APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2005, p. 2979–2986 Vol. 71, No. 6
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.6.2979–2986.2005

Biomass Production and Assimilation of Dissolved Organic Matter by SAR11 Bacteria in the Northwest Atlantic Ocean

Rex R. Malmstrom, Matthew T. Cottrell, Hila Elifantz, and David L. Kirchman*

College of Marine Studies, University of Delaware, Lewes, Delaware 19958

Received 7 October 2004/Accepted 21 December 2004

Members of the SAR11 clade often dominate the composition of marine microbial communities, yet their contribution to biomass production and the flux of dissolved organic matter (DOM) is unclear. In addition, little is known about the specific components of the DOM pool utilized by SAR11 bacteria. To better understand the role of SAR11 bacteria in the flux of DOM, we examined the assimilation of leucine (a measure of biomass production), as well as free amino acids, protein, and glucose, by SAR11 bacteria in the Northwest Atlantic Ocean. We found that when SAR11 bacteria were >25% of total prokaryotes, they accounted for about 30 to 50% of leucine incorporation, suggesting that SAR11 bacteria were major contributors to bacterial biomass production and the DOM flux. Specific growth rates of SAR11 bacteria either equaled or exceeded growth rates for the total prokaryotic community. In addition, SAR11 bacteria were typically responsible for a greater portion of amino acid assimilation (34 to 61%) and glucose assimilation (45 to 57%) than of protein assimilation (≤34%). These data suggest that SAR11 bacteria do not utilize various components of the DOM pool equally and may be more important to the flux of low-molecular-weight monomers than to that of high-molecular-weight polymers.

The SAR11 clade is one of the most abundant bacterial phylogenetic groups in the ocean. About 25% of the 16S rRNA gene sequences retrieved from uncultured marine bacteria belong to the SAR11 bacteria (13), and representatives of the SAR11 clade have been found in clone libraries from around the world (28). Investigations using fluorescence in situ hybridization (FISH) confirm that SAR11 bacteria often make up 25 to 35% of the total prokaryotic community in the surface waters of both coastal and open-ocean systems (22, 24). The high abundance and global distribution of SAR11 bacteria suggest that they mediate a significant fraction of the dissolved organic matter (DOM) flux in the ocean. As with most marine bacterial groups, however, little is known about the contribution of SAR11 bacteria to the flux of DOM.

The uptake of some DOM components by SAR11 bacteria was examined previously in the Gulf of Maine and the Sargasso Sea. Malmstrom el al. (22) found that the SAR11 clade dominated the assimilation of dissolved free amino acids and dimethylsulfoniopropionate, supporting the hypothesis that SAR11 bacteria mediate a large portion of the DOM flux. However, the assimilation of high-molecular-weight (HMW) DOM, which is a major source of C for bacterial communities (2), was not examined. In the Delaware Bay, Cottrell and Kirchman (5) found differences between the assimilation of low-molecular-weight (LMW) monomers and that of HMW polymers by the alpha-proteobacteria. The SAR11 bacteria, which are an abundant subgroup of the alpha-proteobacteria (13), may also contribute differently to the fluxes of LMW and HMW components of the DOM pool. To better understand their contribution to the flux of DOM, the assimilation of LMW monomers and HMW polymers by SAR11 bacteria needs to be examined.

The contribution of SAR11 bacteria to the flux of DOM can also be estimated from their biomass production rates. By following incorporation of leucine, a measure of biomass production, into different bacterial groups, Cottrell and Kirchman (4) found that the alpha-proteobacteria dominated bacterial biomass production in the Delaware Bay. The SAR11 bacteria can often account for most alpha-proteobacteria in the Delaware Bay (19), suggesting that SAR11 bacteria may also be major contributors to biomass production and thus to the total DOM flux. However, direct estimates of SAR11 bacterial biomass production are necessary to clarify the role of SAR11 bacteria in oceanic DOM flux.

The goal of this study was to examine biomass production and the assimilation of low- and high-molecular-weight DOM by SAR11 bacteria. To accomplish this goal, we used a combination of microautoradiography and FISH (Micro-FISH) (5, 21, 27) to follow the assimilation of radiolabeled substrates by SAR11 bacteria. The contribution of the SAR11 clade to bacterial biomass production was determined by incorporation of [^{3}H]leucine (4, 18). The assimilation of [^{3}H] glucose, dissolved free amino acids, and protein was also examined because these compounds can each supply a large portion (ca. 5 to 50%) of the total C demand of microbial communities (16, 29, 34). Protein and dissolved free amino acids can also supply an equally large fraction of total bacterial N demand (15, 16). We found that the SAR11 bacteria dominated the assimilation of amino acids and glucose and contributed as much or more to biomass production than would be predicted based on their abundance.

MATERIALS AND METHODS

Sample collection and preparation. Seawater was collected at five stations in the mid-North Atlantic in July (station 5) and August (stations 1 to 4) 2003 (Table 1). Whole seawater was collected from three to six depths and immediately fixed with paraformaldehyde (2% final concentration) and preserved over-

* Corresponding author. Mailing address: College of Marine Studies, University of Delaware, Lewes, DE 19958. Phone: (302) 645-4375. Fax: (302) 645-4028. E-mail: kirchman@cms.udel.edu.

TABLE 1. Abundance and average cell volume of SAR11 bacteria in the Northwest Atlantic Ocean

Station	Latitude (N)	Longitude (W)	Depth (m)	SAR11 abundance (10^5 cells ml^{-1})[a]	% of total cells[b]	Avg cell vol (μm^3)[c]	
						SAR11	Other cells
1	35°54.9′	73°57.6′	0	3.84 ± 0.32	32 ± 3	0.039 ± 0.001	0.031 ± 0.002*
			7	3.71 ± 0.43	35 ± 3	0.045 ± 0.002	0.039 ± 0.002*
			13	3.17 ± 0.58	28 ± 3	0.033 ± 0.001	0.030 ± 0.002*
			20	1.83 ± 0.26	42 ± 6	0.047 ± 0.002	0.035 ± 0.002*
			50	0.65 ± 0.15	26 ± 4	0.037 ± 0.002	0.038 ± 0.003
			80	0.71 ± 0.28	28 ± 7	0.060 ± 0.004	0.056 ± 0.006
2	36°00.1′	72°59.5′	0	1.71 ± 0.31	26 ± 4	0.037 ± 0.002	0.034 ± 0.003
			8	1.58 ± 0.31	20 ± 4	0.044 ± 0.002	0.039 ± 0.004*
			15	1.85 ± 0.27	24 ± 3	0.040 ± 0.002	0.033 ± 0.003*
			30	1.34 ± 0.33	19 ± 3	0.043 ± 0.002	0.038 ± 0.003*
			50	1.49 ± 0.36	21 ± 4	0.038 ± 0.002	0.034 ± 0.002*
			100	0.33 ± 0.16	11 ± 5	0.060 ± 0.005	0.052 ± 0.009*
3	36°48.6′	73°36.0′	0	1.81 ± 0.37	27 ± 4	0.050 ± 0.002	0.051 ± 0.004
			9	0.74 ± 0.13	28 ± 3	0.052 ± 0.002	0.049 ± 0.004
			17	0.91 ± 0.25	29 ± 5	0.054 ± 0.003	0.050 ± 0.004
			27	1.51 ± 0.38	22 ± 3	0.057 ± 0.003	0.047 ± 0.005*
			50	1.60 ± 0.43	20 ± 4	0.044 ± 0.002	0.030 ± 0.003*
			100	0.32 ± 0.14	14 ± 4	0.046 ± 0.004	0.046 ± 0.007
4	38°00.0′	74°26.2′	0	2.50 ± 0.57	31 ± 3	0.050 ± 0.002	0.039 ± 0.003*
			5	3.29 ± 0.62	32 ± 3	0.047 ± 0.002	0.039 ± 0.003*
			9	3.87 ± 0.92	28 ± 4	0.050 ± 0.002	0.033 ± 0.002*
			15	6.70 ± 1.05	34 ± 5	0.054 ± 0.002	0.035 ± 0.003*
			35	2.89 ± 0.36	19 ± 3	0.040 ± 0.002	0.033 ± 0.003*
			45	3.26 ± 0.60	27 ± 4	0.036 ± 0.002	0.035 ± 0.003
5	38°33.3′	74°35.6′	2	4.31 ± 1.64	28 ± 3	0.038 ± 0.002	0.040 ± 0.002
			8	8.37 ± 2.27	37 ± 4	0.038 ± 0.002	0.040 ± 0.002
			20	12.20 ± 5.09	37 ± 5	0.040 ± 0.002	0.045 ± 0.002*

[a] Mean ± standard error; $n = 10$ fields of view (1,100 to 4,000 bacteria per sample).
[b] Percentage of total prokaryotes that were SAR11 bacteria; mean ± standard error.
[c] Mean ± 95% confidence interval. Statistically significant differences (*t* test, $P < 0.05$) between the size of SAR11 bacteria and that of the other prokaryotes are indicated (*).

night at 4°C. Fixed samples were filtered (20 to 100 ml) onto white polycarbonate filters (0.2 μm), rinsed with deionized water, and stored at −20°C for later analysis using FISH.

Bacterial production was measured by incorporation of [^{3}H]leucine into trichloroacetic acid-insoluble material (18). Triplicate 1.5-ml samples were incubated in the dark with 20 nmol $liter^{-1}$ [^{3}H]leucine (150 Ci $mmol^{-1}$) for 0.5 to 1 h at in situ temperatures. The trichloroacetic acid-insoluble fraction was collected by centrifugation (32), and samples were radioassayed using liquid scintillation counting. Bacterial biomass production was calculated using a conversion factor of 1.5 kg C mol^{-1} of assimilated leucine (18), and specific growth rates were calculated assuming 10 fg C $cell^{-1}$ (10).

To measure the assimilation of radiolabeled compounds, 60-ml aliquots of seawater were collected from depths corresponding to the 30% surface light level (2 to 17 m) and incubated separately with trace additions of [^{3}H]glucose (0.5 nmol $liter^{-1}$; 40 Ci $mmol^{-1}$), a mixture of 15 ^{3}H-amino acids (0.5 nmol $liter^{-1}$; 47 Ci $mmol^{-1}$), and ^{3}H-protein (20 ng/ml; 0.7 to 0.9 Ci g^{-1}). Tritiated protein was derived from *Vibrio alginolyticus* grown on [^{3}H]leucine (25), and protein of ≥10 kDa was recovered using a centrifugal filter device (Micron). Samples were incubated for 24 h at in situ temperature and 30% surface irradiance. Incubation conditions for determining [^{3}H]leucine incorporation by SAR11 bacteria were the same as those for measuring bacterial production. Paraformaldehyde was added (2% final concentration) to terminate the incubations, and samples were preserved as described for FISH samples. Killed controls were treated with paraformaldehyde 10 min prior to addition of radiolabeled substrates and incubated simultaneously with live samples. The assimilation of radioactive compounds was negligible in the killed controls.

Identification of SAR11 bacteria with FISH and Micro-FISH. SAR11 bacteria were identified by hybridization with four Cy3-labeled oligonucleotide probes designed to bind specifically to members of the SAR11 clade (24). The hybridization conditions and buffer compositions followed those described previously (22, 24). The hybridization reactions were performed overnight at 42°C with a buffer containing 30% formamide. After hybridization, filters were washed at 55°C and mounted on a glass slide with a coverslip. The mounting solution contained 4′,6′-diamidino-2-phenylindole (DAPI) (0.5 ng μl^{-1}) and a 4:1 mixture of Citifluor (Ted Pella) and Vectashield (Vector Labs). A negative control probe (5′-CCT AGT GAC GCC GTC GA-3′; 8 ng μl^{-1}) was used to monitor nonspecific binding of probes (14). The negative control probe bound to <5% of cells.

Micro-FISH preparations followed a method described in greater detail elsewhere (22). Briefly, FISH-treated filters were placed on emulsion-coated (Kodak NTB-2 emulsion) glass slides with the bacteria in contact with the emulsion. Slides were exposed in the dark at 4°C for 10 days for protein or 2 to 3 days for leucine, amino acids, and glucose (time was determined as described below). After exposure, the slides were developed with Dektol developer (Kodak) for 2 min, rinsed with deionized water for 10 seconds, fixed with Kodak fixer for 6 min, and rinsed again in deionized water. Slides were dipped in 1% glycerol for 1 minute and stored overnight in a vacuum desiccator. After drying, the FISH-treated filter was peeled away, leaving bacteria attached to the slide. Bacteria were stained with DAPI by mounting a coverslip on the samples with a mounting solution (4:1 Citifluor to Vectashield) containing DAPI (0.5 ng μl^{-1}).

The length of exposure time was determined empirically by following changes in number of substrate-assimilating cells over time. Exposure times were selected once the number of substrate-assimilating cells reached a maximum and did not increase with longer exposure times. For protein assimilation at stations 1 and 4, exposure times were selected before the autoradiography background affected counts in the killed controls. Differences in the specific activities of the substrates used in this study could be accounted for by varying the autoradiography exposure times.

Image analysis and cell size determination. FISH and Micro-FISH slides were examined with a semiautomated microscopy and image analysis system described

TABLE 2. Biomass production and specific growth rates of SAR11 bacteria and the total prokaryotic community in the Northwest Atlantic Ocean

Station	Depth (m)	% of Leu-assimilating cells[a]	% Total silver grain area[a]	Bacterial production (ng C liter^{-1} h^{-1})[b]	SAR11 production (ng C liter^{-1} h^{-1})[c]	Total growth rate (day^{-1})	SAR11 growth rate (day^{-1})[c]
1	13	34 ± 2	23 ± 2	75 ± 4	17–26	0.16	0.13–0.20
3	17	40 ± 2	36 ± 3	63 ± 3	23–25	0.39	0.61–0.66
4	9	40 ± 2	33 ± 3	171 ± 3	56–69	0.30	0.35–0.43
5	2	50 ± 1	46 ± 3	259 ± 1	118–130	0.40	0.65–0.72

[a] Mean ± 95% confidence interval.
[b] Mean ± standard deviation.
[c] Range determined from percent silver grain area (lower bound) and percent Leu-assimilating cells (upper bound).

previously (4). For FISH samples, images of DAPI fluorescence, Cy3 fluorescence, and phycoerythrin fluorescence were captured from 10 fields of view and overlaid. Objects with overlapping signals in the DAPI and Cy3 images were counted as probe positive. Autofluorescent signals from *Synechococcus* were removed from the analysis by deleting cells that appeared in both the Cy3 and phycoerythrin images. The total number of bacteria counted per sample ranged from 1,100 to 4,000.

For Micro-FISH samples, images of DAPI fluorescence, Cy3 fluorescence, and silver grain clusters were captured from 10 to 30 fields of view and overlaid. Probe-positive cells were determined as described above. Substrate-assimilating cells were identified by silver grain clusters that formed during autoradiography. The area of silver grain clusters associated with substrate-assimilating cells was recorded. The total number of bacteria counted per sample ranged from 580 to 4,000.

Cell size data were calculated from images of DAPI fluorescence by using three different algorithms described elsewhere (23, 30, 31), although only cell volumes from one algorithm (31) are reported. Cell volumes were recorded from probe-positive and probe-negative cells in FISH-treated samples, but not from samples treated with Micro-FISH. In addition, we found that the Cy3 fluorescence of probe-positive cells did not enhance DAPI fluorescence and thereby artificially increase the size of probe-positive cells. Cell volumes were logarithmically transformed for statistical analysis.

RESULTS

Abundance and size of the SAR11 bacteria. Previous studies found that SAR11 bacteria can account for 25 to 35% of prokaryotes in the surface waters of both coastal waters and the open ocean of the North Atlantic (22, 24). The abundance of SAR11 bacteria was similarly high in this study, with SAR11 bacteria comprising 27% ± 7% (mean ± standard deviation; $n = 27$) of total prokaryotic communities in surface waters (Table 1). The SAR11 clade was most abundant in the shallow coastal waters of station 5 (4.3×10^5 to 12.2×10^5 cells ml^{-1}), while the lowest numbers occurred off the continental shelf at station 3 (0.32×10^5 to 1.81×10^5 cells ml^{-1}). Total prokaryotic abundance, however, was also highest and lowest at these stations.

The average cell volumes for the SAR11 clade ranged from 0.033 to 0.060 μm^3 among all locations (Table 1). At stations 1 to 4, the average size of SAR11 bacteria was equal to, or slightly (12 to 50%) larger than, that of the other prokaryotes (Table 1). In the shallow coastal waters of station 5, members of the SAR11 clade were the same size, or slightly (12%) smaller than, the rest of the prokaryotic community. The sizes of SAR11 bacteria and the rest of the prokaryotic community varied greatly within each sample, as indicated by high coefficients of variation (51 to 80%). Although the cell volumes calculated from only one algorithm are reported (31), the statistically significant differences and similarities indicated in Table 1 were also found using volumes calculated from two additional algorithms (23, 30) at 24 of 27 depths (data not shown).

Contribution of SAR11 bacteria to bacterial production. The fraction of total bacterial production mediated by the SAR11 clade was estimated from the fraction of leucine-assimilating cells identified as SAR11 bacteria. SAR11 bacteria consistently made up a large proportion of the leucine-assimilating community at all stations (Table 2). The SAR11 clade comprised the largest fraction (50%) of the leucine-assimilating community in shallow coastal waters (station 5) and the smallest fraction (34%) in the Gulf Stream (station 1).

Biomass production by the SAR11 clade was also estimated from the fraction of total silver grain area associated with SAR11 bacteria. Silver grain area was used to estimate production in order to account for variation in per-cell activity, which was suggested by differences in the size (μm^2) of silver grain clusters associated with leucine-assimilating cells. Previous work shows that larger silver grain clusters indicate more assimilation per cell than smaller clusters (26). Between 23 and 45% of the total silver grain area was associated with the SAR11 clade (Table 2). Estimates of the contribution of SAR11 bacteria to biomass production based on silver grain area were lower (paired t test, $P < 0.05$) than estimates using the fraction of leucine-assimilating cells at stations 1 and 4, but SAR11 bacteria still accounted for a large portion of biomass production at these stations (Table 2).

Using both estimates, SAR11 bacterial production ranged from 17 to 26 ng C liter^{-1} h^{-1} in the Gulf Stream (station 1) to 118 to 130 ng C liter^{-1} h^{-1} in coastal waters (station 5) (Table 2). From these production rates, specific growth rates for the total community and SAR11 bacteria were calculated, assuming 10 fg C cell^{-1} (10). The specific growth rates of SAR11 bacteria either equaled or exceed growth rates of the entire community (Table 2). Growth rates were greatest in the coastal waters of station 5 (0.66 to 0.73 day^{-1}) and lowest at station 1 in the Gulf Stream (0.13 to 0.20 day^{-1}) (Table 2).

The fraction of the leucine-assimilating community accounted for by SAR11 bacteria exceeded the fraction of total prokaryotes identified as SAR11 bacteria (Fig. 1A). Similarly, the fraction of total silver grain area associated with SAR11 bacteria was greater than or equal to the fraction of total prokaryotes made up by SAR11 bacteria (Fig. 1B). These data suggest that SAR11 bacteria contributed as much, or more, to bacterial production than would be predicted based on their overall abundance. Since SAR11 bacteria were abundant at all stations, their contribution to total bacterial production was large in these areas of the northwest Atlantic Ocean.

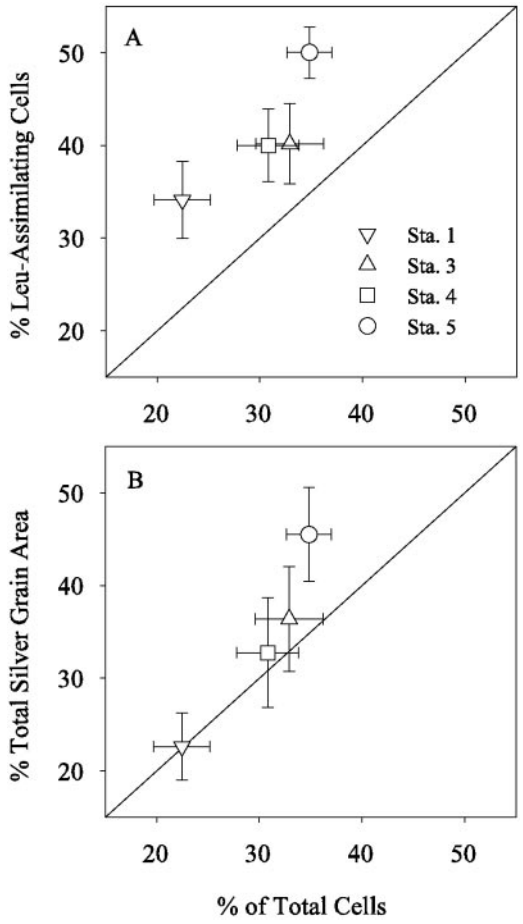

FIG. 1. Contribution of the SAR11 clade to bacterial production in the Northwest Atlantic Ocean. A) The abundance of SAR11 bacteria in the leucine-assimilating community versus the abundance of SAR11 bacteria in the total prokaryote community (DAPI-positive cells). B) The percentage of total silver grain area associated with SAR11 bacteria versus the abundance of SAR11 bacteria in the total prokaryote community. Error bars indicate 95% confidence intervals based on 580 to 4,000 bacteria per sample; 1:1 lines bisect the graphs. Sta., station.

Assimilation of DOM components. Similar to bacterial production, the contribution of SAR11 bacteria to the assimilation of glucose, amino acids, and protein was examined using the percentage of the assimilating community as well as the percentage of total silver grain area. At all stations, SAR11 bacteria made up 47 to 54% of the glucose-assimilating community (Fig. 2A). The SAR11 bacteria also accounted for 45 to 57% of total silver grain area in glucose incubations, though they represented only 25 to 34% of the total prokaryotic community (Fig. 2B). These data indicate that the SAR11 clade dominated the assimilation of glucose and contributed more to total glucose assimilation than would be predicted by their overall abundance (25 to 34%) (Fig. 2 A and B).

SAR11 bacteria also often dominated the assimilation of dissolved free amino acids. Members of the SAR11 clade comprised 34 to 49% of the amino acid-assimilating community at four locations in the Northwest Atlantic Ocean (Fig. 2C). An even greater proportion (45 to 61%) of total silver grain area was associated with SAR11 bacteria (Fig. 2D). The SAR11 bacteria, however, accounted for only 25 to 34% of the total prokaryotic community. Based on either estimate of assimilation, SAR11 bacteria contributed more to total amino acid assimilation at each station than expected from their overall abundance (paired t test, $P < 0.05$), which agrees with previous findings for the Gulf of Maine and the Sargasso Sea (22).

The SAR11 clade appeared to play a smaller role in protein assimilation than in amino acid and glucose assimilation. The SAR11 clade accounted for 13 to 28% of protein assimilation at stations 3 to 5 (Fig. 2E and F), whereas SAR11 bacteria accounted for ≥34% of amino acid and glucose assimilation at these stations (Fig. 2A to D). Within each station, the contribution of SAR11 bacteria to protein assimilation was significantly lower than their contribution to glucose assimilation, based on estimates from both silver grain area and fraction of substrate-assimilating cells (Tukey test, $P < 0.05$). The SAR11 clade also accounted for a significantly smaller portion of protein assimilation than of amino acid assimilation at stations 3 to 5, based on both estimates (Tukey test, $P < 0.05$), although there was no statistically significant difference at station 1. In contrast to amino acid and glucose assimilation, SAR11 bacteria assimilated only as much, or considerably less, protein as would be predicted by their abundance at stations 3 to 5 (Fig. 2E and F). In the Gulf Steam (station 1), however, the SAR11 bacteria were responsible for a larger fraction of protein assimilation than would be predicted based on the abundance of the SAR11 clade.

Differential utilization of DOM components. Variation in the utilization of glucose, amino acids, and protein was examined by calculating the fraction of the SAR11 community that assimilated these compounds. These values differ from those reported in Fig. 2, which gives the fraction of substrate-assimilating cells identified as SAR11 bacteria. For example, 75% of the SAR11 bacteria assimilated amino acids at station 5 (Fig. 3A), but only 49% of amino-acid-assimilating cells belonged to the SAR11 clade (Fig. 2C).

Roughly half (45 to 59%) of SAR11 bacteria assimilated glucose at all stations, but a significantly greater proportion of the SAR11 community assimilated dissolved free amino acids (75 to 81%) at all stations (Tukey test, $P < 0.05$) (Fig. 3A). In contrast to the case for free amino acid assimilation, a much smaller fraction (15 to 49%) of SAR11 bacteria utilized protein (Fig. 3A). The difference between the proportions of SAR11 bacteria assimilating amino acids and protein was greatest in the coastal waters (station 5) and smallest in the Gulf Stream (station 1), and these differences were significantly different at all stations (Tukey test, $P < 0.05$). The differences in the proportions of SAR11 bacteria assimilating glucose, amino acids, and protein indicate that SAR11 bacteria did not utilize these components of the DOM pool equally.

Differential utilization of glucose, amino acids, and protein by the total prokaryote community was also examined. As with SAR11 bacteria, a larger fraction of the total community (53 to 69%) assimilated amino acids than assimilated glucose or protein at each station (Tukey test, $P < 0.05$) (Fig. 3B). These data suggest that the prokaryotic community did not utilize glucose, amino acids, and protein equally. In addition, DOM utilization by SAR11 bacteria differed from DOM utilization by the total prokaryotic community. At each station, the percentage of SAR11 bacteria assimilating amino acids and glucose was

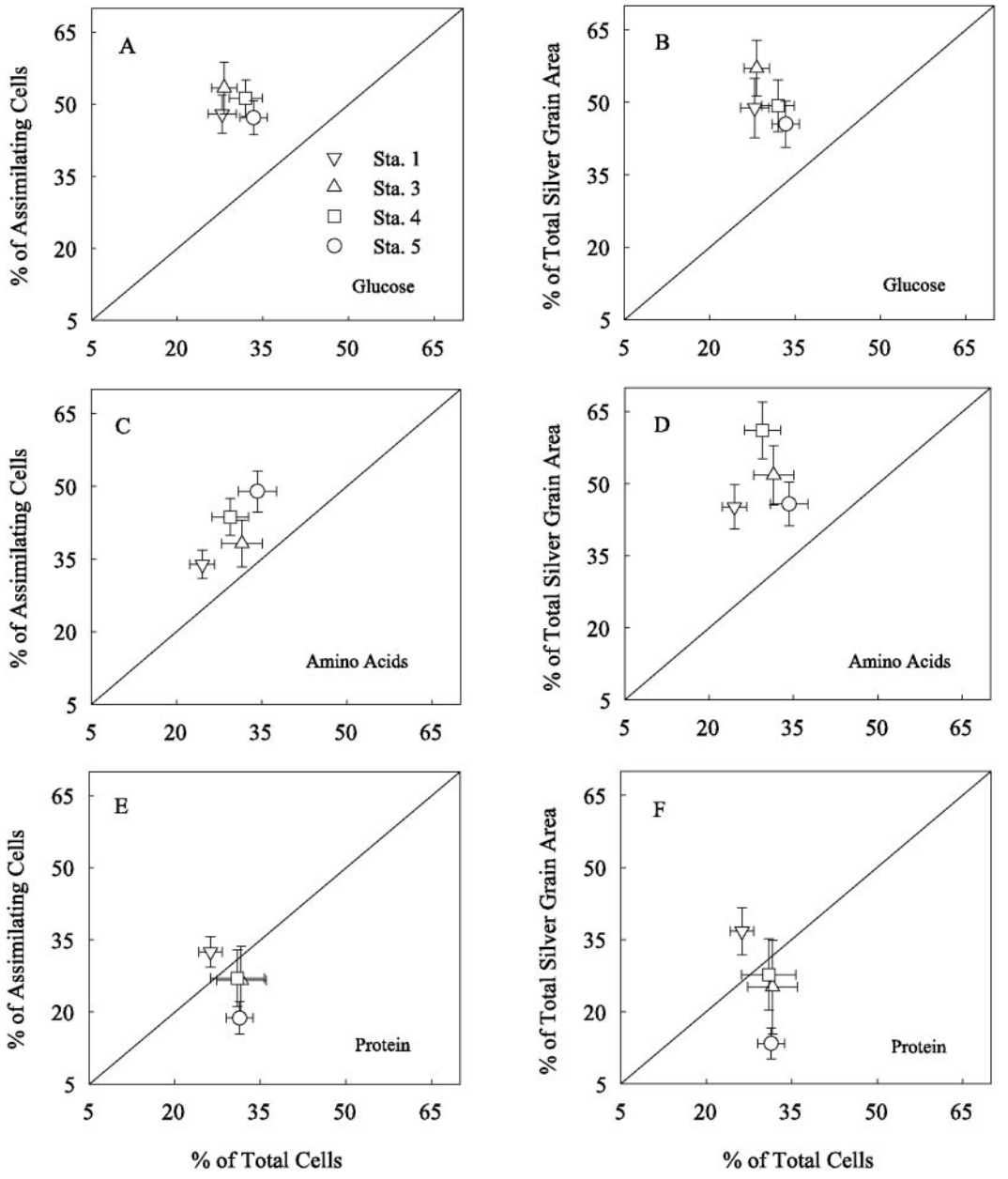

FIG. 2. Contribution of SAR11 bacteria to glucose, amino acid, and protein assimilation in the Northwest Atlantic Ocean. The abundance of SAR11 bacteria in the glucose-, amino acid-, and protein-assimilating community versus the abundance of SAR11 bacteria in the total prokaryote community (A, C, E). The percentage of total silver grain area associated with SAR11 bacteria and the abundance of SAR11 bacteria in the total prokaryote community (B, D, F). Error bars represent 95% confidence intervals based on 580 to 4,000 bacteria per sample; 1:1 lines bisect the graphs. Sta., station.

greater than the percentage of the total community assimilating these compounds (paired t test, $P < 0.05$), but there were no significant differences between the assimilation of protein by SAR11 bacteria and by the total prokaryotic community at three of four stations.

DISCUSSION

The SAR11 bacteria may be one of the most abundant groups of prokaryotes in the ocean, yet little is known about their contribution to marine biogeochemical processes. Estimates of the abundance, cell size, growth rates, biomass production, and utilization of different components of the DOM pool are necessary in order to better understand the importance of SAR11 bacteria to the flux of DOM in the ocean and to help explain the abundance of these bacteria. We found that SAR11 bacteria had high growth rates and were major contributors to both biomass production and the turnover of HMW and LMW DOM in the Northwest Atlantic Ocean. These data suggest that SAR11 bacteria mediate a substantial fraction of the total DOM flux when abundant but that their contribution to the flux of glucose, amino acids, and protein varies.

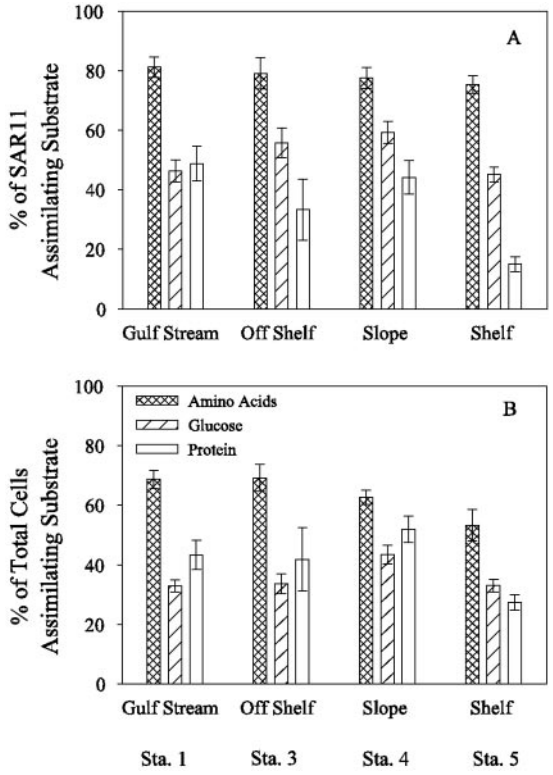

FIG. 3. Percentage of SAR11 bacteria (A) and total prokaryotes (B) assimilating amino acids, glucose, and protein at four stations in the Northwest Atlantic Ocean. Error bars represent 95% confidence intervals. Sta., station.

The abundance of SAR11 bacteria, and their consumption of DOM, is influenced by their growth rate. Specific growth rates for the SAR11 clade ranged from about 0.15 day^{-1} in the oligotrophic waters of the Gulf Stream to about 0.70 day^{-1} in Delaware coastal waters. No other estimates of in situ growth rates of SAR11 bacteria are available, although the growth of alpha-proteobacteria, of which the SAR11 bacteria are an abundant subgroup, was examined previously using dilution techniques (9, 35). Growth rates of SAR11 bacteria in Delaware coastal waters were similar to growth rates of alpha-proteobacteria in German coastal waters (1.0 day^{-1}) (9) but were typically lower than growth rates of alpha-proteobacteria in the Delaware Bay (0 to 5.5 day^{-1}) (35). Except for in the oligotrophic waters of the Gulf Stream, we found that in situ growth rates of natural SAR11 populations were similar to growth rates of an isolated representative of the SAR11 clade, *Pelagibacter ubique*, in pure culture (0.40 to 0.58 day^{-1}) (28). The specific growth rates of SAR11 bacteria either equaled or exceeded growth rates of the total prokaryotic communities in this study and were near the higher estimates of total community growth rates reported for other marine environments (0.05 to 0.30 day^{-1}) (8). Based on the high growth rates of SAR11 bacteria relative to the total community, the high abundances of SAR11 bacteria are likely the result of fast growth rather than low mortality.

The success of SAR11 bacteria, as indicated by high biomass and growth rates, is probably due to their capacity to outcompete other prokaryotes for DOM, if growth is limited by C availability (20). The SAR11 bacteria accounted for either as much or more of total biomass production and of the DOM flux, assuming their growth efficiency was similar to that of the total community, than would be predicted from their abundance. These data support the hypothesis that SAR11 bacteria out-competed other bacterial groups for C. In addition, SAR11 bacteria were more important to the turnover of amino acids and glucose than their abundance would suggest, whereas SAR11 bacteria typically contributed only as much as, or less, to protein turnover as expected. The capacity to out-compete other bacterial groups for C, especially amino acids and glucose, helps explain the high abundance and growth rates of SAR11 bacteria in the ocean.

The differences in their contributions to amino acid, glucose, and protein turnover may indicate that SAR11 bacteria are more important to the biogeochemical flux of LMW monomers than to that of HMW polymers. This hypothesis is consistent with previous observations in the Delaware Bay, where the alpha-proteobacteria, which can be primarily SAR11 bacteria (19), dominated the turnover of amino acids and *N*-acetylglucosamine but contributed less to the turnover of protein and chitin (5). In addition, SAR11 bacteria often dominate free-living microbial communities, yet in particle-attached communities, where rates of HMW organic matter hydrolysis are high (33), SAR11 bacteria appear to be rare (6, 7). Potential differences in the contributions of SAR11 bacteria to LMW and HMW DOM turnover have important consequences for examining the impact of bacterial community composition on DOM composition and flux.

We found that a large number of SAR11 bacteria took up amino acids, protein, and glucose (Fig. 3A). Since these compounds can supply over half of the total C and N needs of bacterial communities (16, 29, 34), the consumption of amino acids, protein, and glucose probably supplies a large fraction of C and N needs of SAR11 bacteria. It is also appears that amino acids provided C to more SAR11 bacteria than did glucose or protein (Fig. 3A). It seems unlikely that the numbers of SAR11 bacteria assimilating glucose and protein were underestimated, because the autoradiography exposures were extended until the number of substrate-assimilating cells reached a maximum or until the exposures could be run no further (stations 1 and 4, protein). The predominance of amino acids as a C source for SAR11 bacteria is consistent with previous observations in the Gulf of Maine and the Sargasso Sea (22) and suggests that dissolved free amino acids are particularly important to the growth and metabolism of SAR11 bacteria. It also appears that SAR11 bacteria utilized DOM differently than the rest of the prokaryotic community, with a larger fraction of the SAR11 bacteria obtaining C and N from amino acids and glucose than did the overall prokaryotic community.

The differential utilization of DOM components by SAR11 bacteria may result from a variety of biogeochemical, physiological, and genetic factors. First, the flux of amino acids into the environment may be greater than the flux of glucose or protein (15, 17). If the flux is greater, then SAR11 bacteria may focus on the amino acid pool because it is a more abundant source of C. Second, it may be more energetically efficient for SAR11 bacteria to utilize amino acids than the other compounds. Amino acids may be a more attractive DOM source

than glucose because amino acids are both C and N sources and can be used directly for biosynthesis as well as metabolized for energy (34). Protein can also provide C and N, but bacteria must expend energy to synthesize and transport proteases before protein can be assimilated. Third, the SAR11 bacteria may have equal capacities to utilize glucose, amino acids, and proteins but vary the uptake of these different compounds depending on ecological conditions. Finally, the genes necessary for amino acid assimilation could be more common in the SAR11 clade than the genes necessary to assimilate glucose or protein. Genomic analysis of the isolated SAR11 bacterium *Pelagibacter ubique* will provide valuable insight into the capacity of SAR11 bacteria to hydrolyze proteins, as well as to assimilate amino acids and glucose, and could provide clues to the specific mechanisms behind the differential DOM utilization by the SAR11 clade.

The SAR11 clade is a phylogenetically, and possibly metabolically, diverse group of bacteria (13). A recent study of Massachusetts coastal waters found that 16S rRNA sequence richness was higher in the SAR11 clade than in other phylogenetic groups, but all SAR11 sequences could be assigned to four microdiverse clusters by grouping sequences at a 99% similarity level (1). It has been hypothesized that these clusters may represent groups of related organisms that share similar genes and ecological functions (3, 12). This hypothesis implies that there should be ecologically distinct subgroups within the SAR11 clade. In this study, the differential utilization of the DOM components suggests that there is some functional diversity within the SAR11 clade. For example, 75% of SAR11 bacteria assimilated amino acids at station 5, whereas 45% and 15% assimilated glucose and protein, respectively (Fig. 3A). Since the percentages add up to >100%, some fraction of the SAR11 community apparently utilized two or three of the DOM components simultaneously, whereas another fraction utilized only amino acids. It appears that within the same environment some SAR11 bacteria utilized different C sources than other SAR11 bacteria and that some SAR11 bacteria made different contributions to the flux of DOM components. These results are consistent with the hypothesis that the SAR11 clade is comprised of ecologically distinct subgroups, but it is unclear if the functional diversity we observed is related to coherent phylogenetic clusters. Further investigations of the microdiverse phylogenetic clusters within the SAR11 clade are necessary to determine the possible ecological differences among subgroups.

Our current understanding of the role of different bacterial phylogenetic groups in the flux of DOM is incomplete, although some patterns may be emerging with the SAR11 clade. The SAR11 clade often dominated the flux of free amino acids in this study and previously (22), and it appear to out-compete other bacteria for this labile component of the DOM pool. Assuming that the diversity of SAR11 bacteria in our samples is similar to that previously observed (1, 11), it appears that the capacity to assimilate and possibly to out-compete other bacteria for dissolved free amino acids is widespread within the SAR11 clade. The SAR11 bacteria can also assimilate glucose and protein, but it is unclear if these capacities are as common among the members of the SAR11 clade as is the capacity to utilize amino acids. To understand differences in the utilization of these compounds, as well as other components of the DOM pool, a variety of approaches will be necessary. Through a combination of isolation techniques, genomic analysis, and environmental observations, the role of SAR11 bacteria in oceanic biogeochemistry, and the adaptations responsible for their success, will be revealed.

ACKNOWLEDGMENTS

This study was supported by grants from the DOE-BIOMP (DF-FG02-97 ER 62479) and Delaware Sea Grant (R/M-42). An NSF Graduate Research Fellowship provided support for R.R.M.

REFERENCES

1. **Acinas, S. G., V. Klepac-Ceraj, D. E. Hunt, C. Pharino, I. Ceraj, D. L. Distel, and M. F. Polz.** 2004. Fine-scale phylogenetic architecture of a complex bacterial community. Nature **430:**551–554.
2. **Amon, R. M. W., and R. Benner.** 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnol. Oceanogr. **41:**41–51.
3. **Cohan, F. M.** 2002. What are bacterial species? Annu. Rev. Microbiol. **56:**457–487.
4. **Cottrell, M. T., and D. L. Kirchman.** 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnol. Oceanogr. **48:**168–178.
5. **Cottrell, M. T., and D. L. Kirchman.** 2000. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. **66:**1692–1697.
6. **Crump, B. C., E. V. Armbrust, and J. A. Baross.** 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean. Appl. Environ. Microbiol. **65:**3192–3204.
7. **Delong, E. F., D. G. Franks, and A. L. Alldredge.** 1993. Phylogenetic diversity of aggregate-attached vs free-living marine bacterial assemblages. Limnol. Oceanogr. **38:**924–934.
8. **Ducklow, H. W.** 2000. Bacterial production and biomass in the oceans, p. 85–120. *In* D. L. Kirchman (ed.), Microbial ecology of the oceans. Wiley-Liss, Inc., New York, N.Y.
9. **Eilers, H., J. Pernthaler, and R. Amann.** 2000. Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. Appl. Environ. Microbiol. **66:**4634–4640.
10. **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.
11. **García-Martínez, J., and F. Rodríguez-Valera.** 2000. Microdiversity of uncultured marine prokaryotes: the SAR11 cluster and the marine archaea of group I. Mol. Ecol. **9:**935–948.
12. **Giovannoni, S.** 2004. Oceans of bacteria. Nature **430:**515–516.
13. **Giovannoni, S. J., and M. S. Rappé.** 2000. Evolution, diversity, and molecular ecology of marine prokaryotes, p. 47–84. *In* D. L. Kirchman (ed.), Microbial ecology of the oceans. Wiley-Liss, Inc., New York, N.Y.
14. **Karner, M., and J. A. Fuhrman.** 1997. Determination of active marine bacterioplankton: a comparison of universal 16S rRNA probes, autoradiography, and nucleoid staining. Appl. Environ. Microbiol. **63:**1208–1213.
15. **Keil, R. G., and D. L. Kirchman.** 1991. Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. Mar. Ecol. Prog. Ser. **73:**1–10.
16. **Keil, R. G., and D. L. Kirchman.** 1999. Utilization of dissolved protein and amino acids in the northern Sargasso Sea. Aquat. Microb. Ecol. **18:**293–300.
17. **Kirchman, D. L.** 2003. The contribution of monomers and other low-molecular weight compounds to the flux of dissolved organic material in aquatic ecosystems, p. 218–237. *In* S. E. Findlay and R. L. Sinsabaugh (ed.), Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, San Diego, Calif.
18. **Kirchman, D. L.** 2001. Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. Methods Microbiol. **30:**227–237.
19. **Kirchman, D. L., A. I. Dittel, R. R. Malmstrom, and M. T. Cottrell.** Biogeography of major bacterial groups in the Delaware estuary. Limnol. Oceanogr., in press.
20. **Kirchman, D. L., and J. H. Rich.** 1997. Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific Ocean. Microb. Ecol. **33:**11–20.
21. **Lee, N., P. H. Nielsen, K. H. Andreasen, S. Juretschko, J. L. Nielsen, K. H. Schleifer, and M. Wagner.** 1999. Combination of fluorescent in situ hybridization and microautoradiography: a new tool for structure-function analyses in microbial ecology. Appl. Environ. Microbiol. **65:**1289–1297.
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. Appl. Environ. Microbiol. **70:**4129–4135.

23. **Massana, R., J. M. Gasol, P. K. Björnsen, N. Blackburn, Å. Hagström, H. Susanna, B. H. Hygum, J. Kuparinen, and C. Pedrós-Alió.** 1997. Measurement of bacterial size via image analysis of epifluorescence preparations: description of an inexpensive system and solutions to some of the most common problems. Sci. Mar. **61:**397–407.
24. **Morris, R. M., M. S. Rappé, S. A. Connon, K. L. Vergin, W. A. Siebold, C. A. Carlson, and S. J. Giovannoni.** 2002. SAR11 clade dominates ocean surface bacterioplankton communities. Nature **420:**806–810.
25. **Nagata, T., R. Fukuda, I. Koike, K. Kogure, and D. L. Kirchman.** 1998. Degradation by bacteria of membrane and soluble protein in seawater. Aquat. Microb. Ecol. **14:**29–37.
26. **Nielsen, J. L., D. Christensen, M. Kloppenborg, and P. H. Nielsen.** 2003. Quantification of cell-specific substrate uptake by probe-defined bacteria under in situ conditions by microautoradiography and fluorescence in situ hybridization. Environ. Microbiol. **5:**202–211.
27. **Ouverney, C. C., and J. A. Fuhrman.** 1999. Combined microautoradiography-16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types in situ. Appl. Environ. Microbiol. **65:**1746–1752.
28. **Rappé, M. S., S. A. Connon, K. L. Vergin, and S. J. Giovannoni.** 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature **418:**630–633.
29. **Rich, J. H., H. W. Ducklow, and D. L. Kirchman.** 1996. Concentrations and uptake of neutral monosaccharides along 140 degrees W in the equatorial Pacific: contribution of glucose to heterotrophic bacterial activity and the DOM flux. Limnol. Oceanogr. **41:**595–604.
30. **Sieracki, M. E., P. W. Johnson, and J. M. Sieburth.** 1985. Detection, enumeration, and sizing of planktonic bacteria by image-analyzed epifluorescence microscopy. Appl. Environ. Microbiol. **49:**799–810.
31. **Sieracki, M. E., C. L. Viles, and K. L. Webb.** 1989. Algorithm to estimate cell biovolume using image analyzed microscopy. Cytometry **10:**551–557.
32. **Smith, D. C., and F. Azam.** 1992. A simple, economical method for measuring bacterial protein synthesis in seawater using 3H-leucine. Mar. Microb. Food Webs **6:**107–114.
33. **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.
34. **Suttle, C. A., A. M. Chan, and J. A. Fuhrman.** 1991. Dissolved free amino acids in the Sargasso Sea—uptake and respiration rates, turnover times, and concentrations. Mar. Ecol. Prog. Ser. **70:**189–199.
35. **Yokokawa, T., T. Nagata, M. T. Cottrell, and D. L. Kirchman.** 2004. Growth rate of the major phylogenetic bacterial groups in the Delaware estuary. Limnol. Oceanogr. **49:**1620–1629.