

Major bacterial contribution to the ocean reservoir of detrital organic carbon and nitrogen

Karl Kaiser¹ and Ronald Benner

Marine Science Program, University of South Carolina, Columbia, South Carolina 29208

Abstract

Bacterial biomarkers (D-amino acids and muramic acid) were measured in various organic matter size fractions collected in the North Pacific and North Atlantic, and they were used to quantitatively estimate bacterial contributions to particulate and dissolved organic carbon and nitrogen reservoirs. The origins and yields of biomarkers were determined in cultured marine bacteria, and the results indicated that D-amino acids are derived from numerous macromolecules in addition to peptidoglycan and are not solely from peptidoglycan. Bacterial detritus was a major component of particulate organic matter (POM) and is an important source of submicron-size particles and colloids in the ocean. Peptidoglycan was a substantial component of POM but not of dissolved organic matter (DOM). Compositional differences between POM and DOM primarily reflected the selective incorporation of specific bacterial components into these reservoirs. Autotrophic and heterotrophic bacterial sources were not quantified separately, but the presence of D-aspartic acid (D-Asx) and D-serine (D-Ser) suggested that heterotrophic sources were substantial. The average reactivity of bacterial organic matter was comparable to that of the bulk organic carbon pool. Bacteria were important sources of labile, semilabile, and refractory dissolved organic carbon. Bacterial organic matter accounted for ~25% of particulate and dissolved organic carbon and ~50% of particulate and dissolved organic nitrogen. These results demonstrate the importance of bacteria in regulating the ocean carbon and nitrogen cycles.

The global cycles of carbon and nitrogen are driven by photosynthetic carbon fixation and heterotrophic metabolism. Diverse populations of Bacteria, Archaea, and Eukarya control these processes, which are also the main sources of nonliving organic matter in the ocean. The pool of nonliving organic matter in the ocean is one of the largest and most dynamic reservoirs of organic carbon on Earth, yet the relative contributions from Bacteria, Archaea, and Eukarya are unknown. Furthermore, it is unclear whether autotrophic or heterotrophic organisms are dominant contributors to marine organic matter. Eukaryotic plankton dominate autotrophic production in many regions of the oceans, and bacteria play a major role in the autotrophic production in the vast subtropical gyres. Heterotrophic bacteria utilize and transform a large fraction of autotrophic production and thereby contribute to the reservoir of nonliving organic matter in the ocean.

Recent reports have presented molecular evidence in support of a major bacterial contribution to nonliving organic matter in the oceans (Tanoue et al. 1995; McCarthy et al. 1998; Benner and Kaiser 2003). Most of the characterized biochemicals specifically occur in the bacterial cell wall–membrane complex. Peptidoglycan is a unique bacterial cell wall heteropolymer, and several studies have

indicated that this biopolymer is a ubiquitous constituent of organic matter from freshwater, estuarine, and open-ocean environments (McCarthy et al. 1998; Dittmar et al. 2001; Jones et al. 2005) and sedimentary organic matter (Lomstein et al. 2006 and references therein).

Biomarkers provide a powerful tool for tracing biogenic sources and diagenetic alterations of organic matter. However, the effective use of biomarkers requires intimate knowledge of the abundance and molecular distribution of biomarkers among source organisms and the sensitivity of biomarkers to diagenetic alterations (Hedges and Prahl 1993). Bacteria synthesize a variety of unique organic compounds that can be used as indicators of bacterial contributions to particulate organic matter (POM) and dissolved organic matter (DOM) in the oceans. Many bacterial biomarkers, including D-amino acids and muramic acid (Mur), are commonly found in the cell wall complex of bacteria. Although there is considerable literature available about the occurrence of D-amino acids and Mur in pathogenic bacteria, not much is known about the abundance and distribution of these biomarkers in marine bacteria. A comprehensive survey is needed before D-amino acids and Mur can be applied to estimate bacterial contributions in marine environments.

The most useful biomarkers for quantitative determination of bacterial detritus are of similar reactivity as bulk bacterial carbon (C) and nitrogen (N). Few studies provide information on the reactivity of biomarkers relative to bacterial C and N, but existing studies indicate that D-amino acids are fairly representative of total bacterial amino acids and bulk C and N (Kawasaki and Benner 2006; Middelboe and Jørgensen 2006). Other laboratory experiments with a mixture of algal and bacterial DOM have shown that D-amino acids and bacterial DOM are less bioreactive than algal DOM (Jørgensen et al. 1999; Amon

¹ Corresponding author (karl@biol.sc.edu).

Acknowledgments

We gratefully acknowledge Nobuyuki Kawasaki for providing field samples and data from the HOT cruise in 2005. We thank Hila Elifantz and Dave Kirchman for providing bacterial cultures, Jenny Davis for insightful discussions, and the crew aboard the *Kai-ma-kai O'Kanaloa* and *Cape Hatteras* for assistance with sampling. We thank two anonymous reviewers for helpful comments on an earlier draft of this manuscript. This work was supported by National Science Foundation grant OCE-0080782.

et al. 2001). Likewise, Nagata et al. (2003) found that a purified peptidoglycan degraded at a much lower rate than a protein. This suggests that refractory DOM in the ocean could be relatively enriched in bacterial DOM compared to algal sources.

In this study, we used a biomarker approach to provide quantitative estimates of the bacterial contributions to nonliving organic C and N pools in the ocean. Samples were collected at the Bermuda Ocean Time Series Station (BATS) and the Hawaii Ocean Time Series (HOT) station ALOHA. The abundance and molecular distribution of several biomarkers (D-amino acids and Mur) were measured in cultures of marine and freshwater bacteria and seawater incubation experiments to establish average C- and N-normalized yields. Our results indicate that ~25% of particulate organic carbon (POC) and dissolved organic carbon (DOC) is of bacterial origin. Bacterial contributions to particulate organic nitrogen (PON) and dissolved organic nitrogen (DON) were ~50%, indicating that bacteria are a major source of the nonliving organic matter in the ocean.

Materials and methods

Sample collection—Water samples were collected at the HOT site station ALOHA (R/V *Kai-ma-kai O'Kanaloa*, November 1999; R/V *Moana Wave*, March 2005) and at BATS (R/V *Cape Hatteras*, June 2001). All samples were collected in Niskin bottles with epoxy-coated closure springs. Niskin bottles were drained with acid-washed silicone tubing and passed through a 60- μm Nitex screen before isolation of POM and DOM. Water samples for total organic carbon (TOC), total hydrolyzable amino acids (THAA), and muramic acid (Mur) were collected directly from the Niskin bottles into 60-mL polyethylene screw-cap bottles (Nalgene) and frozen at -20°C until analysis. In 2005, a set of samples from HOT was filtered through precleaned 0.2- μm Supor filters (Whatman) for measurement of dissolved organic carbon (DOC) and dissolved THAA.

Tangential-flow ultrafiltration—Ultrafiltration procedures followed the methods of Benner et al. (1997). POM (0.1–60 μm) was isolated using a polysulfone hollow-fiber membrane (H5MP01), and high-molecular-weight (HMW) DOM was collected with polysulfone spiral filters (Amicon S10N1) with a nominal pore size of ~1 nm and molecular-weight cutoff of 1,000 Dalton. The ultrafiltrate (<1,000 Dalton) was sampled for measurement of low-molecular-weight (LMW) DOC. Sea salts were removed from ultrafiltration concentrates by diafiltration with deionized water. Desalted concentrates were frozen (-20°C) immediately after collection and dried in a Savant Speed-Vac evaporator upon return to the laboratory.

Cultures—Several bacterial cultures were analyzed to determine yields of D-amino acids and muramic acid. *Synechococcus bacillaris* CCMP 1333 was obtained from the Bigelow laboratory and grown in an artificial seawater medium (Biddanda and Benner 1997). An axenic culture of

Synechococcus sp. CCMP 1334 was also obtained from the Bigelow culture collection. A wild strain of *Trichodesmium* was collected from surface waters of the Gulf of Mexico (Biddanda 1995). Cultures of five heterotrophic marine bacteria, *Pseudoalteromonas piscicida* (coastal Atlantic), *Cytophaga* sp. IRI113, *Erythrobacter longus* ATCC 333941, *Roseobacter litoralis* ATCC 49566, and *Pseudomonas* sp. (Baltic Sea), and four heterotrophic freshwater bacteria, *Acidovorax* sp., *Comamonas* sp., *Cytophaga* sp., and *Janthinobacterium* sp., were provided by David Kirchman (University of Delaware). Heterotrophic marine and freshwater bacteria were grown axenically at 25°C and harvested in early stationary phase by filtration (GFF-filter, Whatman). Filters were rinsed with copious amounts of organic-free isotonic solution. The growth medium for marine bacteria contained 0.5 g yeast, 0.5 g casamino acid, 0.5 g proteose peptone, 0.5 g glucose, and 0.3 g pyruvic acid sodium salt in 750 mL of aged 0.2- μm -filtered and sterilized deep water from the North Atlantic adjusted to 1 liter with deionized water. For freshwater bacteria, the same medium was made up in deionized water. Freeze-dried cultures of three soil bacteria, *Pseudomonas fluorescens* ATCC 13430, *Azotobacter vinlandii* ATCCC 12518, and *Bacillus subtilis* ATCC 6633, were purchased from Sigma. Axenic cultures of marine algae (*Heterocapsa niei* CCMP 447, *Nannochloris* sp. CCMP 518, *Emiliania huxleyi* CCMP 373, *Thalassiosira oceanica* CCMP 1005) were obtained from Bigelow.

Bacterial DOM incubations—Deep water (3,000 m) was collected at HOT and filtered through 0.2- μm Nuclepore polycarbonate filters. In one experiment (North Pacific 1), surface water was filtered through 0.8- μm Nuclepore polycarbonate filters and inoculated into filtered deep water at a 1:10 dilution. Glucose was amended to a final concentration of 25 $\mu\text{mol L}^{-1}$. In another experiment (North Pacific 2), unfiltered surface water was inoculated into 0.2- μm -filtered deep water at a 1:10 dilution. The glucose concentration was the same as in the North Pacific 1 experiment. Both experiments were conducted aboard ship and in the dark at room temperature. Subsamples for TOC, TON, TN, and molecular analyses were taken at the start of the experiments and on day 5.

Molecular analyses—Concentrations of total and dissolved organic carbon (TOC and DOC) were determined using a high-temperature combustion method and a Shimadzu TOC 5000 or Shimadzu TOC-V analyzer. The organic carbon and nitrogen contents of dried POM and HMW-DOM samples were measured after vapor-phase acidification using a Carlo Erba 1108 CHN analyzer.

The amino sugar Mur was quantified after derivatization with a mixture of N-isobutryl-L-cysteine and o-phthalaldehyde by reverse-phase chromatography with fluorescence detection. Samples were hydrolyzed in 3 mol L^{-1} HCl and neutralized with a self-absorbed ion retardation resin (Kaiser and Benner 2000). Derivatization procedures were similar to procedures described by Kaiser and Benner (2005). A Superspher 100 RP-18 (4 μm , 4 \times 125 mm) column coupled to a Lichrospher 100 RP-18 (5 μm , 4 \times

Table 1. Molecular sources of D-amino acids and muramic acid (Mur).

	Molecular sources							References
	Peptidoglycan	Teichoic acid	LPS	Polypeptide	Lipopeptide	Siderophore	Free	
D-Asx	+	–	+	–	+	–	+	Schleifer and Kandler 1972; Morikawa et al. 1993; Asano and Lübbenhüsen 2000; Vater et al. 2002; Kocharova et al. 2004.
D-Glx	+	–	–	+	–	+	+	Schleifer and Kandler 1972; Hanby and Rydon 1946; Thorne et al. 1954; Troy 1973; Asano and Lübbenhüsen 2000; Martinez et al. 2000.
D-Ser	+	–	–	–	+	+	+	Schleifer and Kandler 1972; Demange et al. 1990; Morikawa et al. 1993; Bernardini et al. 1996; Asano and Lübbenhüsen 2000; Martinez et al. 2000; Vater et al. 2002.
D-Ala	+	+	+	–	+	+	+	Schleifer and Kandler 1972; Vanittanakom et al. 1986; Demange et al. 1990; Hanniffy et al. 1999; Asano and Lübbenhüsen 2000; Vater et al. 2002; Hashii et al. 2003; Neuhaus and Baddiley 2003.
Mur	+	–	–	–	–	–	–	Schleifer and Kandler 1972

Abbreviations: D-Asx, D-aspartic acid and D-asparagine; D-Glx, D-glutamic acid and D-glutamine; D-Ser, D-serine; D-Ala, D-alanine; Mur, muramic acid; LPS, lipopolysaccharide; +, positive finding; –, negative finding.

50 mm) guard column at 20°C was used with a linear gradient from 100% 29 mmol L⁻¹ sodium acetate (pH = 6.10) to 18% methanol in 37 min and to 60% methanol in 42 min. Excitation and emission wavelengths were set at 350 and 420 nm, respectively.

D- and L-amino acids were analyzed according to Kaiser and Benner (2005). Briefly, water samples were hydrolyzed in a CEM Mars 5000 microwave equipped with a protein hydrolysis accessory kit. Liquid-phase hydrolysis was employed to hydrolyze particulate samples. After neutralization, free-amino-acid enantiomers were derivatized with a mixture of N-isobutyryl-L-cysteine and o-phthalaldehyde or N-isobutyryl-D-cysteine and o-phthalaldehyde and separated on a reversed-phase column. Samples were run with both reagents to allow for correction of co-eluting peaks. Measured values of enantiomeric amino acids were corrected for acid-catalyzed racemization using the mean of the racemization observed in proteins and free amino acids (Kaiser and Benner 2005). Acid hydrolysis converted D- and L-isomers of asparagine and glutamine to D- and L-isomers of aspartic acid and glutamic acid, and reported concentrations of these amino acids include both (D- and L-Asx, D- and L-Glx).

Results

Literature survey of D-amino acid and Mur sources—There are multiple macromolecular sources of D-amino acids in bacteria, whereas Mur only occurs in the glycan backbone of peptidoglycan (Table 1). Peptide bridges in peptidoglycan are the predominant source of D-amino acids. Peptides in peptidoglycan of Gram-negative bacteria exclusively contain D-Glx, D-alanine (D-Ala), and L-

alanine (L-Ala) in a molar ratio of 1:1:1 if peptides are cross-linked. In non-cross-linked peptides, D-Ala can be split off, reducing molar ratios of D-Ala:Mur and D-Ala:D-Glx to less than 1 (Schleifer and Kandler 1972). The average degree of cross-linking is about 40–50% in peptidoglycan of Gram-negative bacteria (Vollmer and Höltje 2004). Peptide linkages in peptidoglycan of Gram-positive bacteria vary greatly in composition and structural arrangement. D-amino acids that occur in these peptides are not limited to D-Glx and D-Ala as in Gram-negative bacteria, but can also include D-Asx, D-Ser, and D-ornithine. Growth conditions can alter the composition of peptide linkages in Gram-positive bacteria (Schleifer and Kandler 1972).

D-amino acids occur in several other bacterial macromolecules besides peptidoglycan (Table 1). D-Ala appears to be more widely distributed among biopolymers than other D-amino acids. It occurs in teichoic acids (Neuhaus and Baddiley 2003), lipopolysaccharides (Schleifer and Kandler 1972; Hanniffy et al. 1999; Hashii et al. 2003), lipopeptides (Vanittanakom et al. 1986; Vater et al. 2002), and siderophores (Demange et al. 1990). D-Asx, D-Glx, and D-Ser are found in fewer bacterial components. A recent report identified D-Asx in the O-antigen of lipopolysaccharides (Kocharova et al. 2004). D-Asx is also found in bacterial lipopeptides (Morikawa et al. 1993; Vater et al. 2002). Some Gram-positive *Bacilli* form a capsular polypeptide composed of D-Glx (Hanby and Rydon 1946; Troy 1973), whereas other *Bacilli* produce an extracellular D-glutamyl polypeptide (Thorne et al. 1954). D-Ser frequently occurs in iron-scavenging siderophores (Demange et al. 1990; Bernardini et al. 1996; Martinez et al. 2000) and lipopeptides (Morikawa et al. 1993; Vater et al.

Table 2. Yields of D-amino acids and muramic acid in pure cultures and freshly produced bacterial dissolved organic matter (Bacterial DOM).

Culture	C:N atom	AA yield		D-Asx	D-Glx	D-Ser	D-Ala	Mur	D-Asx D-Glx D-Ser D-Ala			
		(% OC)	(% ON)						(nmol mg C ⁻¹)			
Phototrophic marine												
bacteria												
<i>Trichodesmium</i> sp.	6.0	53.3	87.5	0.0	10.2	0.0	12.2	11.0	0	100	0	68
<i>Synechococcus</i> sp.	7.4	53.5	78.6	0.0	28.8	0.0	24.3	33.9	0	100	0	100
<i>Synechococcus bacillaris</i>	7.8	42.2	95.6	0.0	7.9	0.0	11.5	15.1	0	100	0	100
Heterotrophic marine												
bacteria												
<i>Pseudomonas</i> sp.	5.1	53.4	76.5	5.2	57.0	0.1	51.0	27.4	0	48	0	40
<i>Cytophaga</i> sp. IRI113	5.0	50.7	60.5	0.0	37.2	3.9	53.7	36.6	0	98	0	51
<i>Erythrobacter longus</i>	4.7	57.4	73.4	0.0	101.3	8.3	94.6	52.5	0	52	0	42
<i>Roseobacter litoralis</i>	5.2	64.1	90.7	20.4	63.2	3.4	62.7	17.3	0	27	0	21
<i>Pseudoalteromonas piscidia</i>	4.6	58.0	67.1	65.6	23.4	3.1	32.6	15.2	0	65	0	35
Heterotrophic freshwater & soil bacteria												
<i>Acidovorax</i> sp.	4.3	51.0	64.2	8.2	3.9	2.7	99.6	21.1	0	53	0	16
<i>Comamonas</i> sp.	6.1	48.4	80.0	0.0	45.7	1.4	72.0	24.6	0	54	0	26
<i>Cytophaga</i> sp.	5.1	57.4	75.8	7.9	78.9	2.6	96.8	43.7	0	55	0	34
<i>Janthinobacterium</i> sp.	5.8	53.0	71.6	11.1	34.1	0.0	45.8	15.7	0	46	0	26
<i>Pseudomonas fluorescens</i>	4.1	54.0	63.4	6.7	37.1	0.0	29.3	44.6	0	100	0	100
<i>Azotobacter vinlandii</i>	3.8	68.0	73.1	14.7	36.2	0.0	36.7	20.9	0	58	0	43
<i>Bacillus subtilis</i>	4.5	52.2	64.9	18.9	206.7	0.0	381.9	166.7	0	81	0	33
Phototrophic algae												
<i>Heterocapsa niei</i>	7.6	35.6	78.3	0.0	0.0	0.0	0.0	0.0	0	0	0	0
<i>Nannocloris</i> sp.	7.0	41.7	84.7	0.0	0.0	0.0	0.0	0.0	0	0	0	0
<i>Emiliania huxleyi</i>	8.6	29.6	69.0	0.0	0.0	0.0	0.0	0.0	0	0	0	0
<i>Thalassiosira oceanica</i>	7.4	35.1	69.2	0.0	0.0	0.0	0.0	0.0	0	0	0	0
Bacterial DOM												
Coastal Atlantic 1†	8.9	10.3	28.7	21.1	17.6	1.6	27.0	trace	0	<10	0	<10
North Pacific 1	6.4	5.0	9.9	24.9	8.9	4.9	38.0	trace	0	<10	0	<10
North Pacific 2	6.5	8.7	21.8	26.9	23.0	4.7	40.1	trace	0	<10	0	<10

Abbreviations as in Table 1; AA, amino acid; OC, organic carbon; ON, organic nitrogen.

* The occurrence of D-amino acids in peptidoglycan was calculated based on D-Glx : Mur and D-Ala : Mur ratios of 1 and 0.75, respectively (Schleifer and Kandler 1972).

2002). Bacterial siderophores also contain D-Glx (Martinez et al. 2000).

D-amino acids and Mur in bacterial cells and bacterial DOM—The chemical composition of a diverse group of algae and bacteria was analyzed to determine the occurrence and yields of molecular biomarkers (Table 2). The survey included phototrophic marine algae and bacteria and heterotrophic marine, freshwater, and soil bacteria. With the exception of *Bacillus subtilis*, all selected bacteria were Gram-negative bacteria because they are dominant in marine waters (Giovannoni and Rappe 2002). No D-amino acids were detected in any of the axenically grown algal cultures. Overall, D-Ala and D-Glx were the dominant D-amino acids in all analyzed bacteria and occurred in fairly similar molar ratios (D-Ala : D-Glx; 1.3 ± 0.4). Yields of D-Ala and D-Glx were up to tenfold higher in the Gram-positive bacterium *Bacillus subtilis* than in Gram-negative bacteria. The cell wall is much thicker in Gram-positive bacteria than in Gram-negative bacteria (Schleifer and Kandler 1972). Yields of D-Ala and D-Glx were generally higher in heterotrophic bacteria than in phototrophic

bacteria. Yields of D-Asx and D-Ser were highly variable among heterotrophic bacteria, and they were not found in phototrophic bacteria.

Mur was abundant in all bacterial cultures. The yields of Mur averaged 26.5 ± 12.9 nmol mg C⁻¹ in all marine Gram-negative bacteria. Mur yields were about a factor of two lower than D-Ala and D-Glx yields in heterotrophic bacteria, whereas Mur yields in phototrophic bacteria were comparable to D-Ala and D-Glx yields. Mur yields determined in this study generally agree with previous analyses of Mur in terrestrial and marine bacteria (Moriarty 1977; Mimura and Romano 1985; Benner and Kaiser 2003). Mur was not present in any of the axenically grown algal cultures.

Macromolecular sources of D-amino acids were investigated by using characteristic distributions of Mur and various D-amino acids in peptidoglycan. Ratios of D-Glx : Mur and D-Ala : Mur in peptidoglycan from Gram-negative bacteria are 1 and 0.75, respectively (Schleifer and Kandler 1972). Thus, yields of D-Glx and D-Ala that exceed Mur yields in a Gram-negative bacterium indicate the occurrence of these D-amino acids in other macro-

Table 3. Concentrations of total organic carbon (TOC), total hydrolyzable amino acids (THAA), D- and L-enantiomers of aspartic acid and asparagine (D- and L-Asx), glutamic acid and glutamine (D- and L-Glx), serine (D- and L-Ser), alanine (D- and L-Ala), and muramic acid (Mur) at sta. BATS (2001) and HOT (1999).

	Depth (m)	TOC ($\mu\text{mol L}^{-1}$)	THAA (nmol L^{-1})	(nmol L ⁻¹)									
				D-Asx	L-Asx	D-Glx	L-Glx	D-Ser	L-Ser	D-Ala	L-Ala	Mur*	%D†
BATS 01	20	70	167	6	10	4	12	3	10	9	20	0.11	13
unfiltered	100	60	150	5	10	4	15	2	7	7	17	0.08	12
	350	53	232	4	16	3	45	1	10	9	26	0.08	7
	500	54	142	4	10	2	25	2	6	8	18	ND	12
	1,360	46	92	4	9	2	10	2	5	4	9	0.06	13
	2,000	46	90	5	8	2	5	2	5	5	9	0.06	14
	3,250	43	67	4	5	2	4	1	2	2	6	0.06	14
	4,200	45	80	4	6	2	10	1	5	3	7	0.09	13
HOT 99	20	83	266	7	26	4	34	5	19	12	28	0.50	12
unfiltered	80	83	229	8	21	4	29	4	16	11	25	0.60	13
	110	78	257	6	20	3	39	4	21	12	27	0.93	12
	150	63	192	5	14	3	25	4	13	10	21	0.31	13
	200	62	120	4	8	2	14	2	7	7	12	0.30	13
	250	51	132	5	12	2	18	2	11	7	14	0.19	13
	300	53	99	4	7	2	9	3	6	7	12	0.28	18
	500	40	92	3	8	2	10	2	6	5	10	0.25	15
	750	41	68	3	6	2	6	1	3	5	9	0.33	19
	2,500	36	44	2	3	2	3	1	1	4	6	0.09	21
	4,000	38	52	2	5	1	5	1	1	4	7	0.22	18

Abbreviation: ND, not determined.

* Values represent the sum of measured concentration in particulate organic matter (POM) and high-molecular-weight dissolved organic matter (HMW-DOM).

† %D = $100 \times (D/[D + L])$.

molecules besides peptidoglycan. With the exception of phototrophic bacteria and the soil bacterium *Pseudomonas fluorescens*, less than half of the D-Ala in analyzed bacteria occurred in peptidoglycan (Table 2). D-Glx was contained in peptidoglycan and other bacterial macromolecules in similar amounts. All of the D-Asx and D-Ser resided in bacterial macromolecules besides peptidoglycan because these D-amino acids are not constituents of peptidoglycan of Gram-negative bacteria.

Yields of D-amino acids and Mur in bacterially derived DOM were determined by growing natural assemblages of marine bacteria from the coastal Atlantic and North Pacific Ocean (HOT) in the dark with glucose as the sole carbon source (Kawasaki and Benner 2006). Bacterial cells and the DOM produced in these incubation experiments were compositionally different. Dissolved Mur was below the limit of quantification in these experiments, indicating a decoupling of Mur and D-amino acid release during bacterial cell growth (Kawasaki and Benner 2006). Bacterial DOM contained similar yields of D-Asx and D-Ala as bacterial cells but lower yields of D-Glx (Table 2). Peptidoglycan was a minor component of bacterial DOM.

D-amino acids and Mur in marine organic matter—The concentrations of total hydrolyzable amino acids (THAA) and Mur were measured in unfiltered seawater, POM, 0.2- μm -filtered seawater, and HMW-DOM from the Sargasso Sea (BATS) and North Pacific (HOT). THAA concentrations in unfiltered seawater ranged from 67 to 232 nmol L^{-1} at BATS and 44 to 266 nmol L^{-1} at HOT (Tables 3, 4). Dissolved THAA concentrations ranged from 46 to 184

nmol L^{-1} at HOT (Table 4). Four D-amino acids, D-Asp, D-Glx, D-Ser, and D-Ala, were present in all particulate and dissolved samples. D-Ala was the most abundant D-amino acid at both stations (2–12 nmol L^{-1}), followed by D-Asx, D-Glx, and D-Ser. The percentage of D-enantiomers (% D) increased with depth in unfiltered seawater (Table 3) but remained fairly constant in filtered seawater at HOT (Table 4). No depth trend was observed in unfiltered seawater from BATS (Table 3). Even though the samples from HOT were collected 6 yr apart, the measured D- and L-amino acid concentrations in deep water were nearly identical.

Muramic acid concentrations in unfiltered water were below the limit of detection (LOD = 1.2 nmol L^{-1}). Reported concentrations in Table 3 represent the sum of muramic acid in POM and HMW-DOM collected by ultrafiltration. Concentrations were higher at HOT than at BATS at all depths and ranged from 0.09 to 0.93 nmol L^{-1} and 0.06 to 0.11 nmol L^{-1} , respectively.

Concentrations of THAA, total hydrolyzable D-amino acids (D-AA), and Mur were highest in surface waters and decreased with depth at BATS and HOT (Fig. 1). Surface concentrations of biochemicals were significantly greater at HOT compared to BATS (ANOVA *F*-test, $p < 0.05$). A reversed pattern was observed when deep waters from the two stations were compared, with generally higher concentrations of amino acids at BATS.

Total hydrolyzable L-amino acid (L-AA) and D-AA yields (nmol mg C^{-1}) exhibited different trends at both stations (Fig. 2A). L-AA yields decreased from surface to deep water to a much greater extent than D-AA yields. D-

Table 4. Concentrations of total organic carbon (TOC), dissolved organic carbon (DOC), total hydrolyzable amino acids (THAA), D- and L-enantiomers of aspartic acid and asparagine (D- and L-Asx), glutamic acid and glutamine (D- and L-Glx), serine (D- and L-Ser), alanine (D- and L-Ala), and muramic acid (Mur) in unfiltered and filtered seawater at HOT (2005).

	Depth (m)	TOC or DOC ($\mu\text{mol L}^{-1}$)	THAA (nmol L^{-1})	(nmol L ⁻¹)								
				D-Asx	L-Asx	D-Glx	L-Glx	D-Ser	L-Ser	D-Ala	L-Ala	%D
HOT 05 unfiltered	5	78	268	10	19	3	17	4	17	12	26	11
	80	73	239	8	20	5	18	3	13	11	23	11
	150	63	183	6	14	4	13	3	10	8	16	12
	300	50	103	5	8	2	7	1	5	5	9	13
	750	44	67	3	6	1	5	1	4	4	8	15
	3,000	39	50	2	4	1	5	1	2	3	5	16
HOT 05 0.2- μm - filtered	5	76	184	10	12	3	9	4	12	12	17	15
	80	72	158	8	11	4	10	3	8	11	15	17
	150	61	123	6	8	3	7	3	6	8	11	17
	300	49	79	5	6	2	5	1	4	4	6	16
	750	42	58	3	5	1	4	1	3	4	7	17
	3,000	38	46	2	4	1	4	1	2	3	4	18

Abbreviations are the same as in Table 3.

AA yields were remarkably similar at BATS and HOT. In contrast, L-AA yields were more variable between the two locations. Carbon-normalized dissolved D-AA and dissolved L-AA yields at HOT followed the patterns observed for D-AA and L-AA yields in unfiltered water; however, the decrease of dissolved L-AA with depth was much smaller (Fig. 2B).

The majority of D-amino acids resided in the smallest size class of marine organic matter (Fig. 3). Values for LMW-DOM (<1 kDa) were calculated as the difference between measured amino acids in unfiltered water and the sum of POM and HMW-DOM. D-Ala did not follow the same trend as the other D-enantiomers, and this could indicate different sources or diagenetic trends for D-Ala. At BATS, most D-Ala was measured in HMW-DOM, whereas at HOT, D-Ala was equally distributed between HMW-DOM and LMW-DOM.

Ratios of D-Glx:Mur and D-Ala:Mur were used to compare compositional relationships between biomarker sources and bulk organic matter. Peptidoglycan from Gram-negative bacteria, marine phototrophic and heterotrophic bacteria, POM, and DOM were clearly resolved into nonoverlapping areas in a plot of D-Glx:Mur versus D-Ala:Mur (Fig. 4). For calculation of D-Glx:Mur and D-Ala:Mur ratios in DOM, Mur concentrations measured in HMW-DOM were used. Marine bacteria, POM, and DOM had elevated ratios of D-Glx:Mur and D-Ala:Mur compared to peptidoglycan. Ratios of D-Glx:Mur and D-Ala:Mur were dramatically higher in DOM compared to POM.

Quantification of the bacterial contribution to marine organic matter—D-amino acids and Mur were used to estimate the bacterial contribution to POC, particulate organic nitrogen (PON), DOC, and dissolved organic nitrogen (DON). Calculations for POM were based on C- and N-normalized yields of D-Ala, D-Glx, and Mur. Calculations for DOM were based on C- and N-normalized yields of D-Asx, D-Glx, and D-Ala. Concentrations of

DON at BATS were $6.2 \mu\text{mol L}^{-1}$ in the surface, $3.5 \mu\text{mol L}^{-1}$ in the mesopelagic zone, and $2.1 \mu\text{mol L}^{-1}$ in the deep water (Hansell and Carlson 2001). DON concentrations at HOT were $4.8 \mu\text{mol L}^{-1}$ in the surface, $3.8 \mu\text{mol L}^{-1}$ in the mesopelagic zone, and $3.1 \mu\text{mol L}^{-1}$ in the deep water (HOT station report 1999). The bacterial contribution was calculated according to:

$$\text{Bacterial C or N (\%)} = \frac{\text{Biomarker}_{\text{OM}}}{\text{Biomarker}_{\text{Bacteria, bacterial DOM}}} \times 100$$

where $\text{Biomarker}_{\text{OM}}$ and $\text{Biomarker}_{\text{Bacteria, bacterial DOM}}$ are the C- or N-normalized yields of a specific biomarker in marine organic matter (OM) and bacterial cells (Bacteria) or freshly produced bacterial DOM (bacterial DOM). Representative yields of biomarkers in bacteria were obtained from the three phototrophic and five heterotrophic marine bacteria listed in Table 2 assuming a mixture of 80% heterotrophic bacteria and 20% phototrophic bacteria. The bacterial biomarker yields in bacterial cells used for these calculations were $48.3 \text{ nmol mg C}^{-1}$ D-Glx, $206.7 \text{ nmol mg N}^{-1}$ D-Glx, $50.3 \text{ nmol mg C}^{-1}$ D-Ala, $215 \text{ nmol mg N}^{-1}$ D-Ala, $28.1 \text{ nmol mg C}^{-1}$ Mur, and $121.3 \text{ nmol mg N}^{-1}$ Mur. Biomarker yields in freshly produced bacterial DOM were the averages of values for the three incubation experiments presented in Table 2.

The average C- and N-normalized yields of bacterial biomarkers in POM and DOM at both stations and in bacteria and bacterial DOM are shown in Figs. 5 and 6. C- and N-normalized yields of Mur, D-Ala, and D-Glx in POM were consistently higher at HOT than BATS (Fig. 5). No consistent depth trend was observed, indicating that all three D-amino acids exhibited reactivities similar to bulk POC. Carbon- and nitrogen-normalized yields of D-Ala, D-Glx, and D-Asx in DOM were very similar at the two stations and did not vary much with depth. Again, yields of all three biomarkers indicated similar reactivities as bulk DOM (Fig. 6).

As summarized in Fig. 7, biomarkers indicated that 12–22% and 28–32% of the POC at BATS and HOT was

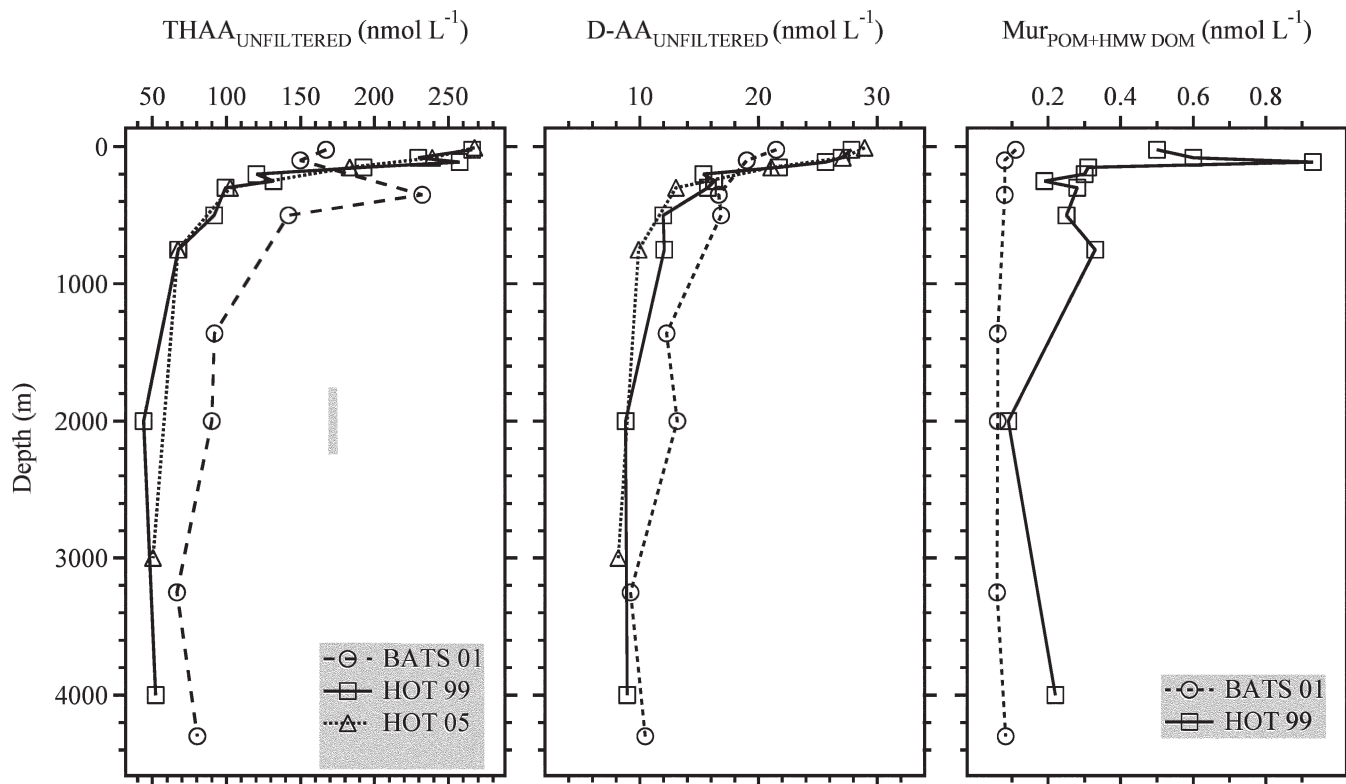


Fig. 1. Concentrations (nmol L^{-1}) of total hydrolyzable amino acids (THAA), total D-amino acids (D-AA), and muramic acid (Mur) in unfiltered seawater from the Sargasso Sea (BATS) and North Pacific (HOT). Mur concentrations represent the sum of Mur measured in particulate organic matter (POM) and high-molecular-weight dissolved organic matter (HMW-DOM).

derived from bacteria. The bacterial contribution to PON was larger, ranging from 20% to 32% at BATS and 49% to 64% at HOT. About 24–29% of the DOC at BATS and 21–29% of the DOC at HOT were of bacterial origin. The bacterial contribution to DON ranged from 45% to 54% at BATS and 47% to 50% at HOT (Fig. 7). There was little variation in the estimated contributions of bacteria to organic matter among biomarkers. A comparison of BATS and HOT indicates that bacterial C and N contributions to DOM were nearly identical, but bacterial contributions to POM were lower at BATS than HOT. We are unsure whether these differences in POM were due to varying recoveries during sampling. Ultrafiltration membranes can adsorb bacteria and POM and cause variable and incomplete recoveries (Benner 1991; Benner et al. 1997). Bacterial contributions to organic matter at the two stations did not show much variation with depth.

Discussion

Biomarker sources—Molecular biomarkers have tremendous potential for investigations into the origins and transformations of organic matter, but it is critical to know the biological sources and diagenetic reactivity of biomarkers for quantitative applications. D-amino acids and Mur are major and specific components of bacterial cell wall complexes and thus are valuable biomarkers for bacterial-derived organic matter in the environment. In the past several years, evidence has emerged that D-amino

acids also occur in archaea (Nagata et al. 1998, 1999) and eukaryotic organisms (Asano and Lübbelhusen 2000). Free D-amino acids are the most common form of D-amino acids reported in archaea and eukaryotes. Nagata et al. (1998, 1999) also detected trace amounts of covalently bound D-amino acids in a small number of hyperthermophilic archaea. However, accurate measurement of trace amounts of bound D-amino acids in the presence of large L-amino acid concentrations is challenging because acid-catalyzed racemization leads to optical inversion of the respective amino-acid enantiomer. The approach Nagata et al. (1998) used to quantify bound D-amino acids relied on a linear behavior of amino-acid racemization over the time of the acid hydrolysis. Many variables, such as the structure of the amino-acid polymer, the position of the amino acid in the polymer, and the sample matrix, determine the rate of racemization during hydrolysis and lead to nonlinear racemization kinetics (Smith and De Sol 1980; Frank et al. 1981; Bada 1984).

The most direct approach to investigate archaeal and eukaryotic contributions to D-amino acids in marine organic matter is to compare bacterial POM estimates based on D-amino acids with estimates derived from Mur yields. Mur has not been found in any organism besides bacteria. Bacterial POM estimates based on Mur and D-amino acids are not statistically different (ANOVA F -test, $p < 0.05$), indicating that archaea are not likely sources of particulate D-amino acids. In addition, our analysis of several axenic eukaryotic phytoplankton cultures shows

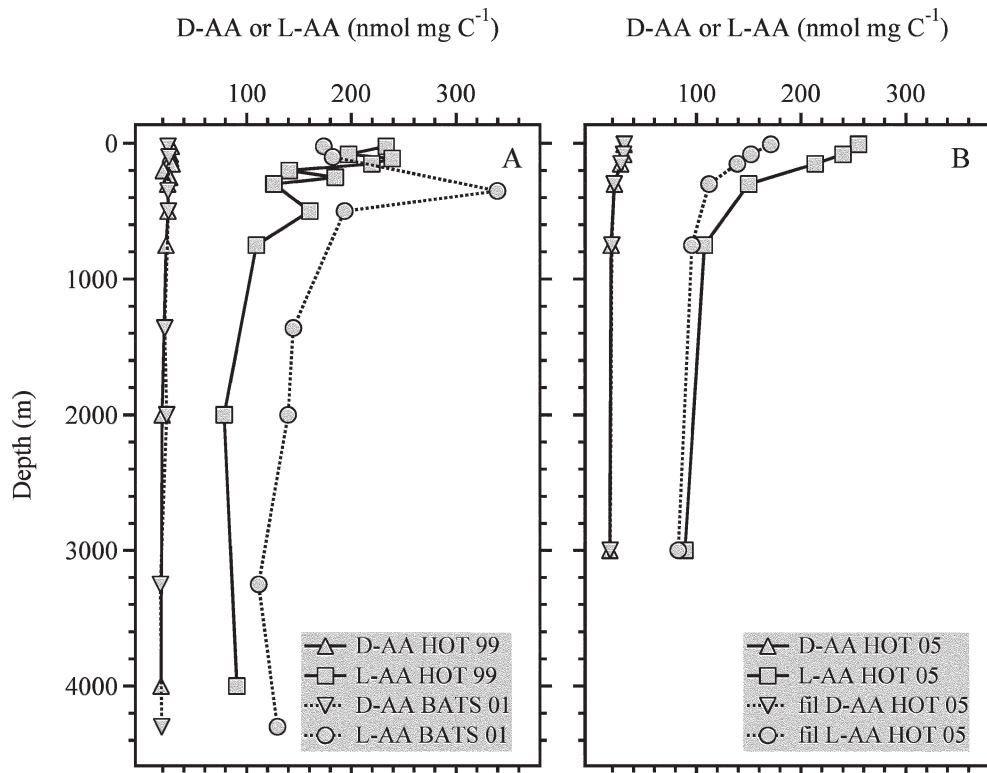


Fig. 2. (A) Yields of total hydrolyzable L-amino acids (L-AA) and total hydrolyzable D-amino acids (D-AA) in unfiltered seawater collected at BATS in 2001 and HOT in 1999. (B) Yields of L-AA and D-AA in unfiltered or filtered seawater collected at HOT in 2005.

that these organisms do not contain measurable D-amino acids or Mur. Consequently, although D-amino acids might be found in unstudied taxa in the future, archaea and eukaryotes are not considered to be sources of these biomarkers. Finally, contributions of free D-amino acids from archaea or eukaryotes can be ruled out because free-amino-acid concentrations in surface water at HOT and BATS were below the limit of detection (Kaiser unpubl. data).

The distribution of D-amino acids in bacterial cultures confirms earlier observations (Table 1) that these biomarkers occur in numerous macromolecules. Less than half of the D-amino acids were associated with peptidoglycan in most of the analyzed heterotrophic marine and freshwater bacteria. Marine phototrophic bacteria were notable exceptions, where most D-amino acids resided in peptidoglycan. Potential molecular sources of D-amino acids besides peptidoglycan include lipopolysaccharides, polypeptides, lipopeptides, or siderophores, which are all integral constituents of the bacterial cell wall-membrane complex. High yields of D-Asx in the marine Gram-negative *P. piscicida* and *R. litoralis* could be indicative of a D-aspartyl polypeptide similar to D-glutamyl polypeptides produced by a few Gram-positive *Bacilli* (Hanby and Rydon 1946; Troy 1973).

The molecular composition of bacterial DOM is quite different from bacterial POM. Bacterial DOM contains only trace concentrations of Mur and has a much higher %D-AA than bacterial POM (Kawasaki and Benner 2006).

These compositional differences indicate that specific bacterial macromolecules are preferentially incorporated into bacterial DOM. Bacteria release D-amino acids during cell growth but not Mur, which is recycled in the cell

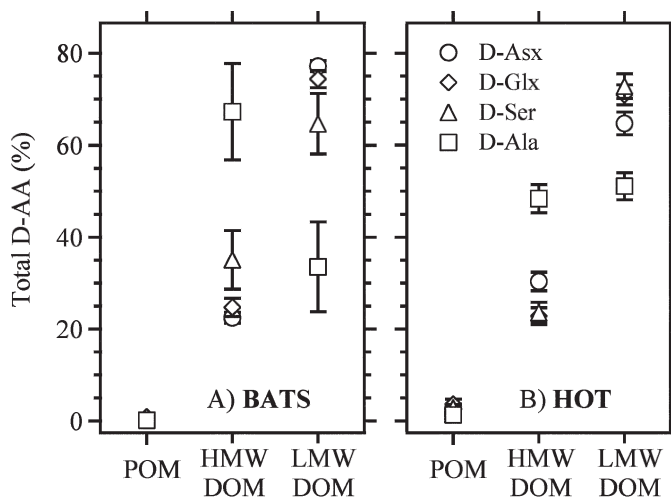


Fig. 3. Distribution of D-amino acids (D-AA) in the different size fractions of marine organic matter at (A) BATS and (B) HOT. D-AA abbreviations are as in Table 1; POM, particulate organic matter; HMW-DOM, high-molecular-weight dissolved organic matter; LMW-DOM, low-molecular-weight dissolved organic matter. Error bars represent ranges from depth integration.

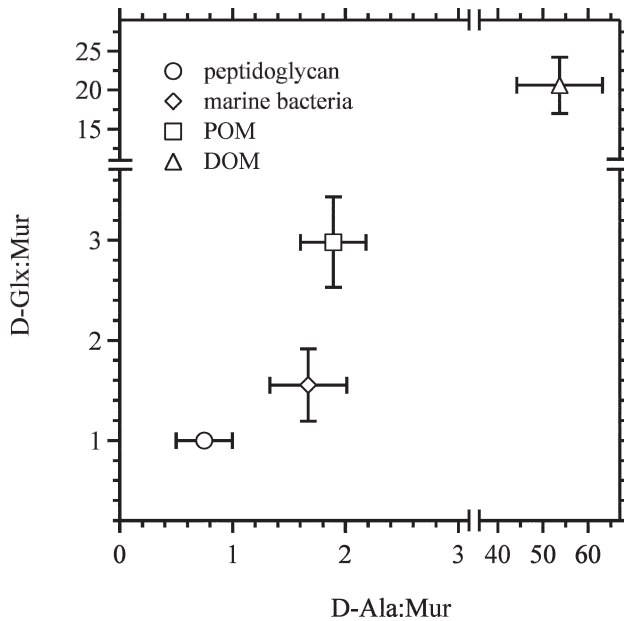


Fig. 4. Molar ratios of D-Glx:Mur versus molar ratios of D-Ala:Mur in peptidoglycan (Gram-negative bacteria; Schleifer and Kandler 1972), marine bacteria (Table 1), POM, and DOM. Mur concentrations in HMW-DOM were used to calculate ratios for DOM.

(Kawasaki and Benner 2006). Bacteria also release capsular material (Stoderegger and Herndl 1998) and dissolved lipopolysaccharides (Cadieux et al. 1983 and references therein) during normal growth.

Bacterivory also leads to the production of DOM that is compositionally distinct from POM. Protozoan bacterivory results in the enzymatic digestion of cells, and only a few eukaryotic organisms are known to express D-amino acid-digesting enzymes (Asano and Lübbelhusen 2000). As a result, protozoan bacterivory leads to assimilation of L-amino acids and release of DOM enriched in D-amino acids. In contrast, viral lysis results in DOM that is compositionally similar to that of bacterial cells (Middelboe and Jørgensen 2006).

Numerous macromolecular sources of D-amino acids were also recognizable in marine POM and DOM. Characteristic biomarker ratios suggested that intact peptidoglycan subunits were a substantial component of marine POM, but not of DOM. The fact that most D-amino acids in marine DOM are not derived from peptidoglycan is consistent with the observed distribution of D-amino acids in HMW-DOM and LMW-DOM. The majority of D-Ala resided in HMW-DOM, while all other D-amino acids mostly occurred in LMW-DOM.

Diagenetic reactivity of biomarkers—The effective use of biomarkers for quantitative applications requires a fundamental understanding of their reactivity. Ideal biomarkers exhibit similar reactivities as bulk C and N. The early stages of diagenesis are typically selective (Hedges and Prahl 1993), and bacteria contain numerous macromolecules with potentially different diagenetic reactivities. Biodegradation experiments indicate that bacterial DOM is generally more resistant to degradation than algal DOM, which only have

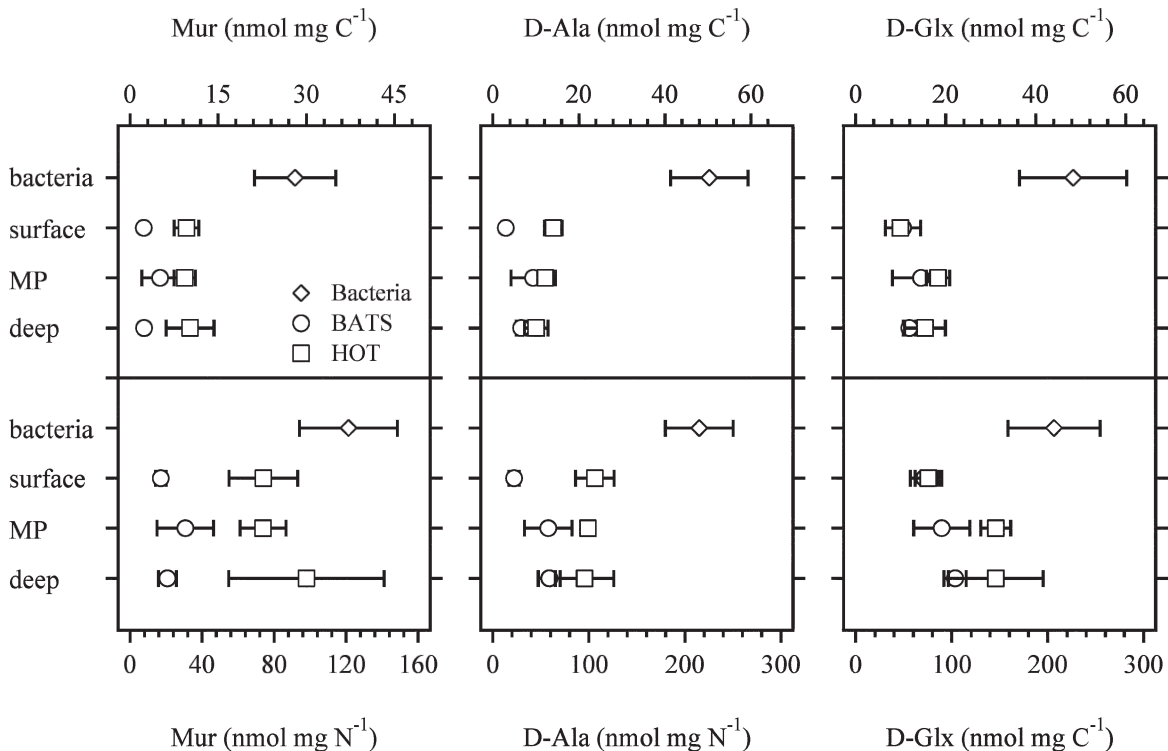


Fig. 5. Average C- and N-normalized yields of muramic acid (Mur), D-alanine (D-Ala), and D-glutamic acid or D-glutamine (D-Glx) in bacteria (Table 3) and particulate organic matter (POM) from surface (0–150 m), mesopelagic (MP, 200–1,000 m), and deep waters (>1,000 m) at BATS and HOT. Error bars represent standard deviations.

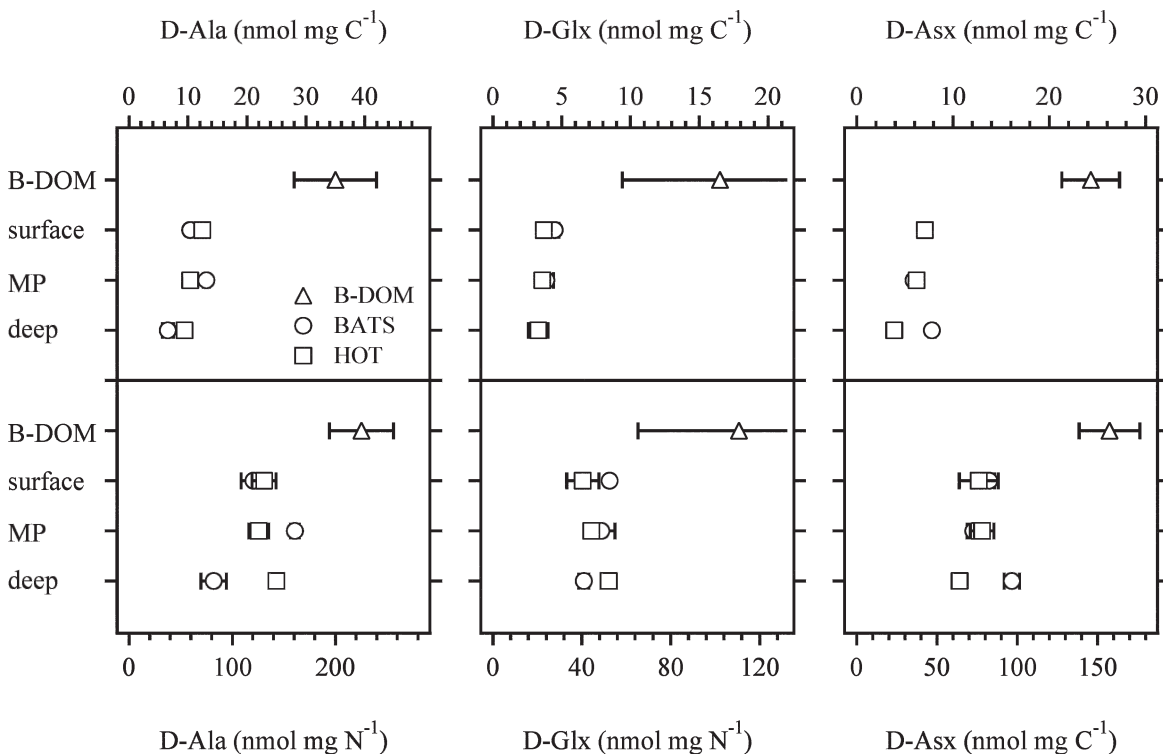


Fig. 6. Average C- and N-normalized yields of D-alanine (D-Ala), D-glutamic acid or D-glutamine (D-Glx), and D-aspartic acid or D-asparagine (D-Asx) in bacterial dissolved organic matter (B-DOM) and DOM from surface (0–150 m), mesopelagic (MP, 200–1,000 m), and deep waters (>1,000 m) at BATS and HOT. Error bars represent standard deviations.

L-amino acids (Jørgensen et al. 1999; Amon et al. 2001). These studies included complex mixtures of eukaryotic and prokaryotic DOM, and, therefore, they do not reflect the degradation of bacterial DOM alone. Kawasaki and Benner (2006) characterized the degradation of bacterial POM and DOM and found that the relative proportions of D- and L-amino acids in bacterial POM did not vary much during decomposition. Decay coefficients of bacterial cells and bacterial biomarkers (D-Asx, D-Glx, D-Ser, D-Ala, Mur) were not statistically different, indicating that these biomarkers have similar reactivities as bacterial C and N.

The relative proportions of D- and L-amino acids in bacterial DOM fluctuated during exponential growth and cell decline but stayed relatively constant during the latter stages of decomposition (Kawasaki and Benner 2006). Muramic acid only occurred in trace quantities throughout the experiment, indicating that it was a minor component of bacterial DOM and that dissolved D-amino acids were not derived primarily from peptidoglycan. The selective degradation of the glycan chain in peptidoglycan would result in the enrichment of D-amino acids in bacterial DOM, but Nagata et al. (2003) found the peptide component of peptidoglycan degrades three times faster than the glycan chain.

Middelboe and Jørgensen (2006) observed fairly constant proportions of dissolved D- and L-amino acids in a similar experiment, further indicating that D- and L-amino acids have similar reactivities in bacterial DOM. Degradation rates of dissolved D- and L-amino acids in estuarine environments (Jørgensen et al. 2003; Middelboe

and Jørgensen 2006) and North Atlantic deep water (Teira et al. 2006) have been shown to be similar. Thus, it appears that the reactivity of dissolved D-amino acids reflects the reactivity of bacterial C and N in DOM.

Most organic matter in the ocean, including the bacterially derived component, has undergone extensive decomposition and is resistant to biological utilization. Molecular analyses can only identify a small fraction of marine DOM as recognizable biomolecules (<10%), demonstrating that most DOM has been chemically altered (Benner 2002). Modifications of bacterial biomarkers by diagenetic processes compromise their molecular and source identification, resulting in conservative or minimal estimates of bacterial contributions. Recently, carboxyl-rich alicyclic molecules (CRAM) were identified as major components of refractory marine DOM (Hertkorn et al. 2006). CRAM show high structural diversity, but the core molecular structures still resemble hopanoid structures, which are only found in bacteria. It is possible that considerable portions of bacterial remnants in marine DOM have lost their molecular source identity and our biomarker approach leads to an underestimation of bacterial contributions.

Bacterial contributions to marine organic matter—Station- and depth-averaged estimates indicate that ~25% of the POC and DOC and ~50% of PON and DON in the ocean is of bacterial origin. Multiple biomarkers were employed to quantify the bacterial contributions to POM and DOM, greatly increasing the degree of confidence in

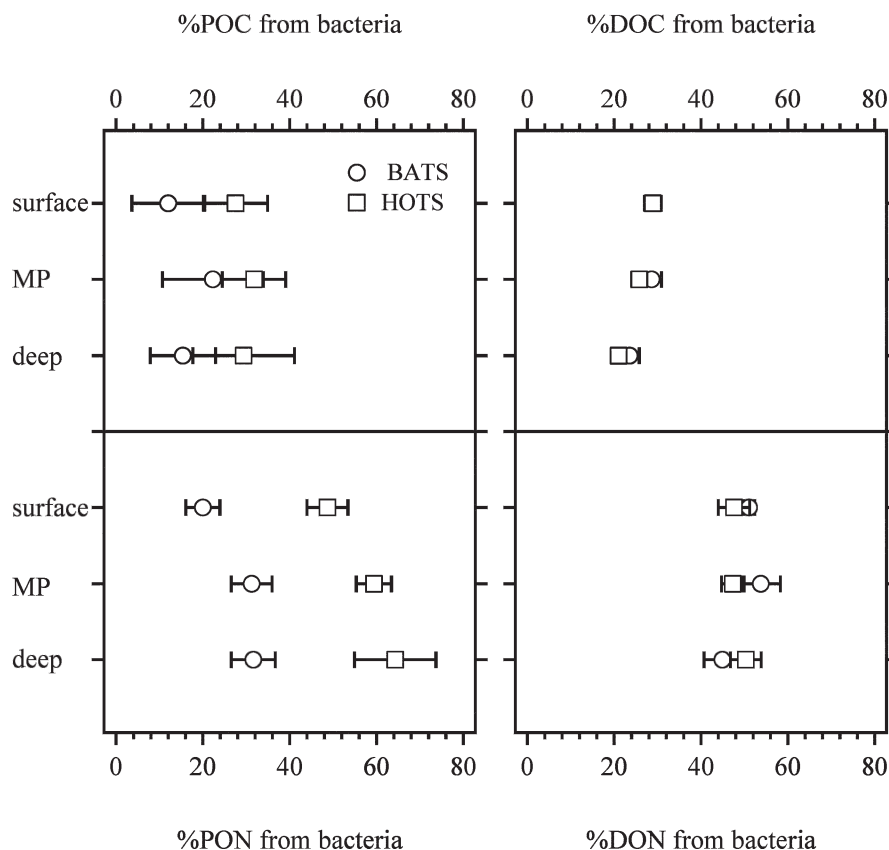


Fig. 7. Average percentages of bacterial contributions to POC, PON, DOC, and DON. Error bars represent standard deviations among depth and biomarkers. MP, mesopelagic; POC, particulate organic carbon; PON, particulate organic nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen.

these estimates. POM contains living bacteria and cellular debris. Calculations using published bacterial abundances at HOT (Karner et al. 2001) and ultrafiltration recovery efficiencies (Benner et al. 1997) suggest that $\leq 15\%$ of biomarkers in surface POM and $\leq 7\%$ in deep POM are associated with living cells. The carbon content of marine bacterial cells was assumed to be $12.4 \text{ fg C cell}^{-1}$ (Fukuda et al. 1998), and biomarker yields for marine bacteria were taken from Table 2. These estimates are similar to earlier ones based on muramic acid in POM (Benner and Kaiser 2003), and they demonstrate that $\geq 85\%$ of bacterial POM is associated with detrital particles. Bacterial remnants comprise a major fraction of the submicron particles (Koike et al. 1990) and colloids (Wells and Goldberg 1991) in the ocean.

Previous reports have suggested that peptidoglycan could be an important source of DON in the open ocean, comparable to that of hydrolyzable protein (McCarthy et al. 1998; Dittmar et al. 2001; Perez et al. 2003), accounting for 2–5% of the DON. These estimates were based on the assumption that all D-Ala is exclusively derived from peptidoglycan, which, as shown in the present study, is incorrect. Based on Mur as an indicator of peptidoglycan, the maximal contribution of intact peptidoglycan subunits to PON and DON was calculated to be $\sim 1\%$ and $< 0.1\%$, respectively. Peptidoglycan N was estimated as $6.7 \times \text{Mur}$,

assuming a typical Gram-negative peptidoglycan architecture (Schleifer and Kandler 1972), and it included N contributions from the glycan chain and the interpeptide bridge.

Bacterial abundance and production, both autotrophic and heterotrophic, decrease exponentially with depth in the ocean. Cyanobacteria, in particular *Prochlorococcus*, dominate primary productivity in the euphotic zone at HOT (Campbell et al. 1997), and both *Synechococcus* and *Prochlorococcus* become dominant in late spring and early summer at BATS (Steinberg et al. 2001). McCarthy et al. (2004) suggested, based on isotopic evidence, that cyanobacteria are substantial sources of DON in the ocean. Seawater concentrations of bacterial organic matter were typically twofold higher in the surface compared to deep waters, reflecting the magnitude of bacterial sources. The relative contributions of bacterial organic matter to bulk organic matter, however, did not show a clear depth trend. A possible explanation for this pattern may be linked to the bioreactivity of bacterial organic matter produced in the surface ocean. A portion of bacterial organic matter is probably consumed rapidly, whereas another portion is more resistant to microbial degradation and is subsequently exported. This view is consistent with the observed tight linkage between production and consumption processes of organic matter in the surface ocean. The fraction of

bacterial organic matter that escapes remineralization contributes to the large pool of slowly cycling organic matter in the ocean.

Bacterial detritus could be also supplied to the deep ocean by sinking particles. The flux of sinking particles rapidly decreases with depth as a result of disaggregation, dissolution, and heterotrophic consumption (Wakeham et al. 1997; Lee et al. 2000). Sinking POC could be a significant source of deep water DOC (Smith et al. 1992).

The bioavailability of marine bacterial C can be analyzed by comparing concentrations in surface waters to those in deep water, assuming that deep-water concentrations are representative of refractory C. Deep-water concentrations of C at HOT probably approximate refractory C pools best because radiocarbon ages indicate that the oldest DOC is at HOT (Druffel et al. 1992). By such a calculation, ~65% of bacterial C in the surface ocean is identified as bioavailable (i.e., labile and semilabile). This indicates that bacterial organic matter is of slightly higher reactivity than bulk organic C (52%). Furthermore, such a comparison reveals that ~40% of labile and semilabile DOC in the surface ocean is derived from bacteria. Taken together, these observations clearly indicate that bacterial organic matter represents a continuum of biological reactivity that ranges from labile to refractory.

Implications for carbon and nitrogen cycling—Our data suggest that bacteria are major sources of marine organic matter. However, compared to their importance as producers and consumers of organic matter in the ocean, the presented bacterial contributions could appear to be lower than expected. The biomarker approach used in this study could lead to conservative estimates of bacterial contributions as the molecular structures of molecules are altered by diagenesis. Extensive diagenesis probably transforms some bacterial remnants into refractory “geopolymers” that lose their molecular identity. The mechanisms by which such “geopolymers” are formed are not well understood, but it has been suggested that abiotic condensation and polymerization reactions could play an important role (Hedges 1988). Ogawa et al. (2001) speculated that nonspecific or secondary enzyme activities could be responsible for the production of altered biomolecules that are resistant to biological utilization. Photochemical transformations may also provide an important mechanism for the formation of structurally altered biorefractory DOM in the ocean (Zika 1981; Benner and Biddanda 1998).

The large contribution of bacteria to marine organic N (~50%) underscores the importance of these organisms in the cycling of marine N. Assuming that diagenetic alterations of bacterial biomarkers occur, bacterial contributions to organic N are probably even larger. Vast regions of the surface ocean are characterized by very low inorganic-nutrient concentrations and tightly linked production and regeneration processes. Most nutrients in these ecosystems are in organic form and are largely of bacterial origin. Thus, marine bacteria play a dominant role in ocean productivity and the global carbon cycle.

The biomarkers applied in this study also provide clues about the extent of autotrophic and heterotrophic bacterial sources. Analyzed cultured and field-collected autotrophic marine bacteria did not contain D-Asx and D-Ser, whereas these D-amino acids were prominent constituents of marine heterotrophic bacteria, marine POM, and DOM. The ubiquitous distribution of D-Asx and D-Ser in POM and DOM suggests that heterotrophic bacteria are a dominant source and that they play an important role in the composition and chemical characteristics of marine organic matter.

References

- AMON, R. M. W., H. P. FITZNER, AND R. BENNER. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnol. Oceanogr.* **46**: 287–297.
- ASANO, Y., AND T. L. LÜBBEHÜSEN. 2000. Enzymes acting on peptides containing D-amino acid. *J. Biosci. Bioeng.* **89**: 295–306.
- BADA, J. L. 1984. In vivo racemization in mammalian proteins. *Methods Enzymol.* **106**: 98–115.
- BENNER, R. 1991. Ultra-filtration for the concentration of bacteria, viruses, and dissolved organic matter, p. 181–185. *In* D. C. Hurd and D. W. Spencer [eds.], *Marine particles: Analysis and characterization*. Geophysical Monograph 63. American Geophysical Union.
- . 2002. Chemical composition and reactivity, p. 59–85. *In* D. A. Hansell and C. A. Carlson [eds.], *Biogeochemistry of marine dissolved organic matter*. Academic Press.
- , AND B. BIDDANDA. 1998. Photochemical transformations of surface and deep marine dissolved organic matter: Effects on bacterial growth. *Limnol. Oceanogr.* **43**: 1373–1378.
- , ———, B. BLACK, AND M. MCCARTHY. 1997. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Mar. Chem.* **57**: 243–263.
- , AND K. KAISER. 2003. Abundance of amino sugars and peptidoglycan in marine particulate and dissolved organic matter. *Limnol. Oceanogr.* **48**: 118–128.
- BERNARDINI, J. J., C. LINGET-MORICE, F. HOH, S. K. COLLINSON, P. KYSLIK, W. J. PAGE, A. DELL, AND M. A. ABDALLAH. 1996. Bacterial siderophores: Structure elucidation, and H-1, C-13 and N-15 two-dimensional NMR assignments of azoverdin and related siderophores synthesized by *Azomonas macrocytogenes* ATCC 12334. *Biomaterials* **9**: 107–120.
- BIDDANDA, B. 1995. *Trichodesmium* bloom in the Gulf of Mexico, summer 1995. *IOC/UNESCO Harmful Algae News* **12/13**: 2.
- , AND R. BENNER. 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. *Limnol. Oceanogr.* **42**: 506–518.
- CADIEUX, J. E., J. KUZIO, F. H. MILAZZO, AND A. M. KROPINSKI. 1983. Spontaneous release of lipopolysaccharide by *Pseudomonas aeruginosa*. *J. Bacteriol.* **155**: 817–825.
- CAMPBELL, L., H. B. LIU, H. A. NOLLA, AND D. VAULOT. 1997. Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at station ALOHA during the 1991–1994 ENSO event. *Deep-Sea Res. I* **44**: 167–192.
- DEMANGE, P., A. BATEMAN, C. MERTZ, A. DELL, Y. PIEMONT, AND M. A. ABDALLAH. 1990. Bacterial siderophores—structures of *Pyoverdins-Pt*, siderophores of *Pseudomonas tolaasii* NCPPB-

- 2192, and *Pyoverdins pf*, siderophores of *Pseudomonas fluorescens* CCM-2798 —identification of an unusual natural amino-acid. *Biochemistry* **29**: 11041–11051.
- DITTMAR, T., H. P. FITZNER, AND G. KATTNER. 2001. Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D- and L-amino acids. *Geochim. Cosmochim. Acta* **65**: 4103–4114.
- DRUFFEL, E. R. M., P. M. WILLIAMS, J. E. BAUER, AND J. R. ERTEL. 1992. Cycling of dissolved and particulate organic-matter in the open ocean. *J. Geophysical. Res. [Ocean]* **97**: 15639–15659.
- FRANK, H., W. WOIWODE, N. NICHOLSON, AND E. BAYER. 1981. Determination of the rate of acidic catalyzed racemization of protein amino acids. *Liebigs Ann. Chem.* **3**: 354–365.
- FUKUDA, R., H. OGAWA, T. NAGATA, AND I. KOIKE. 1998. Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. *Appl. Environ. Microbiol.* **64**: 3352–3358.
- GIOVANNONI, S., AND M. RAPPE. 2000. Evolution, diversity, and molecular ecology of marine prokaryotes, p. 47–84. *In* D. L. Kirchman [ed.], *Microbial ecology of the oceans*. Wiley.
- HANBY, W. E., AND H. N. RYDON. 1946. The capsular substance of *Bacillus-Anthraxis*. *Biochem. J.* **40**: 297–307.
- HANNIFFY, O. M., A. S. SHASHKOV, S. N. SENCHENKOVA, S. V. TOMSHICH, N. A. KOMANDROVA, L. A. ROMANENKO, Y. A. KNIREL, AND A. V. SAVAGE. 1999. Structure of an acidic O-specific polysaccharide of *Pseudoalteromonas haloplanktis* type strain ATCC 14393 containing 2-acetamido-2-deoxy-D- and -L-galacturonic acids and 3-(N-acetyl-D-alanyl)amino-3,6-dideoxy-D-glucose. *Carbohydr. Res.* **321**: 132–138.
- HANSELL, D. A., AND C. A. CARLSON. 2001. Biogeochemistry of total organic carbon and nitrogen in the Sargasso Sea: Control by convective overturn. *Deep-Sea Res. II* **48**: 1649–1667.
- HASHII, N., Y. ISHIKI, T. IGUCHI, AND S. KONDO. 2003. Structural characterization of the carbohydrate backbone of the lipopolysaccharide of *Vibrio parahaemolyticus* O-untypable strain KX-V212 isolated from a patient. *Carbohydr. Res.* **338**: 2711–2719.
- HEDGES, J. 1988. Polymerization of humic substances in natural environments, p. 44–58. *In* F. H. Frimmel and R. F. Christman [eds.], *Humic substances and their role in the environment*. Wiley.
- , AND F. G. PRAHL. 1993. Early diagenesis: Consequences for applications of molecular biomarkers, p. 237–253. *In* M. H. Engel and S. A. Macko [eds.], *Organic geochemistry: Principles and applications*. Plenum.
- HERTKORN, N., R. BENNER, M. FROMMBERGER, P. SCHMITT-KOPPLIN, M. WITT, K. KAISER, A. KETTRUP, AND J. I. HEDGES. 2006. Characterization of a major refractory component of marine dissolved organic matter. *Geochim. Cosmochim. Acta* **70**: 2990–3010.
- HOT STATION REPORT. 1999. HOT station report [Internet]. Available from <http://hahana.soest.hawaii.edu/hot/hot-dogs/bextraction.html>. Manoa, The University of Hawaii, Laboratory for Microbial Oceanography. Accessed August 2006.
- JONES, V., M. J. COLLINS, K. E. H. PENKMAN, R. JAFFE, AND G. A. WOLFF. 2005. An assessment of the microbial contribution to aquatic dissolved organic nitrogen using amino acid enantiomeric ratios. *Org. Geochem.* **36**: 1099–1107.
- JØRGENSEN, N. O. G., R. STEPANUKAS, A. G. U. PEDERSEN, M. HANSEN, AND O. NYBRØE. 2003. Occurrence and degradation of peptidoglycan in aquatic environments. *FEMS Microbiol. Ecol.* **46**: 269–280.
- , L. J. TRANVIK, AND G. M. BERG. 1999. Occurrence and bacterial cycling of dissolved nitrogen in the Gulf of Riga, the Baltic Sea. *Mar. Ecol. Prog. Ser.* **191**: 1–18.
- KAISER, K., AND R. BENNER. 2000. Determination of amino sugars in environmental samples with high salt content by high performance anion exchange chromatography and pulsed amperometric detection. *Anal. Chem.* **72**: 2566–2572.
- , AND ———. 2005. Hydrolysis induced racemization of amino acids. *Limnol. Oceanogr. Meth.* **3**: 318–325.
- KARNER, M. B., E. F. DELONG, AND D. M. KARL. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* **409**: 507–510.
- KAWASAKI, N., AND R. BENNER. 2006. Bacterial release of dissolved organic matter during cell growth and decline: Molecular origin and composition. *Limnol. Oceanogr.* **51**: 2170–2180.
- KOCHAROVA, N. A., S. N. SENCHENKOVA, A. N. KONDAKOVA, A. I. GREMYAKOV, G. V. ZATONSKY, A. S. SHASHKOV, Y. A. KNIREL, AND A. N. K. KOCHETKOV. 2004. D- and L-aspartic acids: New non-sugar components of bacterial polysaccharides. *Biochemistry (Mosc.)* **69**: 103–107.
- KOIKE, I., S. HARA, K. TREAUCHI, AND K. KOGURE. 1990. Role of sub-micrometer particles in the ocean. *Nature* **345**: 242–244.
- LEE, C., S. G. WAKEHAM, AND J. I. HEDGES. 2000. Composition and flux of particulate amino acids and chloropigments in equatorial Pacific seawater and sediments. *Deep-Sea Res. I* **47**: 1535–1568.
- LOMSTEIN, B. A., B. B. JØRGENSEN, C. J. SCHUBERT, AND J. NIGGEMANN. 2006. Amino acid biogeo- and stereochemistry in coastal Chilean sediments. *Geochim. Cosmochim. Acta* **70**: 2970–2989.
- MARTINEZ, J. S., G. P. ZHANG, P. D. HOLT, H. T. JUNG, C. J. CARRANO, M. G. HAYGOOD, AND A. BUTLER. 2000. Self-assembling amphiphilic siderophores from marine bacteria. *Science* **287**: 1245–1247.
- MCCARTHY, M. D., R. BENNER, C. LEE, J. I. HEDGES, AND M. L. FOGEL. 2004. Amino acid carbon isotopic fractionation patterns in oceanic dissolved organic matter: An unaltered photoautotrophic source for dissolved organic nitrogen in the ocean? *Mar. Chem.* **92**: 123–134.
- , J. I. HEDGES, AND R. BENNER. 1998. Major bacterial contribution to marine dissolved organic nitrogen. *Science* **281**: 231–234.
- MIDDELBOE, M., AND N. O. G. JØRGENSEN. 2006. Viral lysis of bacteria: An important source of dissolved amino acids and cell wall compounds. *J. Mar. Biol. Ass. U.K.* **86**: 605–612.
- MIMURA, T., AND J. C. ROMANO. 1985. Muramic acid measurements for bacterial investigations in marine environments by high pressure liquid chromatography. *Appl. Environ. Microbiol.* **50**: 229–237.
- MORIARTY, D. J. W. 1977. Improved method using muramic acid to estimate biomass of bacteria in sediments. *Oecologia* **26**: 317–323.
- MORIKAWA, M., H. DAIDO, T. TAKAO, S. MURATA, Y. SHIMONISHI, AND T. IMANAKA. 1993. A new lipopeptide biosurfactant produced by *Arthrobacter* sp. strain Mis38. *J. Bacteriol.* **175**: 6459–6466.
- NAGATA, T., B. MEON, AND D. L. KIRCHMAN. 2003. Microbial degradation of peptidoglycan in seawater. *Limnol. Oceanogr.* **48**: 745–754.
- NAGATA, Y., T. FUJIWARA, K. KAWAGUCHI-NAGATA, Y. FUKUMORI, AND T. YAMANAKA. 1998. Occurrence of peptidyl D-amino acids in soluble fractions of several eubacteria, archaea and eukaryotes. *Biochim. Biophys. Acta Gen. Subj.* **1379**: 76–82.

- , AND OTHERS. 1999. Occurrence of D-amino acids in a few archaea and dehydrogenase activities in hyperthermophile *Pyrobaculum islandicum*. *Biochim. Biophys. Acta, Prot. Struct. Molec. Enzym.* **1435**: 160–166.
- NEUHAUS, F. C., AND J. BADDILEY. 2003. A continuum of anionic charge: Structures and functions of D-alanyl-teichoic acids in Gram-positive bacteria. *Microbiol. Mol. Biol. Rev.* **67**: 686–723.
- OGAWA, H., Y. AMAGAI, I. KOIKE, K. KAISER, AND R. BENNER. 2001. Production of refractory dissolved organic matter by bacteria. *Science* **292**: 917–920.
- PEREZ, M. T., C. PAUSZ, AND G. J. HERNDL. 2003. Major shift in bacterioplankton utilization of enantiomeric amino acids between surface waters and the ocean's interior. *Limnol. Oceanogr.* **48**: 755–763.
- SCHLEIFER, K. H., AND O. KANDLER. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bact. Rev.* **36**: 407–477.
- SMITH, D. C., M. SIMON, A. L. ALLDREDGE, AND F. AZAM. 1992. Intense hydrolytic enzyme-activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359**: 139–142.
- SMITH, G. G., AND B. S. DE SOL. 1980. Racemization of amino acids in dipeptides shows COOH > NH₂ for non-sterically hindered residues. *Science* **207**: 765–767.
- STEINBERG, D. K., C. A. CARLSON, N. R. BATES, R. J. JOHNSON, A. F. MICHAELS, AND A. H. KNAP. 2001. Overview of the US JGOFS Bermuda Atlantic Time-Series Study (BATS): A decade-scale look at ocean biology and biogeochemistry. *Deep-Sea Res. II* **48**: 1405–1447.
- STODEREGGER, K., AND G. J. HERNDL. 1998. Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. *Limnol. Oceanogr.* **43**: 877–884.
- TANOUE, E., S. NISHIYAMA, M. KAMO, AND A. TSUGITA. 1995. Bacterial membranes—possible source of a major dissolved protein in seawater. *Geochim. Cosmochim. Acta* **59**: 2643–2648.
- TEIRA, E., P. LEBARON, H. VAN AKEN, AND G. J. HERNDL. 2006. Distribution and activity of Bacteria and Archaea in the deep water masses of the North Atlantic. *Limnol. Oceanogr.* **51**: 2131–2144.
- THORNE, C. B., C. G. GOMEZ, H. E. NOYES, AND R. D. HOUSEWRIGHT. 1954. Production of glutamyl polypeptide by *Bacillus subtilis*. *J. Bacteriol.* **68**: 307–315.
- TROY, F. A. 1973. Chemistry and biosynthesis of poly(γ -D-glutamyl) capsule in *Bacillus licheniformis*. 1. Properties of membrane-mediated biosynthetic reaction. *J. Biol. Chem.* **248**: 305–315.
- VANITTANAKOM, N., W. LOEFFLER, U. KOCH, AND G. JUNG. 1986. Fengycin—a novel antifungal lipopeptide antibiotic produced by *Bacillus subtilis* F-29-3. *J. Antibiot.* **39**: 888–901.
- VATER, J., B. KABLITZ, C. WILDE, P. FRANKE, N. MEHTA, AND S. S. CAMEOTRA. 2002. Matrix-assisted laser desorption ionization-time of flight mass spectrometry of lipopeptide biosurfactants in whole cells and culture filtrates of *Bacillus subtilis* C-1 isolated from petroleum sludge. *Appl. Environ. Microbiol.* **68**: 6210–6219.
- VOLLMER, W., AND J. V. HÖLTJE. 2004. The architecture of the murein (peptidoglycan) in Gram-negative bacteria: Vertical scaffold or horizontal layer(s)? *J. Bacteriol.* **186**: 5978–5987.
- WAKEHAM, S. G., C. LEE, J. I. HEDGES, P. J. HERNES, AND M. L. PETERSON. 1997. Molecular indicators of diagenetic status in marine organic matter. *Geochim. Cosmochim. Acta* **61**: 5363–5369.
- WELLS, M. L., AND E. D. GOLDBERG. 1991. Occurrence of small colloids in sea-water. *Nature* **353**: 342–344.
- ZIKA, R. G. 1981. Marine organic photochemistry, p. 299–322. *In* E. K. D. A. R. Dawson [ed.], *Marine organic chemistry*. Elsevier.

Received: 29 March 2007

Accepted: 3 August 2007

Amended: 20 August 2007