences of this relatively small clade is the first cultured non-alphaproteobacterium known to contain a putative *dmdA*, gammaproteobacterium HTCC2080 (Figure 3.2; 2). Based on the range of G+C content and gene neighborhoods, all of the *dmdA* clades except Clade D are taxonomically heterogenous (Figure 3.3; 2). As more bacterioplankton genomes become sequenced, it is likely that the number and diversity of organisms harboring a *dmdA* gene will increase.

Normalizing the number of *dmdA* sequences found in the 2007 GOS to the average number of six, single-copy genes in the GOS (a*tpD*, *rpoB*, *gyrB*, *hsp70*, *tufA* and *recA*), *dmdA* was found to be present in 58±9% of bacterioplankton cells in ocean surface waters (2). This incredible abundance of a biogeochemically relevant gene in seawater is rivaled only by the abundance of the likewise biogeochemically relevant proteorhodopsin gene (43% of cells; Table 3.2; 2, 3). Genes recently discovered to act in the cleavage pathway of DMSP degradation, *dddD* and *dddL*, are calculated to be present in only 0.2% and 0.6% of surface water bacterioplankton cells, respectively (Table 3.2; 2), suggesting that either bacteria are not primarily responsible for DMSP degradation to DMS and/or that the major DMSP cleavage genes are yet to be found.

In Chapter 4, a microcosm study of an induced phytoplankton bloom in Gulf of Mexico seawater over the course of six days revealed changes in the relative distribution of the *dmdA* clades during the development and decline of the bloom. The relative abundance of *dmdA* genes in Clades A and E marginally increased, while those in Clades C and D marginally decreased in relative abundance (Figure 4.4). Further analysis revealed that clusters from Clades A and C were primarily responsible for the dissimilarity between experimental (bloom) and control (non-bloom) conditions (Figure 4.5).

The discovery of the DMSP demethylase *dmdA*, and understanding its genetics, diversity, and distribution both spatially and temporally, reveals the capabilities and dynamics of marine surface water bacterioplankton. This work provides a strong basis for further studies into the regulation of bacterial DMSP demethylation in the ocean.

References

1. Howard, E. C., J. R. Henriksen, A. Buchan, C. R. Reisch, H. Bürgmann, R. Welsh, W. Ye, J. M. González, K. Mace, S. B. Joye, R. P. Kiene, W. B. Whitman, and M. A. Moran. 2006. Bacterial Taxa That Limit Sulfur Flux from the Ocean. *Science* 314:649-652.

2. Howard, E. C., S. Sun, E. J. Biers, and M. A. Moran. 2008. Abundant and diverse bacteria involved in DMSP degradation in marine surface waters. *Environmental Microbiology* 10:2397-2410.

3. Rusch, D. B., A. L. Halpern, G. Sutton, K. B. Heidelberg, S. Williamson, S. Yooseph, D. Wu, J. A. Eisen, J. M. Hoffman, K. Remington, K. Beeson, B. Tran, H. Smith, H. Baden-Tillson, C. Stewart, J. Thorpe, J. Freeman, C. Andrews-Pfannkoch, J. E. Venter, K. Li, S. Kravitz, J. F. Heidelberg, T. Utterback, Y.-H. Rogers, L. I. Falcon, V. Souza, G. Bonilla-Rosso, L. E. Eguiarte, D. M. Karl, S. Sathyendranath, T. Platt, E. Bermingham, V. Gallardo, G. Tamayo-Castillo, M. R. Ferrari, R. L. Strausberg, K. Nealson, R. Friedman, M. Frazier, and J. C. Venter. 2007. The *Sorcerer II* Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. *PLoS Biology* 5:0398-0431.

4. Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y.-H. Rogers, and H. O. Smith. 2004. Environmental Genome Shotgun Sequencing of the Sargasso Sea. *Science* 304:66-74.

APPENDIX A

CHAPTER 2 SUPPLEMENTARY MATERIAL[1]

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[1] Supporting online material for:

Howard, Erinn C., James R. Henriksen, Alison Buchan, Chris R. Reisch, Helmut Burgmann, Rory Welsh, Wenying Ye, Jose M. Gonzalez, Kimberly Mace, Samantha B. Joye, Ronald P. Kiene, William B. Whitman, and Mary Ann Moran. 2006. *Science*. 314: 649-652.

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Methods

Mutant library construction and screening

A random transposon insertion library of *S. pomeroyi* DSS-3 was constructed using the EZ-Tn5 <KAN-2> Tnp Transposome Kit (Epicentre) with the addition of TypeOne Restriction Inhibitor (Epicentre). A collection of 20,000 transformants were screened for lack of MeSH production as follows: to overnight cultures grown in an artificial seawater medium (with 10 mM lactate, 17 mg L-1 yeast extract, 27 mg L-1 tryptone, and 10 mg·L-1 kanamycin), DMSP was added (1 mM final concentration) followed 3 h later by Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid; 1 mM final concentration). Colorless wells indicated lack of MeSH formation. For mutants of interest, DNA flanking the transposon insertion site was determined by the RATE (random amplification of transposon ends) approach (*1*).

Analysis of sulfur compounds

For gas chromatographic analysis of sulfur gases, wild-type *S. pomeroyi* and putative MeSH_ mutants were grown overnight in 60 ml Teflon-sealed serum bottles (with 20 mM glucose and 10 mg L-1 kanamycin in artificial seawater medium). DMSP or MMPA (1 mM) was added, and 500 µl of headspace gas was analyzed after 24 h by FPD gas chromatography (*2*) (SRI 8610-C with a Chromosil 330 column, nitrogen carrier gas at 60 ml min-1, 60°C oven temperature).

Complementation

To construct plasmid pECH01, SPO1913 plus 100 bp upstream was amplified from wildtype *S. pomeroyi* DNA and ligated into plasmid pRK415 (*3*). pECH01 was introduced into *E. coli* S17-1 and delivered to the *dmdA*- mutant 41-H6 via biparental mating. Resultant 41-H6 transconjugants were assayed by GC for restoration of wild-type sulfur gas formation, as described above. Mutant 41-H6 carrying the vector pRK415 alone served as a negative control. Analysis of DMSP demethylase activity in cell-free extracts Cells were grown on 1 mM glucose/artificial seawater medium with 2 mM DMSP. After 2 days, cells were harvested and resuspended in anoxic potassium phosphate buffer (50 mM, pH 7.2) plus 1 mM DTT. Under anaerobic conditions, cells were lysed by passage through a French pressure cell at 20,000 psi. Extracts (~1 mg of protein) were assayed for MMPA formation (*4*) for 90 min at 25oC in the presence of 5 mM DMSP. Reactions were quenched by freezing, and MMPA concentrations were measured by liquid chromatography (*5*) on an Eclipse XDB-C18 column (2.1 x 150 mm; Aligent) by isocratic elution (1.25% acetonitrile and 0.2% phosphoric acid at 0.75 ml min-1) and detected by absorbance at 214 nm.

Identification of *dmdA* homologs

dmdA homologs were identified in cultured organisms using BLAST analysis of the GenBank database and the Moore Foundation Marine Microbial Genome Sequencing Project database https://research.venterinstitute.org/moore/) with *S. pomeroyi* SPO1913 as the query sequence and a maximum E value of <10-80. *dmdA* homologs in the Sargasso Sea metagenomic libraries (*6*) were identified by BLAST analysis of the unassembled trace files (https://research.venterinstitute.org/sargasso/) with SPO1913 as the query sequence and a maximum E value of 10-35. Sargasso Sea homologs were translated and sequences longer than 120 amino acid residues were aligned using ClustalW in five groups, depending on the region of DmdA covered by each sequence, along with sequences of Roseobacter DmdA and related GcvT sequences. Trees were constructed with programs from the PHYLIP package (*7*) using the James-Taylor-Thornton matrix for distance calculation. Based on these trees, sequences were designated orthologs and assigned to one of four putative DmdA clades or otherwise rejected. A final tree with selected sequences was constructed with MEGA version 3.1 using minimum evolution phylogenetic reconstruction tested with 100 bootstrap samplings. Other tree building algorithms (Maximum parsimony, Neighbor Joining) supported the observed topology. *dmdA* homologs in the Station Aloha (*8*) metagenomic libraries were identified by BLAST analysis with SPO1913 and SAR11_0246 as the query sequences and a maximum E value of 10-35. The Sapelo Island metagenomic library was generated from a 0.2-3.0 µm size fraction of surface water collected in November 2005 by pyrosequencing (454 Inc.). *dmdA* homologs were identified by BLAST analysis with SPO1913 and SAR11_0246 as the query sequences and a maximum E value of 10-5. Possible *dmdA* sequences were manually annotated.

Heterologous expression

The *dmdA* homolog in the *P. ubique* genome (*9*), SAR11_0246, was synthesized commercially with *E. coli* codon usage (GenScript Corporation). The synthesized gene was inserted into expression vector pCYB1 (Invitrogen) to generate pABX101 and introduced into *E. coli* Top10F'. Plasmid-bearing *E. coli* cells were grown overnight in Luria-Bertani broth, 70 µM DMSP, and isopropyl-â-D-thiogalactopyranoside (IPTG; 500 µM). After incubation, cell-free extracts were prepared and assayed for MMPA production by HPLC as above. *E. coli* Top10F' carrying the vector pCYB1 alone served as the negative control.

Table 2.3. DMSP demethylation phenotype and presence of *dmdA* orthologs in 12 marine Roseobacter strains. n.d. = not determined. Genome sequences are available at NCBI[a].

Organism	**DMSP demethylation activity**	***dmdA* ortholog**	**identity**
Silicibacter pomeroyi DSS-3	+[b]	+	100%
Silicibacter sp. TM1040	+[c]	+	68%
Roseovarious nubinhibens ISM	+[b]	+	62%
Sulfitobacter sp. EE-36	-[b]	-	
Sulfitobacter sp. NAS-14.1	n.d	-	
Jannaschia sp. CCS1	n.d.	+	60%
Roseobacter sp. MED193	n.d.	+	65%
Roseovarius sp. Str. 217	n.d.	+	64%
Loktanella vestfoldensis SKA53	n.d.	-	
Rhodobacterales sp. HTCC2654	n.d.	-	
Oceanicola batsensis HTCC2597	n.d.	-	
Oceanicola granulosus HTCC2516	n.d.	-	

[a] Accession numbers: NC_006569 (*S. pomeroyi*), AAFG00000000 (*Silicibacter* sp. TM1040), AAIG00000000 (*Jannaschia* sp. CCS1), AALY00000000 (*Roseovarius nubinhibens* ISM), AALV00000000 (*Sulfitobacter* sp. EE-36), AAMS00000000 (*Loktanella vestfoldensis* SKA53), AANB00000000 (*Roseobacter* sp. MED193), AAMV00000000 (*Roseovarius* sp. 217), AAMT00000000 (Rhodobacterales sp. HTCC2654), AAMO00000000 (*Oceanicola batsensis* HTCC2597), and AAOT00000000 (*O. granulosus* HTCC2516).

[b] See reference 10

[c] See reference 11

Figure 2.3. Proposed pathways of dimethylsulfoniopropionate (DMSP) degradation by marine bacterioplankton (10). The gray circle indicates the gene (*dmdA*) responsible for routing DMSP to methylmercaptopropionate (MMPA) and away from the climatically active dimethylsulfide (DMS). Genes SPO1913 from *Silicibacter pomeroyi* DSS-3 and SAR11_0246 from *P. ubique* encode a confirmed DMSP demethylase. THF = tetrahydrofolate. MPA = mercaptopropionate. MeSH = methanethiol.

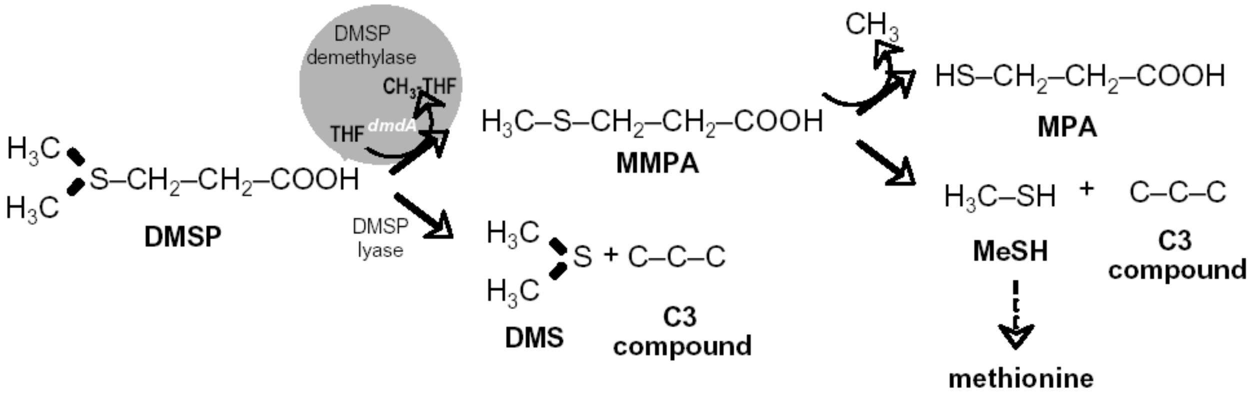
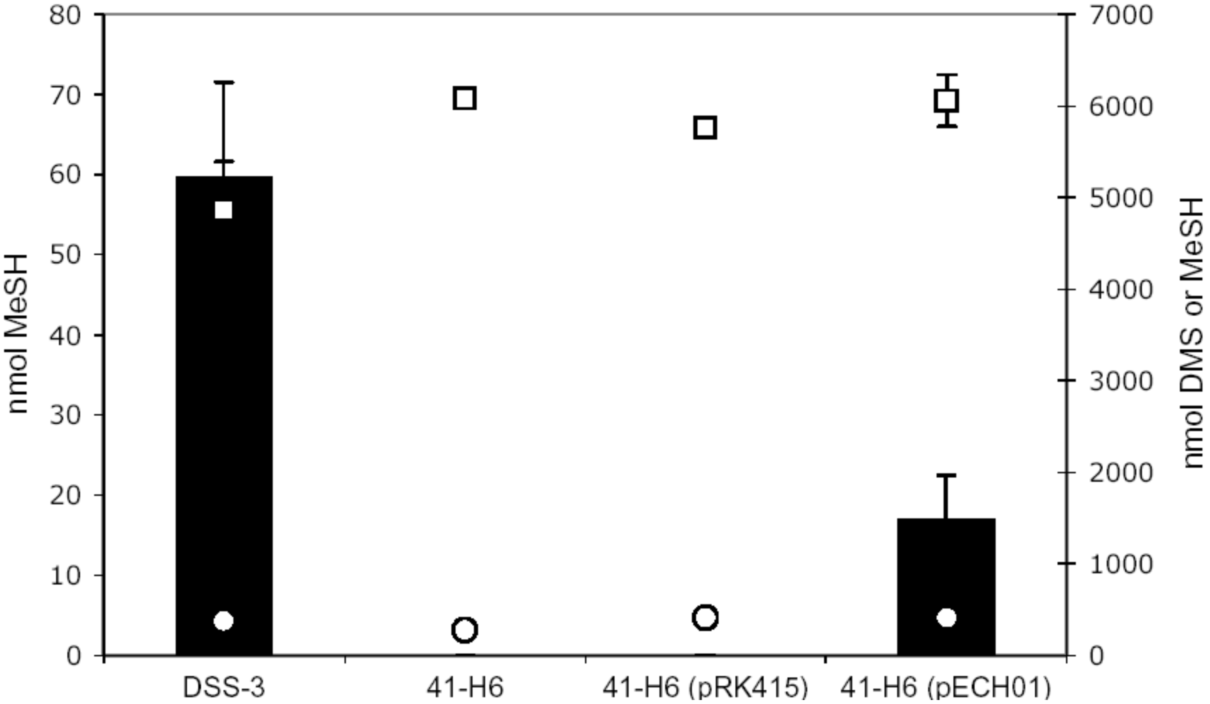

DMSP demethylase
CH3-THF
THF
dmdA
DMSP lyase
DMSP
MMPA
DMS
C3 compound
CH3
MPA
MeSH
C3 compound
methionine

Figure 2.4. DMSP degradation by *Silicibacter pomeroyi* after 24 h of incubation with mM DMSP or MMPA. DSS-3 = wild type; 41-H6 = MeSH– mutant; 41-H6 (pRK415) mutant with the vector pRK415 alone; 41-H6 (pECH01) = mutant with *in trans* copy of SPO1913 in pRK415. Black bars show production of MeSH from DMSP (left axis) and demonstrate the absence of DMSP demethylase activity in mutant 41-H6 and restoration of activity with an intact copy of SPO1913. White squares show production of DMS from DMSP and white circles show production of MeSH from MMPA (right axis), both of which are unaffected by disruption of SPO1913. Standard errors are shown where they exceed the size of the symbol. n=3.


80
70
60
50
40
30
20
10
0
nmol MeSH
7000
6000
5000
4000
3000
2000
1000
0
nmol DMS or MeSH
DSS-3
41-H6
41-H6 (pRK415)
41-H6 (pECH01)

References

1. Ducey, T. F. and D. W. Dyer. 2002. Rapid identification of EZ::TN$^{\mathrm{TM}}$ transposon insertion sites in the genome of *Neisseria gonorrhoeae*. Epicentre Forum 9: 6.

2. de Souza, M.P. and D. C. Yoch. 1995. Purification and Characterization of Dimethylsulfoniopropionate Lyase from an Alcaligenes-Like Dimethyl Sulfide-Producing Marine Isolate. Appl. Environ. Microbiol. 61: 21.

3. Keen, N. T., S. Tamaki, D. Kobayashi, and D. Trollinger. 1988. Improved broad-host-range plasmids for DNA cloning in Gram-negative bacteria. Gene 70: 191.

4. Jansen, M., and T. A. Hansen. 1998. Tetrahydrofolate serves as a methyl acceptor in the demethylation of dimethylsulfoniopropionate in cell extracts of sulfate-reducing bacteria. Archives Microbiology 169: 84-87.

5. Ansede, J. H., P. J. Pellechia, and D. C. Yoch, Appl. 1999. Metabolism of Acrylate to β-Hydroxypropionate and Its Role in Dimethylsulfoniopropionate Lyase Induction by a Salt Marsh Sediment Bacterium, *Alcaligenes faecalis* M3A. Environ. Microbiol. 65: 5075.

6. Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y.-H. Rogers, and H. O. Smith. 2004. Environmental Genome Shotgun Sequencing of the Sargasso Sea. Science 304:66-74.

7. Felsenstein, J. 1989. Cladistics. 5: 164.

8. DeLong, E.F., C.M. Preston, T. Mincer, V. Rich, S.J. Hallam, N.-U. Frigaard, A. Martinez, M.B. Sullivan, R. Edwards, B.R. Brito, S.W. Chisholm, and D.M. Karl. 2006. Science 311: 496.

9. Giovannoni, S. J., J. H. Tripp, S. Givan, M. Podar, K. L. Vergin, D. Baptista, L. Bibbs, J. Eads, T. H. Richardson, M. Noordewier, M. S. Rappe, J. M. Short, J. C. Carrington, and E. J. Mathur. 2005. Genome streamlining in a cosmopolitan oceanic bacterium. Science 309:1242-1245.

10. Kiene, R. P., L. J. Linn, J. M. Gonzalez, M. A. Moran, and J. A. Bruton. 1999. Dimethylsulfoniopropionate and methanethiol are important precursors of methionine and protein-sulfur in marine bacterioplankton. Applied and Environmental Microbiology 65:4549-4558.

11. Miller, T. R., and R. Belas. 2004. Dimethylsulfoniopropionate Metabolism by *Pfiesteria*-Associated *Roseobacter* spp. Applied and Environmental Microbiology 70:3383-3391.

APPENDIX B

CHAPTER 3 SUPPLEMENTARY MATERIAL[1]

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[1] Supporting online material for:

Howard, Erinn C., Shulei Sun, Erin J. Biers, and Mary Ann Moran. 2008. *Environmental Microbiology*. 10(9): 2397-2410.

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Figure 3.5. Correlation analysis of *dmdA* and 16S rDNA counts. (A) Clade A *dmdA* and Roseobacter 16S rRNA gene counts; (B) Clade D *dmdA* and SAR11 16S rRNA gene counts; and (C) Clade C *dmdA* and SAR11 (bottom axis-black), SAR86 (top axis-red) and SAR116 (top axis-green) 16S rRNA gene counts.

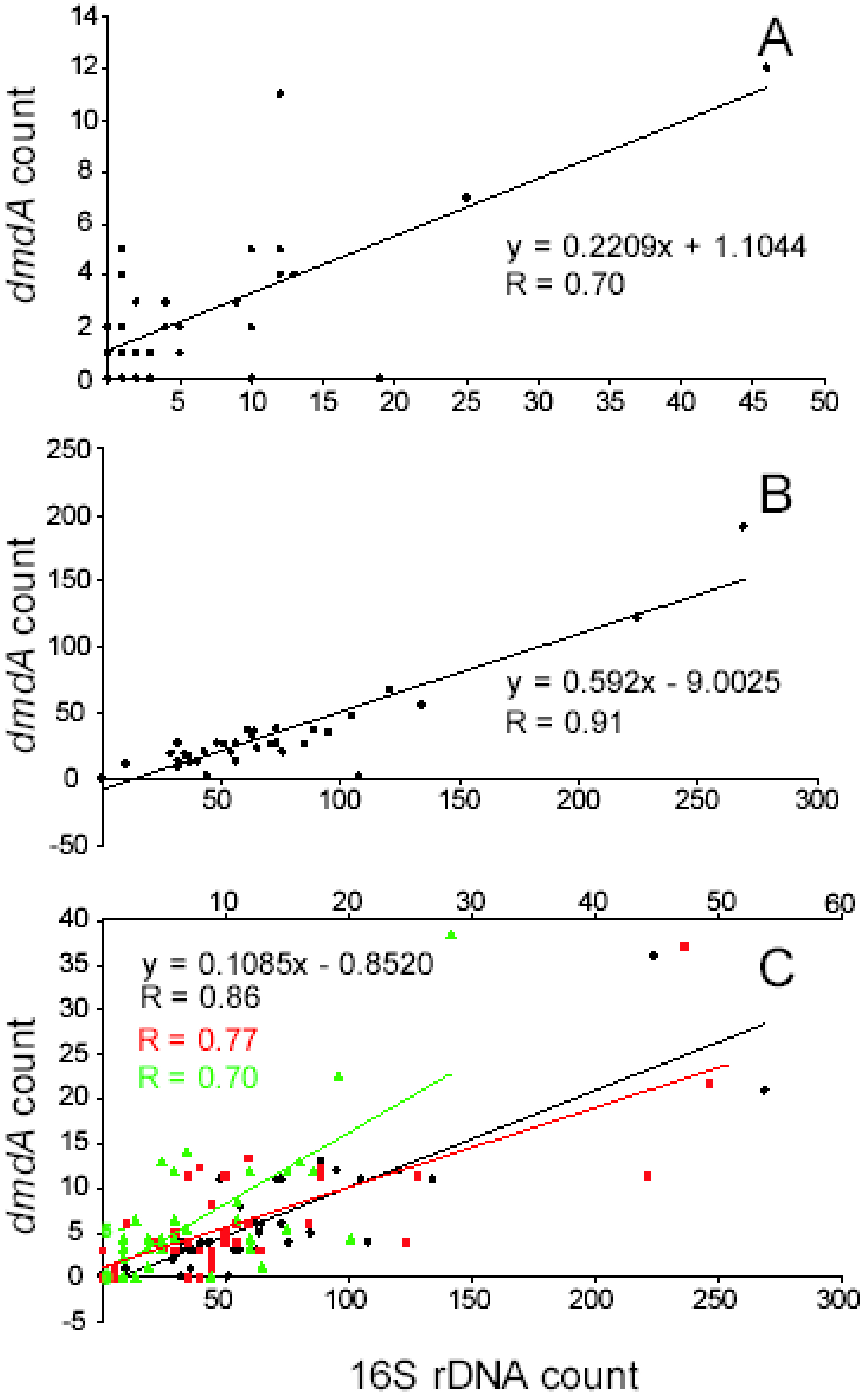

A
y = 0.2209x + 1.1044
R = 0.70
B
y = 0.592x - 9.0025
R = 0.91
C
y = 0.1085x - 0.8520
R = 0.86
R = 0.77
R = 0.70
dmdA count
16S rDNA count
0
2
4
6
8
10
12
14
5
10
15
20
25
30
35
40
45
50
-50
50
100
150
200
250
300
-5
60

Figure 3.6**.** Diversity of *dddD* in the (2007) GOS and cultured organisms. The neighbour-joining tree shows all *dddD* sequences in the (2007) GOS (except JCVI_PEP_1105118567433, which did not overlap). DddD sequences with confirmed function are indicated with a yellow star, and those deposited in GenBank since previous publication (Todd *et al*., 2007) are indicated with a green star. Related *caiB* out-group sequences are indicated.

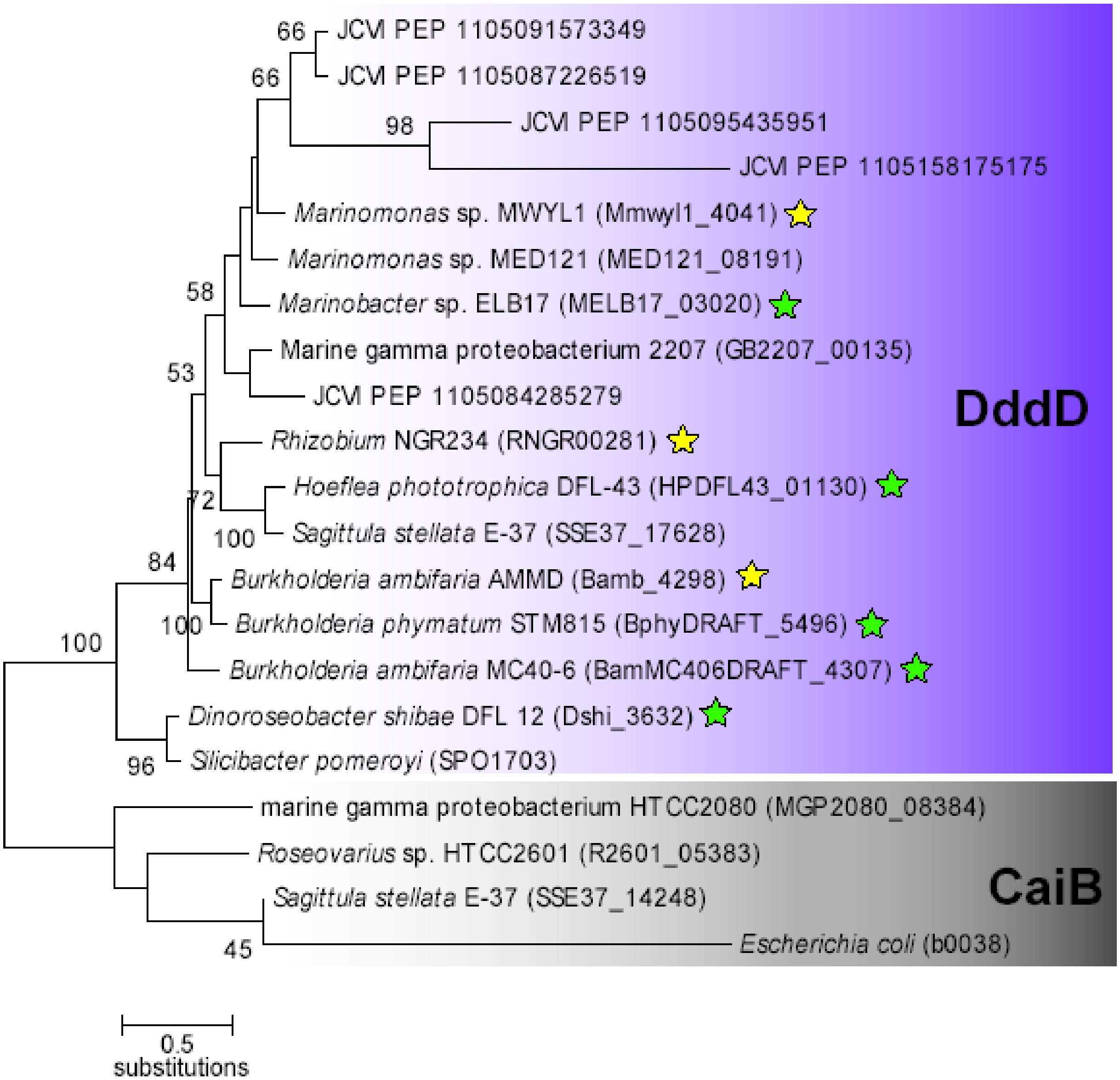

66
JCVI PEP 1105091573349
66
JCVI PEP 1105087226519
98
JCVI PEP 1105095435951
JCVI PEP 1105158175175
Marinomonas sp. MWYL1 (Mmwyl1_4041) ☆
Marinomonas sp. MED121 (MED121_08191)
58
Marinobacter sp. ELB17 (MELB17_03020) ★
Marine gamma proteobacterium 2207 (GB2207_00135)
53
JCVI PEP 1105084285279
DddD
Rhizobium NGR234 (RNGR00281) ☆
72
Hoeflea phototrophica DFL-43 (HPDFL43_01130) ★
100
Sagittula stellata E-37 (SSE37_17628)
84
Burkholderia ambifaria AMMD (Bamb_4298) ☆
100
Burkholderia phymatum STM815 (BphyDRAFT_5496) ★
100
Burkholderia ambifaria MC40-6 (BamMC406DRAFT_4307) ★
Dinoroseobacter shibae DFL 12 (Dshi_3632) ★
96
Silicibacter pomeroyi (SPO1703)
marine gamma proteobacterium HTCC2080 (MGP2080_08384)
Roseovarius sp. HTCC2601 (R2601_05383)
CaiB
Sagittula stellata E-37 (SSE37_14248)
45
Escherichia coli (b0038)
0.5
substitutions

Figure 3.7**.** Diversity of *dddL* in the (2007) GOS and cultured organisms. The neighbour-joining tree shows selected *dddL* sequences in the (2007) GOS. DddL sequences with confirmed function are indicated with a yellow star. Related sequences from a hypothetical protein out-group are indicated.

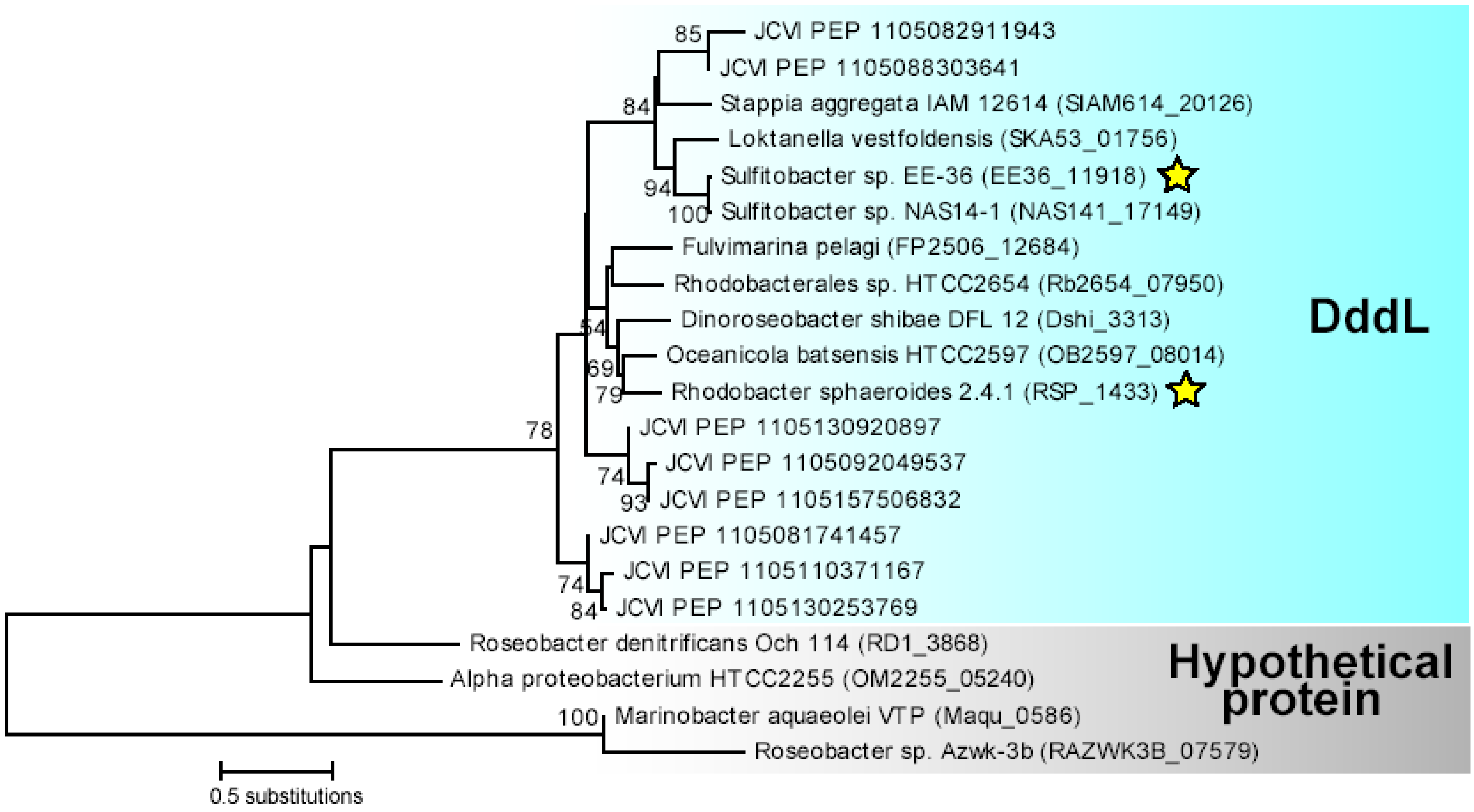
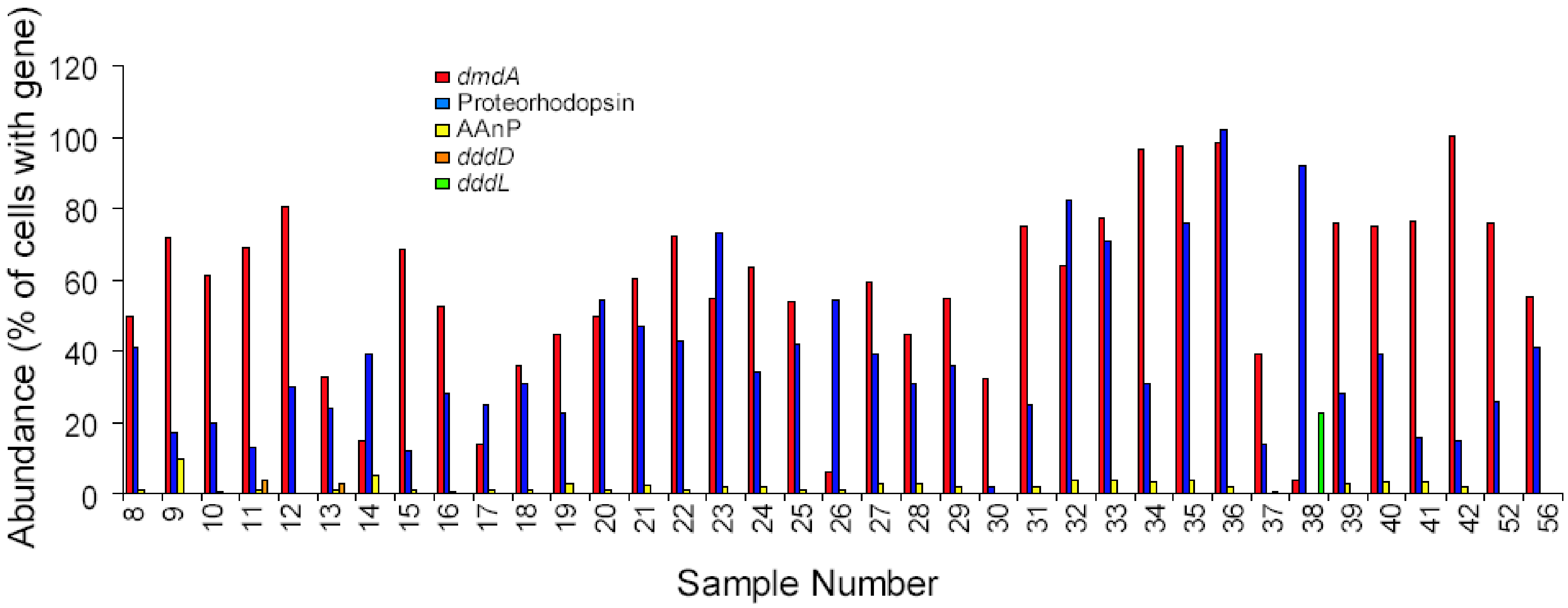

85
JCVI PEP 1105082911943
JCVI PEP 1105088303641
84
Stappia aggregata IAM 12614 (SIAM614_20126)
Loktanella vestfoldensis (SKA53_01756)
94
Sulfitobacter sp. EE-36 (EE36_11918) ☆
100
Sulfitobacter sp. NAS14-1 (NAS141_17149)
Fulvimarina pelagi (FP2506_12684)
Rhodobacterales sp. HTCC2654 (Rb2654_07950)
54
Dinoroseobacter shibae DFL 12 (Dshi_3313)
69
Oceanicola batsensis HTCC2597 (OB2597_08014)
79
Rhodobacter sphaeroides 2.4.1 (RSP_1433) ☆
78
JCVI PEP 1105130920897
74
JCVI PEP 1105092049537
93
JCVI PEP 1105157506832
JCVI PEP 1105081741457
74
JCVI PEP 1105110371167
84
JCVI PEP 1105130253769
Roseobacter denitrificans Och 114 (RD1_3868)
Alpha proteobacterium HTCC2255 (OM2255_05240)
100
Marinobacter aquaeolei VTP (Maqu_0586)
Roseobacter sp. Azwk-3b (RAZWK3B_07579)
DddL
Hypothetical protein
0.5 substitutions

Figure 3.8. Abundance of selected genes in marine surface water by (2007) GOS sample number. AAnP abundances (average of *pufM*, *pufL* and *bchX*) are from fig. 6 in Yutin and colleagues (2007). Proteorhodopsin abundances are from fig. 12 in Rusch and colleagues (2007). The methodology used to determine AAnP and proteorhodopsin abundance is based on de-convolution of assembled data, rather than direct analysis of predicted proteins from unassembled reads, as used in this study.



dmdA
Proteorhodopsin
AAnP
dddD
dddL
Abundance (% of cells with gene)
0
20
40
60
80
100
120
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
52
56
Sample Number

Table 3.3. Single-copy gene counts, genome equivalents and *dmdA* abundance by sampling site. Homologues of six single-copy genes were identified in the (2007) GOS metagenome using a cut-off *E*-value of 10^{-20} (‘original count’ column). These counts were corrected by removal of duplicates from paired reads (‘paired reads deleted’ column) and size-normalized based on the length of *recA* (1062 bp; ‘size norm.’ column).

				recA			*tufA*			*atpD*			*dnaK*			*gyrB*			*rpoB*					
Site ID Number	Sample Number	Site Name	Original count	Paired Reads Deleted	Size Norm.^{a}	Original count	Paired Reads Deleted	Size Norm.^{a}	Original count	Paired Reads Deleted	Size Norm.^{a}	Original count	Paired Reads Deleted	Size Norm.^{a}	Original count	Paired Reads Deleted	Size Norm.^{a}	Original count	Paired Reads Deleted	Size Norm.^{a}	Total Reads	DmdA Reads	Average Genome Equivalents^b	Percent of cells with dmdA
2	8	Gulf of Maine	54	43	43	80	63	56	87	71	55	163	110	61	115	82	36	256	158	42	121590	25	49	49.7
3	9	Browns Bank	32	32	32	48	43	39	46	45	35	101	87	48	60	52	23	106	72	19	61605	24	33	71.6
4	10	Outside Halifax	27	21	21	57	45	40	55	46	35	75	59	33	75	54	24	98	68	18	52959	18	29	61.2
5	11	Bedford Basin	25	21	21	38	31	28	33	27	21	52	43	24	76	59	26	85	59	16	61131	16	22	69.0
6	12	Bay of Fundy	56	48	48	48	45	40	58	53	41	78	62	34	79	63	28	132	99	26	59679	30	36	80.4
7	13	Northern Gulf of Maine	37	27	27	52	41	37	61	51	39	88	67	37	71	47	21	87	63	17	50980	10	30	32.8
8	14	Newport Harbor	79	56	56	245	168	151	140	94	72	164	114	63	199	127	56	386	239	63	129655	12	77	15.2
9	15	Block Island	48	26	26	83	47	42	61	36	28	110	63	35	127	73	32	167	91	24	79303	22	31	68.5
10	16	Cape May	54	49	49	72	59	53	100	80	61	138	109	60	169	127	56	175	112	30	78304	28	52	52.7
11	17	Delaware Bay	76	53	53	136	93	83	128	91	70	174	114	63	228	144	63	286	168	44	124435	9	63	13.9
12	18	Chesapeake Bay	65	49	49	138	95	85	119	80	61	167	106	59	214	142	62	264	158	42	126162	22	60	35.7
13	19	Off Nags Head	71	59	59	83	67	60	72	63	48	121	91	50	149	110	48	208	128	34	138033	23	50	44.6
14	20	South of Charleston	100	86	86	125	107	96	145	110	84	211	166	92	233	169	74	331	219	58	128885	42	82	49.8
15	21	Off Key West	83	65	65	147	104	93	121	87	67	231	168	93	196	141	62	299	206	54	127362	45	72	60.3
16	22	Gulf of Mexico	79	64	64	103	83	74	121	93	71	216	164	91	215	163	72	275	182	48	127122	52	70	72.0
17	23	Yucatan	181	156	156	241	205	184	259	206	158	363	277	153	371	284	125	548	363	96	257581	82	145	54.7
18	24	Rosario Bank	128	119	119	138	117	105	193	157	121	213	173	96	220	172	76	313	205	54	142743	62	96	63.3
19	25	Northeast of Colon	127	97	97	136	92	82	140	103	79	228	164	91	209	159	70	330	218	58	135325	44	79	53.7
20	26	Lake Gatun	211	182	182	229	180	161	237	180	138	339	253	140	449	331	146	494	324	86	296355	9	142	6.1
21	27	Gulf of Panama	88	70	70	109	77	69	144	117	90	137	102	57	188	141	62	323	200	53	131798	41	67	59.6
22	28	250 miles from Panama City	78	61	61	123	90	81	136	96	74	218	149	83	207	147	65	252	157	41	121662	31	67	44.7
23	29	30 miles from Cocos Island	86	72	72	131	106	95	166	138	106	209	164	91	224	172	76	295	188	50	133061	46	82	54.7
25	30	Dirty Rock	18	16	16	24	19	17	39	31	24	59	46	25	38	28	12	85	54	14	120671	6	18	32.1
26	31	134 miles NE of Galapagos	83	56	56	117	77	69	105	72	55	174	111	61	159	109	48	229	134	35	102708	42	54	75.2
27	32	Devil's Crown	171	147	147	205	169	151	268	221	170	326	250	139	371	279	123	562	347	92	222080	90	137	63.8
28	33	Coastal Floreana	161	132	132	187	147	132	214	166	127	278	200	111	347	250	110	457	281	74	189052	91	114	77.2
29	34	North James Bay	107	66	66	133	77	69	147	88	68	208	119	66	220	122	54	322	173	46	131529	61	61	96.5
30	35	Warm Seep	349	197	197	347	193	173	554	307	236	640	351	194	728	404	178	1021	554	146	359152	188	187	97.3
31	36	Upwelling	371	265	265	410	282	253	547	373	286	676	440	244	803	534	235	1164	686	181	436401	247	244	98.2
32	37	Mangrove	84	63	63	111	72	65	135	88	68	190	120	66	180	115	51	288	164	43	148018	24	59	39.3
33	38	Punta Cormorant Hypersaline Lagoon	402	298	298	761	523	469	498	353	271	641	437	242	1037	685	301	1084	662	175	692255	12	293	4.0
34	39	North Seamore Island	85	68	68	134	96	86	136	100	77	225	154	85	209	144	63	319	184	49	134347	56	71	76.1
35	40	Wolf Island	109	72	72	129	83	74	167	109	84	216	130	72	259	169	74	279	161	43	140814	54	70	75.0
36	41	Cabo Marshall	49	42	42	69	57	51	81	69	53	101	79	44	158	117	51	163	98	26	77538	35	45	76.2
37	42	Equatorial Pacific	40	25	25	73	42	38	65	43	33	102	56	31	112	65	29	169	94	25	64670	31	30	100.2
47	52	201 miles from F. Polynesia	47	41	41	69	55	49	89	72	55	111	81	45	100	71	31	145	91	24	66023	32	41	75.8
51	56	Rangirora Atoll	82	68	68	117	90	81	121	93	71	151	111	61	169	120	53	280	175	46	128982	36	63	55.1
		TOTALS	**3943**	**3012**	**3012**	**5448**	**3940**	**3530**	**5788**	**4209**	**3233**	**7894**	**5490**	**3041**	**8964**	**6171**	**2715**	**12277**	**7535**	**1989**	**7688374**	**1701**	**2920**	**58.3^c**

Table 3.4. (2007) GOS *dmdA* homologue PEP numbers and corresponding nucleotide read numbers, sampling sites of origin and assigned clades. The PEP number, nucleotide read number, GOS site of origin and sample number, and assigned clades are given for the 1701 identified *dmdA* homologues. [a]Number begins “JCVI_SMPL_”.

Clade	**JCVI PEP READ**	**JCVI read**	**Sample Numbers[a]**
A	JCVI_PEP_1105078288479	JCVI_READ_1092963395135	1103283000023
A	JCVI_PEP_1105079008849	JCVI_READ_1093018300858	1103283000038
A	JCVI_PEP_1105080071775	JCVI_READ_1092343732701	1103283000032
A	JCVI_PEP_1105081627505	JCVI_READ_1095344024074	1103283000032
A	JCVI_PEP_1105082014523	JCVI_READ_1095898066078	1103283000015
A	JCVI_PEP_1105082820799	JCVI_READ_1092256177530	1103283000013
A	JCVI_PEP_1105084039245	JCVI_READ_1095516040788	1103283000037
A	JCVI_PEP_1105084150227	JCVI_READ_1092963149368	1103283000035
A	JCVI_PEP_1105084394367	JCVI_READ_1095901442127	1103283000008
A	JCVI_PEP_1105087472581	JCVI_READ_1093015754535	1103283000041
A	JCVI_PEP_1105088981997	JCVI_READ_1092963159919	1103283000035
A	JCVI_PEP_1105088991279	JCVI_READ_1092963594504	1103283000019
A	JCVI_PEP_1105089433797	JCVI_READ_1093012093420	1103283000033
A	JCVI_PEP_1105089777585	JCVI_READ_1093017509089	1103283000035
A	JCVI_PEP_1105091056417	JCVI_READ_1093017608985	1103283000035
A	JCVI_PEP_1105092077639	JCVI_READ_1095898027119	1103283000017
A	JCVI_PEP_1105093134687	JCVI_READ_1093018088644	1103283000038
A	JCVI_PEP_1105093503391	JCVI_READ_1095469429005	1103283000036
A	JCVI_PEP_1105094920701	JCVI_READ_1095521403108	1103283000038
A	JCVI_PEP_1105095037479	JCVI_READ_1091140678384	1103283000021
A	JCVI_PEP_1105095133471	JCVI_READ_1092963016858	1103283000034
A	JCVI_PEP_1105095777477	JCVI_READ_1092963428749	1103283000023
A	JCVI_PEP_1105096832571	JCVI_READ_1092977802640	1103283000023
A	JCVI_PEP_1105098649061	JCVI_READ_1092255452018	1103283000013
A	JCVI_PEP_1105099256127	JCVI_READ_1093017878117	1103283000035
A	JCVI_PEP_1105100009511	JCVI_READ_1092955196983	1103283000036
A	JCVI_PEP_1105100394269	JCVI_READ_1092956086054	1103283000036
A	JCVI_PEP_1105101968521	JCVI_READ_1093017183916	1103283000038
A	JCVI_PEP_1105102351249	JCVI_READ_1092256332829	1103283000026
A	JCVI_PEP_1105103798399	JCVI_READ_1091143187299	1103283000027
A	JCVI_PEP_1105103930273	JCVI_READ_1095390181622	1103283000026
A	JCVI_PEP_1105104039623	JCVI_READ_1095403579747	1103283000033
A	JCVI_PEP_1105104050679	JCVI_READ_1092955191006	1103283000035
A	JCVI_PEP_1105104058149	JCVI_READ_1092955183754	1103283000035
A	JCVI_PEP_1105104247761	JCVI_READ_1092351376646	1103283000032
A	JCVI_PEP_1105106214621	JCVI_READ_1095949473236	1103283000008
A	JCVI_PEP_1105106433819	JCVI_READ_1092959713993	1103283000034
A	JCVI_PEP_1105106693799	JCVI_READ_1095898043614	1103283000015
A	JCVI_PEP_1105107717195	JCVI_READ_1091143478450	1103283000011

A	JCVI_PEP_1105108262995	JCVI_READ_1093022077637	1103283000039
A	JCVI_PEP_1105108660345	JCVI_READ_1091138262188	1103283000031
A	JCVI_PEP_1105109112565	JCVI_READ_1095454109471	1103283000031
A	JCVI_PEP_1105109116723	JCVI_READ_1095454109510	1103283000031
A	JCVI_PEP_1105109168119	JCVI_READ_1092344158471	1103283000039
A	JCVI_PEP_1105109169495	JCVI_READ_1092344164615	1103283000039
A	JCVI_PEP_1105109230597	JCVI_READ_1095526022205	1103283000038
A	JCVI_PEP_1105110279963	JCVI_READ_1093016207424	1103283000041
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A	JCVI_PEP_1105111641189	JCVI_READ_1095390093291	1103283000028
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A	JCVI_PEP_1105119294001	JCVI_READ_1091139345087	1103283000022
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A	JCVI_PEP_1105121788701	JCVI_READ_1095526030063	1103283000038
A	JCVI_PEP_1105121807015	JCVI_READ_1095522177093	1103283000038
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A	JCVI_PEP_1105124711495	JCVI_READ_1091143580558	1103283000011
A	JCVI_PEP_1105124789535	JCVI_READ_1092963189556	1103283000034
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A	JCVI_PEP_1105125207349	JCVI_READ_1094338648910	1103283000039
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A	JCVI_PEP_1105133662685	JCVI_READ_1092256147551	1103283000022
A	JCVI_PEP_1105137475849	JCVI_READ_1092404030365	1103283000032
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A	JCVI_PEP_1105139970693	JCVI_READ_1091138288815	1103283000027
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A	JCVI_PEP_1105141850079	JCVI_READ_1092960052773	1103283000056
A	JCVI_PEP_1105143903453	JCVI_READ_1095901238187	1103283000052
A	JCVI_PEP_1105144039859	JCVI_READ_1091143457749	1103283000010
A	JCVI_PEP_1105144137865	JCVI_READ_1092963756310	1103283000040
A	JCVI_PEP_1105144337741	JCVI_READ_1093022075736	1103283000022
A	JCVI_PEP_1105145057351	JCVI_READ_1092351189955	1103283000038
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A	JCVI_PEP_1105157347892	JCVI_READ_1093016049621	1103283000020
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A	JCVI_PEP_1105159247285	JCVI_READ_1093018089988	1103283000038
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A	JCVI_PEP_1105162968531	JCVI_READ_1091143249816	1103283000027
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A	JCVI_PEP_1105164642991	JCVI_READ_1092956045661	1103283000035
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B	JCVI_PEP_1105081186809	JCVI_READ_1095333013600	1103283000039
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B	JCVI_PEP_1105116064561	JCVI_READ_1092955108503	1103283000035
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B	JCVI_PEP_1105117428947	JCVI_READ_1092963826857	1103283000040
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B	JCVI_PEP_1105135300438	JCVI_READ_1095469482764	1103283000036
B	JCVI_PEP_1105135724825	JCVI_READ_1093017832949	1103283000035
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C	JCVI_PEP_1105075689499	JCVI_READ_1095349024939	1103283000039
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C	JCVI_PEP_1105080134701	JCVI_READ_1095462316992	1103283000036
C	JCVI_PEP_1105080776095	JCVI_READ_1092961104068	1103283000036
C	JCVI_PEP_1105080807611	JCVI_READ_1092256082926	1103283000013
C	JCVI_PEP_1105080808129	JCVI_READ_1092256080978	1103283000013
C	JCVI_PEP_1105080915151	JCVI_READ_1093017824441	1103283000035
C	JCVI_PEP_1105081163061	JCVI_READ_1092960023555	1103283000023
C	JCVI_PEP_1105081331521	JCVI_READ_1093018732664	1103283000024
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C	JCVI_PEP_1105083254345	JCVI_READ_1093017729705	1103283000045
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C	JCVI_PEP_1105085529136	JCVI_READ_1091143306872	1103283000008
C	JCVI_PEP_1105086296071	JCVI_READ_1093017468100	1103283000035
C	JCVI_PEP_1105086311047	JCVI_READ_1093018289165	1103283000021
C	JCVI_PEP_1105086373051	JCVI_READ_1095460226933	1103283000031
C	JCVI_PEP_1105086639891	JCVI_READ_1093022093961	1103283000035
C	JCVI_PEP_1105087266985	JCVI_READ_1092256303038	1103283000025
C	JCVI_PEP_1105087479345	JCVI_READ_1092955209154	1103283000034
C	JCVI_PEP_1105087838509	JCVI_READ_1091140882081	1103283000022
C	JCVI_PEP_1105087899375	JCVI_READ_1093015796395	1103283000040
C	JCVI_PEP_1105088427009	JCVI_READ_1095462420327	1103283000036
C	JCVI_PEP_1105088905183	JCVI_READ_1095898019566	1103283000016
C	JCVI_PEP_1105089443281	JCVI_READ_1092343145565	1103283000032
C	JCVI_PEP_1105089804661	JCVI_READ_1092344284890	1103283000029
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Unclassified	JCVI_PEP_1105106890697	JCVI_READ_1092344275811	1103283000029
Unclassified	JCVI_PEP_1105107046397	JCVI_READ_1095901386151	1103283000052
Unclassified	JCVI_PEP_1105107138669	JCVI_READ_1092402508167	1103283000029
Unclassified	JCVI_PEP_1105107488099	JCVI_READ_1091138180006	1103283000022
Unclassified	JCVI_PEP_1105107597519	JCVI_READ_1092963057435	1103283000023
Unclassified	JCVI_PEP_1105108537315	JCVI_READ_1095949463233	1103283000008
Unclassified	JCVI_PEP_1105108557757	JCVI_READ_1093018399007	1103283000035
Unclassified	JCVI_PEP_1105108617303	JCVI_READ_1095901531631	1103283000014
Unclassified	JCVI_PEP_1105109883953	JCVI_READ_1092963096974	1103283000035
Unclassified	JCVI_PEP_1105109918105	JCVI_READ_1092344167505	1103283000032
Unclassified	JCVI_PEP_1105110269049	JCVI_READ_1092343675300	1103283000029
Unclassified	JCVI_PEP_1105110543125	JCVI_READ_1092351295637	1103283000030
Unclassified	JCVI_PEP_1105110793295	JCVI_READ_1092962058135	1103283000034
Unclassified	JCVI_PEP_1105110848685	JCVI_READ_1092963449470	1103283000036
Unclassified	JCVI_PEP_1105111035763	JCVI_READ_1092302012473	1103283000027
Unclassified	JCVI_PEP_1105112366551	JCVI_READ_1092963207266	1103283000056
Unclassified	JCVI_PEP_1105112996207	JCVI_READ_1091140693360	1103283000021
Unclassified	JCVI_PEP_1105113024757	JCVI_READ_1095462393685	1103283000036
Unclassified	JCVI_PEP_1105113072485	JCVI_READ_1091138106770	1103283000021
Unclassified	JCVI_PEP_1105113697317	JCVI_READ_1092351918374	1103283000035
Unclassified	JCVI_PEP_1105113760899	JCVI_READ_1093022098547	1103283000027
Unclassified	JCVI_PEP_1105114012291	JCVI_READ_1095913056440	1103283000052
Unclassified	JCVI_PEP_1105114587597	JCVI_READ_1093018999559	1103283000035
Unclassified	JCVI_PEP_1105115967751	JCVI_READ_1093011965423	1103283000023
Unclassified	JCVI_PEP_1105117252487	JCVI_READ_1095353006327	1103283000039

Unclassified	JCVI_PEP_1105117363449	JCVI_READ_1092963434143	1103283000023
Unclassified	JCVI_PEP_1105117479679	JCVI_READ_1091141198518	1103283000015
Unclassified	JCVI_PEP_1105117918985	JCVI_READ_1093011411295	1103283000023
Unclassified	JCVI_PEP_1105118200497	JCVI_READ_1092959680034	1103283000036
Unclassified	JCVI_PEP_1105118883737	JCVI_READ_1093016301278	1103283000041
Unclassified	JCVI_PEP_1105119521505	JCVI_READ_1091142954019	1103283000009
Unclassified	JCVI_PEP_1105119602347	JCVI_READ_1095403285916	1103283000032
Unclassified	JCVI_PEP_1105119793373	JCVI_READ_1095515646376	1103283000037
Unclassified	JCVI_PEP_1105119917437	JCVI_READ_1092351113109	1103283000032
Unclassified	JCVI_PEP_1105120531021	JCVI_READ_1095462204382	1103283000036
Unclassified	JCVI_PEP_1105121689233	JCVI_READ_1092963394020	1103283000033
Unclassified	JCVI_PEP_1105122438539	JCVI_READ_1095898242350	1103283000016
Unclassified	JCVI_PEP_1105123303773	JCVI_READ_1092215010063	1103283000035
Unclassified	JCVI_PEP_1105123304727	JCVI_READ_1092214954862	1103283000035
Unclassified	JCVI_PEP_1105123315921	JCVI_READ_1093024001865	1103283000035
Unclassified	JCVI_PEP_1105123377829	JCVI_READ_1092215025094	1103283000028
Unclassified	JCVI_PEP_1105123582271	JCVI_READ_1093015544518	1103283000040
Unclassified	JCVI_PEP_1105124756129	JCVI_READ_1092963286482	1103283000023
Unclassified	JCVI_PEP_1105126299413	JCVI_READ_1093012030167	1103283000033
Unclassified	JCVI_PEP_1105126668769	JCVI_READ_1091143192868	1103283000008
Unclassified	JCVI_PEP_1105127257543	JCVI_READ_1093016163245	1103283000040
Unclassified	JCVI_PEP_1105127979695	JCVI_READ_1095913038410	1103283000052
Unclassified	JCVI_PEP_1105128410185	JCVI_READ_1092955336659	1103283000036
Unclassified	JCVI_PEP_1105129162561	JCVI_READ_1092961161630	1103283000036
Unclassified	JCVI_PEP_1105130191225	JCVI_READ_1092344185134	1103283000029
Unclassified	JCVI_PEP_1105130492039	JCVI_READ_1093006415497	1103283000023
unclassified	JCVI_PEP_1105130883367	JCVI_READ_1095901175748	1103283000052
Unclassified	JCVI_PEP_1105131368163	JCVI_READ_1095460163028	1103283000036
Unclassified	JCVI_PEP_1105132442809	JCVI_READ_1092256423441	1103283000028
Unclassified	JCVI_PEP_1105132804809	JCVI_READ_1092344053308	1103283000032
Unclassified	JCVI_PEP_1105133357913	JCVI_READ_1092960187449	1103283000023
Unclassified	JCVI_PEP_1105133614583	JCVI_READ_1092351082526	1103283000032
Unclassified	JCVI_PEP_1105134410628	JCVI_READ_1094428755081	1103283000039
Unclassified	JCVI_PEP_1105135437244	JCVI_READ_1092960096718	1103283000056
Unclassified	JCVI_PEP_1105135642607	JCVI_READ_1093015398782	1103283000040
Unclassified	JCVI_PEP_1105136153795	JCVI_READ_1092955381676	1103283000036
Unclassified	JCVI_PEP_1105136692168	JCVI_READ_1093018480508	1103283000021
Unclassified	JCVI_PEP_1105137153975	JCVI_READ_1095460075633	1103283000036
Unclassified	JCVI_PEP_1105137240045	JCVI_READ_1093018651856	1103283000024
Unclassified	JCVI_PEP_1105137776559	JCVI_READ_1093017682890	1103283000024
Unclassified	JCVI_PEP_1105138689991	JCVI_READ_1092343424058	1103283000027
Unclassified	JCVI_PEP_1105139210797	JCVI_READ_1095403242788	1103283000032
Unclassified	JCVI_PEP_1105139363107	JCVI_READ_1092956048773	1103283000035
Unclassified	JCVI_PEP_1105141071401	JCVI_READ_1093015292166	1103283000033
Unclassified	JCVI_PEP_1105141431783	JCVI_READ_1092256058012	1103283000021
Unclassified	JCVI_PEP_1105142007683	JCVI_READ_1095506218079	1103283000036

Unclassified	JCVI_PEP_1105142015305	JCVI_READ_1091141790382	1103283000014
Unclassified	JCVI_PEP_1105142537099	JCVI_READ_1095901421467	1103283000052
Unclassified	JCVI_PEP_1105142952593	JCVI_READ_1091143541595	1103283000010
Unclassified	JCVI_PEP_1105143234265	JCVI_READ_1091142117885	1103283000026
Unclassified	JCVI_PEP_1105143432407	JCVI_READ_1093012177160	1103283000023
Unclassified	JCVI_PEP_1105144166933	JCVI_READ_1093018457839	1103283000035
Unclassified	JCVI_PEP_1105144540533	JCVI_READ_1095467006325	1103283000036
Unclassified	JCVI_PEP_1105145462177	JCVI_READ_1093023071051	1103283000035
Unclassified	JCVI_PEP_1105145734571	JCVI_READ_1092955192059	1103283000018
Unclassified	JCVI_PEP_1105146491887	JCVI_READ_1092343156226	1103283000032
Unclassified	JCVI_PEP_1105146492625	JCVI_READ_1092343155617	1103283000032
Unclassified	JCVI_PEP_1105146863003	JCVI_READ_1093018974814	1103283000022
Unclassified	JCVI_PEP_1105146875843	JCVI_READ_1095353023949	1103283000039
Unclassified	JCVI_PEP_1105147226891	JCVI_READ_1093011895981	1103283000023
Unclassified	JCVI_PEP_1105147466951	JCVI_READ_1091142095122	1103283000026
Unclassified	JCVI_PEP_1105147865431	JCVI_READ_1092963172057	1103283000023
Unclassified	JCVI_PEP_1105148651617	JCVI_READ_1093016224521	1103283000041
Unclassified	JCVI_PEP_1105148796891	JCVI_READ_1095368013538	1103283000026
unclassified	JCVI_PEP_1105149134023	JCVI_READ_1092961116416	1103283000023
Unclassified	JCVI_PEP_1105149246545	JCVI_READ_1091140402706	1103283000016
Unclassified	JCVI_PEP_1105149720607	JCVI_READ_1091141781684	1103283000025
Unclassified	JCVI_PEP_1105150329723	JCVI_READ_1092301002809	1103283000029
Unclassified	JCVI_PEP_1105151044139	JCVI_READ_1092963141089	1103283000023
Unclassified	JCVI_PEP_1105151740881	JCVI_READ_1093011964696	1103283000034
Unclassified	JCVI_PEP_1105151904559	JCVI_READ_1092963686761	1103283000033
Unclassified	JCVI_PEP_1105152888163	JCVI_READ_1092343563222	1103283000030
Unclassified	JCVI_PEP_1105153380005	JCVI_READ_1095515642861	1103283000036
Unclassified	JCVI_PEP_1105153514137	JCVI_READ_1093018474546	1103283000035
Unclassified	JCVI_PEP_1105153540409	JCVI_READ_1095306074214	1103283000032
Unclassified	JCVI_PEP_1105153564179	JCVI_READ_1093018689865	1103283000024
Unclassified	JCVI_PEP_1105153925155	JCVI_READ_1095390067963	1103283000032
Unclassified	JCVI_PEP_1105154136593	JCVI_READ_1092257166906	1103283000023
Unclassified	JCVI_PEP_1105154704927	JCVI_READ_1093006406212	1103283000034
Unclassified	JCVI_PEP_1105155849011	JCVI_READ_1095460028921	1103283000036
Unclassified	JCVI_PEP_1105156293383	JCVI_READ_1093015402076	1103283000033
Unclassified	JCVI_PEP_1105156325957	JCVI_READ_1093010301671	1103283000034
unclassified	JCVI_PEP_1105156595841	JCVI_READ_1092963342541	1103283000023
Unclassified	JCVI_PEP_1105157731348	JCVI_READ_1093017318004	1103283000035
Unclassified	JCVI_PEP_1105158950649	JCVI_READ_1091141132490	1103283000014
Unclassified	JCVI_PEP_1105159663511	JCVI_READ_1093012156421	1103283000023
Unclassified	JCVI_PEP_1105159683031	JCVI_READ_1092959523734	1103283000036
Unclassified	JCVI_PEP_1105159771321	JCVI_READ_1093018642277	1103283000024
Unclassified	JCVI_PEP_1105160802701	JCVI_READ_1093017425997	1103283000024
Unclassified	JCVI_PEP_1105161182277	JCVI_READ_1092955185342	1103283000034
Unclassified	JCVI_PEP_1105161703519	JCVI_READ_1092955083850	1103283000018
Unclassified	JCVI_PEP_1105161868665	JCVI_READ_1093015240321	1103283000040

Unclassified	JCVI_PEP_1105162373545	JCVI_READ_1092963297298	1103283000023
Unclassified	JCVI_PEP_1105162464079	JCVI_READ_1092216073739	1103283000013
unclassified	JCVI_PEP_1105163735245	JCVI_READ_1092963069383	1103283000023
Unclassified	JCVI_PEP_1105165020927	JCVI_READ_1092351200072	1103283000032
Unclassified	JCVI_PEP_1105165753807	JCVI_READ_1093022168513	1103283000022
	Unclassified	JCVI_READ_1092955139615	

Table 3.5. Station ALOHA fosmid APKI441 annotation. Putative gene function was assigned based on the top three tblastn results against Refseq with a maximum *E*-value of $< 10^{-7}$ or an amino acid identity of > 30%. Taxonomic designations were based on the assignments of the best hits in GenBank. Genes and taxonomic affiliations not meeting the cut-off values are labelled 'n/a'; gene suites with related functions are colour-coded.

APKI441 fosmid gene number	**Putative gene designation**	**Taxonomic Class designation**	**Top BLAST hit Order designation**
1	Transthyretin-like protein	Alpha proteobacteria	Roseobacter
2	***dmdA***	Alpha proteobacteria	*P. torquis* contaminant
3	Major Facilitator Superfamily (MFS) transporter	Gamma proteobacteria	Pseudomonadales
4	Glycerophosphoryl diester phosphodiesterase	Alpha proteobacteria	Rhodospiralles
5	Threonine dehydratase	Alpha proteobacteria	Roseobacter
6	Nitroreductase	Gamma proteobacteria	Oceanospirillales
7	MFS transporter	Alpha proteobacteria	Rhizobiales
8	Multi-drug and toxin efflux (MATE) family protein	Alpha proteobacteria	Roseobacter
9	NADPH-dependent FMN reductase	Alpha proteobacteria	Rhizobiales
10	n/a	n/a	
11	n/a	n/a	
12	Predicted deacylase	Gamma proteobacteria	Oceanospirillales
13	Peptidase family M20	Actinobacteria	Actinomycetales
14	GntR family transcriptional regulator	Alpha proteobacteria	Roseobacter
15	Alkylphosphonate utilization protein, PhnG	Alpha proteobacteria	Roseobacter
16	Alkylphosphonate utilization protein, PhnH	Alpha proteobacteria	Roseobacter
17	Alkylphosphonate utilization protein, PhnI	Alpha proteobacteria	Roseobacter
18	Alkylphosphonate utilization protein, PhnJ	Alpha proteobacteria	Rhizobiales
19	n/a	n/a	
20	ABC-type phosphonate transport system	Beta proteobacteria	Burkholderiales
21	Alkylphosphonate utilization protein, PhnL	Alpha proteobacteria	Roseobacter
22	Putative alkylphosphonate utilization protein, PhnN	Alpha proteobacteria	Roseobacter
23	n/a	n/a	
24	Alkylphosphonate utilization protein PhnM	Alpha proteobacteria	Roseobacter
25	Hypothetical protein	Alpha proteobacteria	Roseobacter
26	n/a	n/a	

27	Cytochrome C biogenesis protein, CycL	Alpha proteobacteria	Rhizobiales
28	Probable thiol-disulfide interchange protein, CycY	Alpha proteobacteria	Roseobacter
29	Cytochrome C biogenesis protein, CcmF	Alpha proteobacteria	Rhodobacterales
30	Aminomethyl transferase family protein	Alpha proteobacteria	SAR11
31	Aldehyde dehydrogenase family protein	Alpha proteobacteria	Rhizobiales
32	Probable alcohol dehydrogenase protein	Alpha proteobacteria	Rhizobiales
33	Glycine betaine/proline ABC transporter, permease protein	Alpha proteobacteria	Roseobacter
34	Glycine betaine/proline ABC transporter, ATP-binding protein	Alpha proteobacteria	Rhizobiales
35	Glycine betaine/proline ABC transporter, ATP-binding protein	Alpha proteobacteria	Rhizobiales
36	Glycine betaine/proline ABC transporter, periplasmic substrate-binding protein	Alpha proteobacteria	*P. torquis* contaminant

Table 3.6. Third-position G+C content of *dmdA* and flanking genes in the (2007) GOS metagenome. Standard deviations are indicated in parentheses.

	Flanking Upstream Gene	**DmdA Ortholog**	**Flanking Downstream Gene**
Clade A[a]	37 (15)	41 (17)	39 (14)
Clade B[b]	42 (9)	41 (14)	42 (14)
Clade C[c]	29 (15)	26 (14)	29 (16)
Clade D[d]	18 (4)	19 (4)	21 (7)
Clade E[e]	37 (23)	49 (13)	60 (5)
Combined Clades	20 (9)	22 (11)	23 (10)

[a] 9 upstream flanking genes, 102 *dmdA*s, and 11 downstream flanking genes were analyzed

[b] 7 upstream flanking genes, 48 *dmdA*s, and 2 downstream flanking genes were analyzed

[c] 29 upstream flanking genes, 263 *dmdA*s, and 27 downstream flanking genes were analyzed

[d] 175 upstream flanking genes, 1202 *dmdA*s, and 138 downstream flanking genes were analyzed

[e] 7 upstream flanking genes, 11 *dmdA*s, and 2 downstream flanking genes were analyzed

Table 3.7. (2007) GOS *dddD* homologue PEP numbers and corresponding nucleotide read numbers, template reads and sampling sites of origin. The PEP file number, nucleotide read number, template number, and GOS site of origin and sample number, are given for the six identified *dddD* homologues.

Site Name	Sample Number	JCVI Read Number	JCVI Pep Read	JCVI Template ID
Bedford Basin, Nova Scotia	11	JCVI_READ_1091143402062	JCVI_PEP_1105118567433	JCVI_TMPL_1061000885712
Bedford Basin, Nova Scotia	11	JCVI_READ_1091145541951	JCVI_PEP_1105084285279	JCVI_TMPL_1061000895474
Northern Gulf of Maine	13	JCVI_READ_1092215112293	JCVI_PEP_1105087226519	JCVI_TMPL_1061001073648
Northern Gulf of Maine	13	JCVI_READ_1092216036910	JCVI_PEP_1105091573349	JCVI_TMPL_1061001047376
Upwelling Fernandina Island	36	JCVI_READ_1092960119971	JCVI_PEP_1105095435951	JCVI_TMPL_1061001976019
Mangrove on Isabela Island	37	JCVI_READ_1095516121784	JCVI_PEP_1105158175175	JCVI_TMPL_1061005983305

Table 3.8. (2007) GOS *dddL* homologue PEP numbers and corresponding nucleotide read numbers, template reads and sampling sites of origin. The PEP file number, nucleotide read number, template number, and GOS site of origin and sample number, are given for the 42 identified *dddL* homologues.

Sample Site	Site ID Number	Site name	JCVI PEP NUMBER	JCVI READ NUMBER	TEMPLATE NUMBER
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105082911943	JCVI_READ_1093016362621	JCVI_TMPL_1061002641879
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105157506832	JCVI_READ_1093017112988	JCVI_TMPL_1061002617164
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105092049537	JCVI_READ_1095522289323	JCVI_TMPL_1061006161091
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105083820790	JCVI_READ_1095527052398	JCVI_TMPL_1061006178661
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105144753569	JCVI_READ_1093017122050	JCVI_TMPL_1061002603081
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105109847599	JCVI_READ_1092397200132	JCVI_TMPL_1061001625022
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105160721229	JCVI_READ_1095521484964	JCVI_TMPL_1061006093847
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105103318705	JCVI_READ_1095516165132	JCVI_TMPL_1061005970858
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105146207143	JCVI_READ_1095516177535	JCVI_TMPL_1061006012987
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105089204191	JCVI_READ_1092344322040	JCVI_TMPL_1061001535308
38	33	Punta Cormorant,	JCVI_PEP_1105139343023	JCVI_READ_1095527004563	JCVI_TMPL_1061006212394

		Hypersaline Lagoon, Floreana Island			
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105130253769	JCVI_READ_1095522286769	JCVI_TMPL_1061006174678
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105123223729	JCVI_READ_1092405883574	JCVI_TMPL_1061001530642
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105078443808	JCVI_READ_1095521066275	JCVI_TMPL_1061006007212
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105114644413	JCVI_READ_1093018143536	JCVI_TMPL_1061002718298
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105110371167	JCVI_READ_1095516038296	JCVI_TMPL_1061005991776
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105131501811	JCVI_READ_1093015904678	JCVI_TMPL_1061002608884
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105160401587	JCVI_READ_1095526056992	JCVI_TMPL_1061006224849
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105121779845	JCVI_READ_1095526032239	JCVI_TMPL_1061006201699
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105111302897	JCVI_READ_1095521696814	JCVI_TMPL_1061005979600
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105133187797	JCVI_READ_1095521436346	JCVI_TMPL_1061006124005
38	33	Punta Cormorant,	JCVI_PEP_1105129601865	JCVI_READ_1093017217978	JCVI_TMPL_1061002710605

		Hypersaline Lagoon, Floreana Island			
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105142141135	JCVI_READ_1095521469546	JCVI_TMPL_1061006127788
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105086003015	JCVI_READ_1095521699328	JCVI_TMPL_1061006125581
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105145273421	JCVI_READ_1092403995155	JCVI_TMPL_1061001620650
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105153463541	JCVI_READ_1093016363183	JCVI_TMPL_1061002609571
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105157446906	JCVI_READ_1095522167819	JCVI_TMPL_1061006125073
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105089224985	JCVI_READ_1093017023071	JCVI_TMPL_1061002606539
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105140088201	JCVI_READ_1095522061243	JCVI_TMPL_1061006022058
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105088303641	JCVI_READ_1093018020224	JCVI_TMPL_1061002641879
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105093483243	JCVI_READ_1092343779643	JCVI_TMPL_1061001534316
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105125182909	JCVI_READ_1095462342154	JCVI_TMPL_1061006102742
38	33	Punta Cormorant,	JCVI_PEP_1105085430841	JCVI_READ_1095522075135	JCVI_TMPL_1061006080729

		Hypersaline Lagoon, Floreana Island			
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105081741457	JCVI_READ_1093017276027	JCVI_TMPL_1061002660197
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105162579259	JCVI_READ_1095527039446	JCVI_TMPL_1061006219832
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105150899145	JCVI_READ_1093018267080	JCVI_TMPL_1061002711030
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105109490407	JCVI_READ_1092402566235	JCVI_TMPL_1061001627532
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105095674883	JCVI_READ_1095527069292	JCVI_TMPL_1061006215506
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105130920897	JCVI_READ_1093018084238	JCVI_TMPL_1061002600970
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105114934121	JCVI_READ_1093017014668	JCVI_TMPL_1061002619782
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105136170691	JCVI_READ_1095516148060	JCVI_TMPL_1061005979362
38	33	Punta Cormorant, Hypersaline Lagoon, Floreana Island	JCVI_PEP_1105081646699	JCVI_READ_1095522123195	JCVI_TMPL_1061006014154