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Bacterial Community Structure Associated with a Dimethylsulfoniopropionate-Producing North Atlantic Algal Bloom

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The bacteria associated with oceanic algal blooms are acknowledged to play important roles in carbon, nitrogen, and sulfur cycling, yet little information is available on their identities or phylogenetic affiliations. Three culture-independent methods were used to characterize bacteria from a dimethylsulfoniopropionate (DMSP)-producing algal bloom in the North Atlantic. Group-specific 16S rRNA-targeted oligonucleotides, 16S ribosomal DNA (rDNA) clone libraries, and terminal restriction fragment length polymorphism analysis all indicated that the marine *Roseobacter* lineage was numerically important in the heterotrophic bacterial community, averaging >20% of the 16S rDNA sampled. Two other groups of heterotrophic bacteria, the SAR86 and SAR11 clades, were also shown by the three 16S rRNA-based methods to be abundant in the bloom community. In surface waters, the *Roseobacter*, SAR86, and SAR11 lineages together accounted for over 50% of the bacterial rDNA and showed little spatial variability in abundance despite variations in the dominant algal species. Depth profiles indicated that *Roseobacter* phylotype abundance decreased with depth and was positively correlated with chlorophyll *a*, DMSP, and total organic sulfur (dimethyl sulfide plus DMSP plus dimethyl sulfoxide) concentrations. Based on these data and previous physiological studies of cultured *Roseobacter* strains, we hypothesize that this lineage plays a role in cycling organic sulfur compounds produced within the bloom. Three other abundant bacterial phylotypes (representing a cyanobacterium and two members of the α *Proteobacteria*) were primarily associated with chlorophyll-rich surface waters of the bloom (0 to 50 m), while two others (representing *Cytophagales* and δ *Proteobacteria*) were primarily found in deeper waters (200 to 500 m).

The bacterial communities associated with oceanic algal blooms play critical roles in carbon and nitrogen cycling through their influence on the formation and fate of dissolved organic matter (4, 7), nutrient availability (24), sinking flux (45), and many other processes. In blooms dominated by algal species that produce dimethylsulfoniopropionate (DSMP), bloom-associated bacteria also play an important role in organic-sulfur cycling. Degradation of DMSP by marine bacteria is one of the primary routes for the formation of dimethyl sulfide (DMS), a volatile sulfur compound that influences global climate through effects on backscatter and cloud formation (6). Recent studies have suggested that marine bacteria may control DMS formation through the expression of a competing pathway that routes the sulfur in DMSP through methanethiol (MeSH) rather than to DMS (21, 27, 46).

New evidence is pointing to one particular lineage of marine bacteria as a key participant in DMSP biogeochemistry in the ocean. Both culture-independent (i.e., 16S rRNA-based) and culture-dependent studies indicate that members of the α *Proteobacteria* belonging to the *Roseobacter* lineage are abundant in coastal and open-ocean environments (15, 17, 18), where they are often found in association with marine algae (2, 3, 25, 35, 38, 47). In contrast to other dominant marine bacterial clades which have no close relatives in culture (15), members of the *Roseobacter* group are readily cultured and have yielded important information about the sulfur physiology of this lineage (18, 23). Laboratory studies of *Roseobacter* isolates show a widespread ability to degrade DMSP and to mediate various other transformations of organic and inorganic sulfur compounds (18, 23, 28). *Roseobacter* isolates express both the DMS-producing pathway and the MeSH-producing pathway during DMSP degradation (18), although the regulation of these two competing pathways is not yet understood. The *Roseobacter* group also harbors the only known cultured bacteria that are able to incorporate DMSP sulfur into cellular proteins (via MeSH), an important fate of reduced sulfur in DMSP that may be regulated by bacterial sulfur demand (22, 23, 40).

Relatively little is known of the identities of the other bacterial groups that may be active in DMSP-producing algal blooms. Recently, Kerkhof et al. (20) identified bacterial 16S rRNA sequences unique to a coastal bloom, including members of the *Roseobacter* group and the γ and ε subdivisions of *Proteobacteria*, although it is not clear whether DMSP was produced during this bloom. Riemann et al. (38) report that heterotrophic bacteria associated with induced diatom blooms (which typically do not produce DMSP) were dominated by *Roseobacter* and *Cytophagales* 16S rRNA gene sequences.

We report here a comprehensive inventory of the dominant heterotrophic bacterioplankton associated with a spatially complex DMSP-producing algal bloom in the North Atlantic. The bloom consisted of a cold core of an eddy dominated by the coccolithophore *Emiliania huxleyi* and surrounding waters characterized by a mixed phytoplankton assemblage dominated by dinoflagellates and small flagellates. Concentrations

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of dissolved plus particulate DMSP were high (30 to 200 nM) throughout the bloom region, and total DMSP:chlorophyll *a* ratios (27 to 107 nmol μg^{-1}) were similar inside and outside the eddy, despite the differences in algal-species composition. Calculations based on short-term variability in DMSP and DMS concentrations and fluxes indicated that heterotrophic bacteria played a major role in determining the fate of DMSP in this bloom (41, 42).

The purpose of this bacterial inventory was twofold: (i) to describe the heterotrophic bacterial community across horizontal and vertical gradients in algal-species composition, chlorophyll *a* concentration, and DMSP dynamics and (ii) to address the emerging hypothesis that bacteria belonging to the *Roseobacter* lineage are key ecological players in DMSP-rich marine environments. We took a methodologically comprehensive approach in this study, using 16S ribosomal DNA (rDNA) clone libraries, group-specific oligonucleotide probe hybridizations, and terminal restriction fragment length polymorphism (T-RFLP) fingerprinting to obtain a robust inventory of the dominant heterotrophic bacteria associated with this DMSP-producing algal bloom.

MATERIALS AND METHODS

Algal-bloom description. Sample collection took place during the Atmospheric Chemistry Studies in the Oceanic Environment North Atlantic experiment onboard the RSS Discovery. Sampling was carried out in June 1998 in the vicinity of an anticyclonic eddy at approximately 59°N, 21°W (400 km south of Iceland). Satellite imagery showed that the eddy core was characterized by lower chlorophyll *a* than the edge and surrounding waters but much higher reflectance at 555 nm, indicating the presence of a bloom of coccolithophorid algae. Microscopic analyses confirmed that *Emiliania huxleyi* constituted 40 to 50% of the total phytoplankton biomass in surface waters inside the eddy (R. Davidson, personal communication). The remaining algal biomass was attributable to picophytoplankters (including cyanobacteria), small flagellates, and dinoflagellates of the genera *Gymnodinium* and *Ceratium*. Outside the eddy core, picoalgae and cyanobacteria, small flagellates, and dinoflagellates (primarily *Gymnodinium*) dominated the phytoplankton assemblage, with a significant contribution from the diatom *Chaetoceros atlanticus*. The surface chlorophyll *a* concentrations ranged from 0.5 to 0.9 $\mu g\ liter^{-1}$ in waters inside the eddy, of which 20 to 25% was associated with cells passing a 2-μm-pore-size filter. Surface chlorophyll *a* concentrations were 1 to 2 $\mu g\ liter^{-1}$ in waters outside the eddy, of which 25 to 30% was associated with <2-μm cells. At all stations, fluorescence-inferred chlorophyll *a* was relatively evenly distributed throughout the seasonal mixed layer (0 to 40 m), and the depth of the euphotic layer averaged 30 m.

Sampling. Water was collected in Niskin bottles attached to a CTD recording continuous depth profiles of temperature, salinity, and fluorescence. A total of 42 water samples were collected inside and outside the eddy for DNA extraction, including 17 surface samples, 5 deep samples (500 m), and 5 depth profiles (0 to 200 m). To collect microbial biomass in the 2- to 0.2-μm size range, approximately 20 liters of seawater was filtered with a peristaltic pump through a 2-μm-pore-size Nuclepore filter and a 0.2-μm-pore-size Sterivex filter (Durapore; Millipore) in succession. After filtration, the Sterivex unit was filled with 1.8 ml of lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose) and stored at −70°C until nucleic acid extraction was done.

Chemical analyses. A fluorometric method was used to measure chlorophyll *a* in 90% acetone extracts of ground GF/F filters (33). Whole-water samples were filtered to determine total chlorophyll *a*, while water samples previously passed through a 2-μm-pore-size Nuclepore filter were refiltered through GF/F-filters to measure chlorophyll *a* in particles <2 μm in diameter.

Concentrations of DMSP, DMS, and dimethyl sulfoxide (DMSO) were determined for the surface water samples (12 out of 42 samples) following reaction, purge, cryotrapping, and sulfur-specific gas chromatography procedures described by Simó et al. (43). Dissolved compounds were measured in GF/F-filtered seawater, and the filters were treated for determination of particulate DMSP and DMSO.

DNA extraction. A lysozyme solution (1 mg ml^{-1} [final concentration]) was added to the Sterivex filters and incubated at 37°C for 45 min. Proteinase K (0.2 mg ml^{-1} [final concentration]) and sodium dodecyl sulfate (1% [final concentration]) were added, and the filters were incubated at 55°C for 1 h. The lysate was extracted twice with equal amounts of phenol-chloroform-isoamyl alcohol (25:24:1; pH 8) and once with chloroform-isoamyl alcohol (24:1; pH 8). The aqueous phase was centrifuged in a microconcentrator (Centricon-100; Millipore), washed with sterile water several times, and reduced to a volume of 100 to 200 μl. The recovered DNA was quantified by a Hoechst dye fluorescence assay. Nucleic acid extracts were stored at −70°C.

Quantitative oligonucleotide hybridizations. Quantitative dot blot hybridizations were carried out to estimate the abundance of the *Roseobacter* bacterial lineage in the region of the algal bloom (all stations and depths; 42 samples). Community DNA from each station was hybridized with a ^{32}P-labeled oligonucleotide probe as previously described (17). The *Roseobacter* group-specific probe (MALF-1) targets positions 488 to 507 (*Escherichia coli* numbering) of the 16S rRNA gene (17). Based on information from the 16S rRNA clone libraries (see below) that two other phylogenetic groups were abundant in the algal-bloom region, quantitative hybridizations with group-specific probes were also carried out for the SAR86 and SAR11 groups, although for a limited number of samples (11 and 9 samples, respectively). The SAR86 group-specific probe (SAR86F; 5′-TCT TCG GAT ATG AGT AG) targets positions 83 to 100 (*E. coli* numbering) and was designed based on the clone sequences obtained in this study and those available in GenBank. The SAR11 group-specific probe (SAR11F; 5′-AAT GAC TGT ACC CGA ATA A) targets positions 477 to 495 and was similarly based on all available sequences. Since culturable members of these groups have not been isolated, a standard curve was generated with various amounts of plasmid DNA from one of the clones (from 10 ng to 0.1 pg). Negative controls consisted of DNA from clones outside the groups. The signals of the group-specific probes were normalized to the signal of universal probe 1406R (26) based on quantification with a laser densitometer (Molecular Dynamics, Sunnyvale, Calif.). The hybridization conditions were as previously described (17).

rDNA clone libraries. 16S rRNA genes were amplified from algal-bloom DNA from three water samples, one collected in surface water outside the eddy (sample 1), one collected from surface water inside the eddy (sample 11), and one collected from a depth of 500 m outside the eddy (sample 60). General bacterial primers 27F and 1522R (14) were used in the amplification. The PCR mixtures contained (in a final volume of 100 μl) 20 ng of community DNA, 10 mM Tris (pH 8.3), 50 mM KCl, 0.2 μM each primer, 50 μM each deoxynucleoside triphosphate, 1.25 mM $MgCl_2$, and 2.5 U of Amplitaq Gold DNA polymerase (Perkin-Elmer [PE] Corporation, Foster City, Calif.). The mixture was preincubated for 9 min at 95°C to activate the polymerase, and then temperature cycles were as follows: 1 min at 95°C, 1 min at 55°C, and 2 min at 72°C for 30 cycles. Following the final cycle, the reaction was extended for 10 min at 60°C. The PCR product was subjected to electrophoresis in a 1% agarose gel, and the band corresponding to the correctly sized product (approximately 1,500 bp) was recovered from the gel as described by Zhen and Swank (49). A minimum of 30 PCR cycles were required to obtain a visible product in the agarose gels. Clone libraries were constructed using a TA cloning kit (Invitrogen Corporation, Carlsbad, Calif.). One hundred clones were obtained from the PCR products for each of the three samples. Twenty random clones were checked for the presence of a 1,500-bp insert by digestion with *Eco*RI followed by electrophoresis in 3% agarose gels.

The clones were screened for phylogenetic affiliation with the *Roseobacter*, SAR11, and SAR86 lineages by colony hybridizations as described by González and Moran (17) using the MALF-1, SAR86F, and SAR11F probes. Because of potential mismatches between some *Roseobacter* group members and the MALF-1 probe, clones known to belong to the *Roseobacter* group (based on partial 16S rRNA gene sequences) but having varying complementarity to the probe (zero, one, or four mismatches) were used in quantitative dot blot hybridizations. For each clone, 100 ng of DNA was spotted on the hybridization membrane along with a standard curve made with DNA from a clone with no mismatch to the MALF-1 probe (clone NAC11-2). A clone not affiliated with the *Roseobacter* group (NAC1-17) served as the negative control. The hybridization conditions and quantification of the signal were as referenced above.

Sequencing 16S rDNA clones. A total of 20 clones were sequenced from each of the three clone libraries using the primer 27F to obtain approximately 500 bp of sequence information. All clones from the original 300 that were positive for the MALF-1 probe were sequenced (6 from sample 1, 8 from sample 11, and 2 from sample 60). The remainder necessary to complete 20 for each sample were chosen at random. Sequences were obtained by capillary electrophoresis on an ABI PRISM 310 genetic analyzer using the BigDye terminator cycle-sequencing kit (PE Corporation). The clones NAC1-2, NAC1-3, NAC1-5, NAC1-6, NAC1-19, NAC11-3, NAC11-6, NAC11-7, NAC11-16, NAC11-19, NAC60-3, and NAC60-12 were completely sequenced (~1,500 bp). Chimeras were detected by generating phylogenetic trees with different regions of the gene. Sequences were aligned using the Genetics Computer Group Inc. package (program manual for the Wisconsin package version 10.0, 1999). Phylogenetic trees were inferred, and bootstrap analysis (100 replicates) was performed with the PHYLIP package (10) using evolutionary distances (Jukes-Cantor distances) and the neighbor-joining method. Only alignment positions for which >50% of the sequences shared the most common base and positions without gaps were considered. The clone designation provides information on the sample from which it originated: clones with the prefixes NAC1 and NAC11 originated in the two surface samples, and clones with the prefix NAC60 originated in the 500-m sample.

T-RFLP analysis. The PCR conditions for T-RFLP analysis were the same as for cloning, except that the concentration of the forward primer (0.2 μM) was reduced to 0.02 μM and 0.18 μM of 8F-FAM (PE Corporation) was added. The primer 8F differs at position 12 (5′-AGA GTT TGA TCC TGG CTC AG [29]) from the primer 27F (5′-AGA GTT TGA TCM TGG CTC AG, where M is A or C). For amplification of algal-bloom DNA, the DNA was further purified with a Sephadex G-75 column (31) and 20 ng of DNA was used in the amplifications.

For amplification of clone and isolate DNA, no further purification step was used and 50 ng of DNA was used in the amplifications. Following amplification, the PCR product was purified with a Wizard PCR DNA Prep purification system column (Promega) and 30 ng of PCR product was digested for 3 h with 10 U of one of the following restriction enzymes with 4-bp recognition sites: *Alu*I, *Hae*III, *Hha*I (Boehringer Mannheim), or *Sau*3AI (Promega). Preliminary experiments with various digestion times (up to 12 h) demonstrated that 3 h was sufficient for complete digestion of the PCR products. A 4-μl aliquot of the 10-μl digest was vacuum dried and resuspended in 12 μl of deionized formamide and 1 μl of the DNA fragment length standard Genescan-2500 (TAMRA; PE Corporation). The length of the terminal restriction fragment was determined on an ABI PRISM 310 genetic analyzer in Genescan mode. Replicate analyses of a single sample on four different days (including separate PCR amplifications and digestions) produced peak areas with an average coefficient of variation of 13%, although the smallest peaks (i.e., those composing less than 4% of the chromatogram area) had coefficients of variation as high as 70%.

Prediction of terminal restriction fragment lengths. A Visual Basic program for Microsoft Word 97 was written to predict the lengths of the T-RFLP fragments for the clone sequences obtained in this study and for 16S rRNA sequences available from the Ribosomal Database Project (RDP) and GenBank. Aligned 16S rRNA sequences from the RDP database were downloaded from the RDP web site (7,008 bacterial sequences; release 7.1 [30]). For sequences that were not in the alignment format of the RDP, alignment was based on that of the most closely related sequence available using the RDP Sequence Aligner program. The aligned sequences were then analyzed with the T-RFLP program, which calculates the length of the terminal restriction fragment from the beginning of the 8F primer to the first restriction site of the enzyme used for digestion (*Alu*I, *Hae*III, *Hha*I, or *Sau*3AI). For sequences that were not complete for the region of the 8F primer (including 3,782 of the 7,008 aligned RDP bacterial sequences), the number of nucleotides in the gap was estimated based on the sequence with the highest percent similarity that was complete for this region.

To empirically evaluate the T-RFLP program, 10 isolates belonging to the *Roseobacter* group and for which 16S rRNA sequence data were available were subjected to T-RFLP analysis, and the resulting fragment lengths were compared to those predicted by the program. In cases where a major peak in a T-RFLP pattern from an algal-bloom sample matched the predicted fragment size of an algal-bloom clone, the fragment size was checked empirically by direct T-RFLP analysis. 16S rDNAs from clones NAC1-1, NAC11-16, NAC60-12, NAC60-3, NAC60-7, NAC11-6, NAC1-20, NAC1-6, NAC1-21, and NAC1-33 were amplified as described above except that a different fluorescent label (TET; PE Corporation) was used on the forward primer. A 4-μl volume of the FAM-labeled community DNA digest was coinjected with 0.5 μl of each TET-labeled clone digest to confirm identification. The variation in size for terminal restriction fragments from the algal-bloom community and coinjected clone digests was <0.1 nucleotide for all fragments assigned an identity.

Nucleotide sequence accession numbers. The sequences determined in this study were given GenBank accession no. AF245614 to AF245657.

RESULTS

Phylogenetic screening of 16S rDNA clones. The 300 clones (100 per library) were screened initially with a group-specific oligonucleotide probe targeting the *Roseobacter* group and later with group-specific probes for the SAR86 and SAR11 clades (based on indications from sequence data that these groups were also abundant in the algal-bloom community; see below). Screens with the *Roseobacter* group-specific probe (MALF-1) showed 14 strong positive hybridization signals (5 from the sample 1 library, 7 from the sample 11 library, and 2 from the sample 60 library). Sequencing of positive clones confirmed that all were affiliated with the *Roseobacter* group. Weaker hybridization signals that were not clearly positive (one each from samples 1 and 11) were also checked by sequencing, and both of these were also found to be members of the *Roseobacter* group, although they had several mismatches to the probe. Thus, *Roseobacter*-affiliated clones could be categorized in three groups based on complementarity with the MALF-1 probe: clones with four mismatches at the 3′ end of the probe that produced hybridization signals that were only 1 to 2% of the fully complementary signal (NAC1-4 and NAC11-7), clones with one mismatch at the 3′ end that produced signals similar to the fully complementary signal (NAC1-1, NAC1-2, NAC1-16, NAC11-1, and NAC11-12), and clones with complete complementarity (all others). Including both strongly and weakly hybridizing clones, the percentages of clones positive for the MALF-1 probe were similar for the surface samples outside (sample 1; 6%) and inside (sample 11; 8%) the eddy and slightly lower in the deep-water sample (sample 60; 2%).

Screens of the clone libraries with the SAR86 group-specific probe resulted in 34 positive hybridization signals. The numbers of positive clones were similar for surface samples outside (16 from sample 1) and inside (18 from sample 11) the eddy, but the group was not detected in the clone library from sample 60 at 500 m. Screens of the clone libraries with the SAR11 group-specific probe resulted in 30 positive hybridization signals (16 from sample 1, 2 from sample 11, and 12 from sample 60). Subsequent sequencing of the clones (see below) confirmed that the probes were accurately identifying clones affiliated with these groups.

Random checks of the clones for the presence of a complete insert (20 clones from each library) indicated that all clones in the sample 1 and 11 libraries contained full 16S rRNA inserts while only 60% of the clones in the sample 60 library contained full inserts. The lower cloning efficiency in this library may have resulted in underestimates of the relative representation of specific taxa in this sample.

Of the 12 clones that were completely sequenced, none were chimeric, since similar tree topologies were found with different regions of the 16S rRNA gene. However, two SAR11 clones from sample 60 that were partially sequenced were chimeric, with regions of the sequences showing affiliations to the SAR11 group and the γ *Proteobacteria*.

Phylogenetic diversity of 16S rDNA clones. The 60 clones sequenced from the three clone libraries (Table 1) were affiliated primarily with the *Roseobacter*, SAR86, and SAR11 groups (39 sequences). A number of *Roseobacter* group clones showed close phylogenetic affinities with several cultured bacteria and environmental clones, clustering with isolates and clones from southeastern U.S. coastal waters (17), western U.S. coastal waters (13), and open-ocean waters (5). The percent similarity among the 16 clone sequences in the *Roseobacter* group was above 90% for the regions 89 to 478 (*E. coli* numbering system). An analysis of nearly complete *Roseobacter* group sequences available in GenBank (positions 49 to 1439; $n = 31$) also showed within-group similarities of ≥90%.

The 11 clones in the SAR86 group showed less within-group sequence variation, with percent similarities above 97% for the regions 60 to 445 (*E. coli* numbering system). An analysis of our SAR86 sequences and those previously reported indicated that the SAR86 clade contains two subgroups supported by relatively high bootstrap values. Both subgroups were well represented in the two surface libraries from which SAR86 clones were retrieved. The sequence similarity among our clones and those previously reported from a variety of marine environments was quite high (≥94%). Nearly complete SAR86 sequences from GenBank (positions 48 to 1405; $n = 6$) also exhibit percent similarities of ≥94%.

Five clones from the SAR11 group fell within subgroup A1, a subcluster previously found to have a primarily surface distribution (11). Four of these clones were retrieved from surface samples, while one was retrieved at 500 m. The remainder of the clones in the SAR11 group did not cluster in the subgroups previously described for SAR11. Several of our clones from the A1 subgroup showed 100% similarity with SAR11 clones previously retrieved from the ocean (Table 1). The percent similarity among all 12 of our clones in the SAR11 group was >87% (region 99 to 449; *E. coli* numbering system), while nearly complete SAR11 sequences from GenBank (positions 60 to 1405; $n = 7$) showed percent similarities of >88%.

The 19 clones not affiliated with the three major groups were

TABLE 1. Phylogenetic affiliations of sequences from 16S rDNA clone libraries[a]

Clone	Group	Closest relative (accession no.)	Similarity (%)
NAC1-9	*Roseobacter*	*Prionitis lanceolata* symbiont (U37762)	97.0
NAC1-16	*Roseobacter*	*P. lanceolata* symbiont (U37762)	96.6
NAC11-2	*Roseobacter*	*P. lanceolata* symbiont (U37762)	96.8
NAC11-6	*Roseobacter*	*P. lanceolata* symbiont (U37762)	97.3
NAC1-1	*Roseobacter*	Strain ISM (AF098495)	95.9
NAC11-1	*Roseobacter*	Strain ISM (AF098495)	96.1
NAC1-4	*Roseobacter*	Clones OM42 (U70680), OCS19 (U78942)	100
NAC11-7	*Roseobacter*	Clones OM42 (U70680), OCS19 (U78942)	99.3
NAC11-12	*Roseobacter*	Strain SRF1 (AJ002563)	100
NAC11-3	*Roseobacter*	Strain GAI-36 (AF007259)	99.8
NAC1-2 NAC60-16	*Roseobacter*	Strain GAI-36 (AF007259)	94.8
NAC1-19 NAC11-18 NAC11-10 NAC60-4	*Roseobacter*	Strain GAI-36 (AF007259)	93.4
NAC11-4	SAR86	Clone OCS5 (AF001651)	100
NAC11-11	SAR86	Clone OCS44 (AF001650)	98.5
NAC1-20	SAR86	Clone OCS44 (AF001650)	99.5
NAC11-19 NAC11-5	SAR86	Clone OCS44 (AF001650)	99.8
NAC1-13 NAC1-8 NAC11-8	SAR86	Clone OCS44 (AF001650)	100
NAC11-15 NAC11-9 NAC11-17	SAR86	Clone OCS5 (AF001651)	99.8
NAC1-7	SAR11	Clone OM136 (U70684)	100
NAC1-15	SAR11	Clone OM188 (U70687)	100
NAC11-13	SAR11	Clone OM136 (U70684)	99.8
NAC60-7	SAR11	Clone SAR11 (X52172)	95.1
NAC60-10	SAR11	Clone OM258 (U70691)	99.0
NAC60-13	SAR11	Clone SAR220 (U75257)	95.1
NAC60-15	SAR11	Clone FL11 (L10935)	93.1
NAC60-17	SAR11	Clone SAR220 (U75257)	94.6
NAC60-19	SAR11	Clone SAR220 (U75257)	96.0
NAC60-11 NAC1-3 NAC1-12	SAR11	Clone OCS12 (U75252)	100
NAC1-6	α *Proteobacteria*	Clone OCS116 (U78944)	99.7
NAC1-14	α *Proteobacteria*	Clone CRE-FL20 (AF141447)	95.8
NAC1-17	α *Proteobacteria*	Clone OCS116 (U78944)	98.2
NAC11-14	α *Proteobacteria*	*Rhodobium marinum* (D30791)	91.5
NAC11-16	α *Proteobacteria*	Clone OCS126 (AF001638), SAR116 clade	100
NAC60-9	α *Proteobacteria*	*H. jannaschiana* (AJ227814)	99.3
NAC60-14	α *Proteobacteria*	*Olavius loisae* symbiont (AF104473)	91.9
NAC1-5 NAC1-10 NAC1-11 NAC11-20	Cyanobacteria	Clone CRO-29 (AF141574), *Synechococcus* group	100
NAC1-18	γ *Proteobacteria*	Clone OM10 (U70693)	85.4
NAC60-1	γ *Proteobacteria*	Clone CRO-FL8 (AF141586)	95.8
NAC60-2	γ *Proteobacteria*	Clone CRE-FL8 (AF141439)	91.4
NAC60-6	γ *Proteobacteria*	*P. haloplanktis* (X82139)	99.8
NAC60-8	γ *Proteobacteria*	Clone CRE-PA14 (AF141501)	98.9
NAC60-5	δ *Proteobacteria*	*Nitrospina gracilis* (L35504)	89.1
NAC60-12	δ *Proteobacteria*	Clone SAR324 (U65908), SAR324 clade	95.2
NAC60-3	*Cytophagales*	NA[b]	

[a] From samples 1 (surface; outside eddy), 11 (surface; inside eddy), and 60 (500 m; outside eddy). Twenty clones were sequenced from each library (60 total); two clones from sample 60 were chimeric. The percent similarities are based on comparisons of 500 to 550 nucleotides, beginning at position 70 (*E. coli* numbering). Duplicate clones are indented.

[b] Phylogenetic analyses did not give a clear close relative.

distributed among *Proteobacteria*, cyanobacteria, and *Cytophagales*. Among the seven α *Proteobacteria* clones, NAC60-9 was similar to the cultured bacterium *Hyphomonas jannaschiana* (99%) and was closely related to other sequences in the genus *Hyphomonas* retrieved from seawater by PCR (1, 37, 47). The α *Proteobacteria* clones NAC1-6 and NAC1-17 were closely related to a clone retrieved from coastal Oregon seawater (OCS116 [44]), and clone NAC11-16 was 100% similar to clone OCS126 and clustered within the major marine clade designated the SAR116 group (44).

Among the γ *Proteobacteria* clones that were not affiliated with the SAR86 group (five clones), NAC60-6 had a sequence identical to that of a cultured bacterium, *Pseudoalteromonas haloplanktis* subsp. *tetraodonis*, except for one mismatch in 400 bases. 16S rDNA clones related to the genus *Pseudoalteromonas* have frequently been retrieved from seawater samples (32, 44). Four identical cyanobacterial clones (NAC1-5, NAC1-10, NAC1-11, and NAC11-20) from the surface libraries had the same sequence as clone CRO-29 reported by Crump et al. (8), were 97% similar to SAR7 (16), and were closely related to cultivated *Synechococcus* isolates. The δ *Proteobacteria* (two clones) were represented by clone NAC60-12, which clusters with the marine SAR324 clade (15), and clone NAC60-5, related to marine ammonia- and nitrite-oxidizing bacteria in the genus *Nitrospina* (>89% similarity). *Cytophagales* was represented by one clone (NAC60-3).

T-RFLP analysis. Prior to optimization of T-RFLP conditions, multiple fragment peaks attributable to incomplete digestion of rDNA were sometimes evident during analysis of isolates or clones, but modifying the ratio of DNA to enzyme and the digestion time eliminated this problem and yielded a single peak. The difference between the expected fragment size (predicted from the T-RFLP program) and experimentally determined fragment sizes for clones and isolates was generally ≤3 nucleotides but was greatest for larger fragment sizes (>350 nucleotides) due to spreading of the chromatogram peaks. Duplicate runs of the same sample, including separate PCRs and separate digestions, consistently produced fragment sizes that differed by no more than a few nucleotides.

Analysis of T-RFLP signatures from algal-bloom samples showed that major peaks could frequently be identified to taxon, based on sequences from the clone libraries. Ambiguities in fragment identity after digestion with *Hha*I were resolved with subsequent digestions with *Alu*I, *Hae*III, and/or *Sau*3AI. Putative matches from the clone libraries were coinjected with algal-bloom DNA digests to confirm coelution, resulting in over 50% of the chromatogram area being assigned to taxa represented in the clone libraries (Fig. 1).

Comparisons of T-RFLP fingerprints for surface samples inside and outside the eddy showed little difference in bacterial community composition, despite clear differences in algal-community composition. *Roseobacter* sequences ranged from 42 to 57% ($n = 7$) of the chromatogram area for surface samples inside the *E. huxleyi*-dominated eddy and 35 to 51% ($n = 3$) for surface samples outside the eddy (Fig. 1 and Table 2). Likewise the percent representations of SAR86 (1.9 to 6% inside versus 1.4 to 5.3% outside) and SAR11 (8.8 to 23% inside versus 5.7 to 26% outside) among surface samples inside and outside the eddy were similar. Comparisons of T-RFLP fragments unaffiliated with the three major groups or that could not be identified to taxon also showed highly similar distributions and abundances inside and outside the eddy (Fig. 1).

Comparisons of T-RFLP patterns over two depth profiles (samples 1, 2, 3, and 5 and samples 47, 48, 50, and 51) showed fragments corresponding to *Roseobacter*, SAR86, and SAR11 clones throughout the water column (Table 2). A subsurface maximum and a slight decrease in abundance with depth were evident for all three groups (Fig. 2). Three other taxa were clearly more abundant in surface waters than at depth, including NAC11-16 (SAR116 clade; α *Proteobacteria*), the cya-

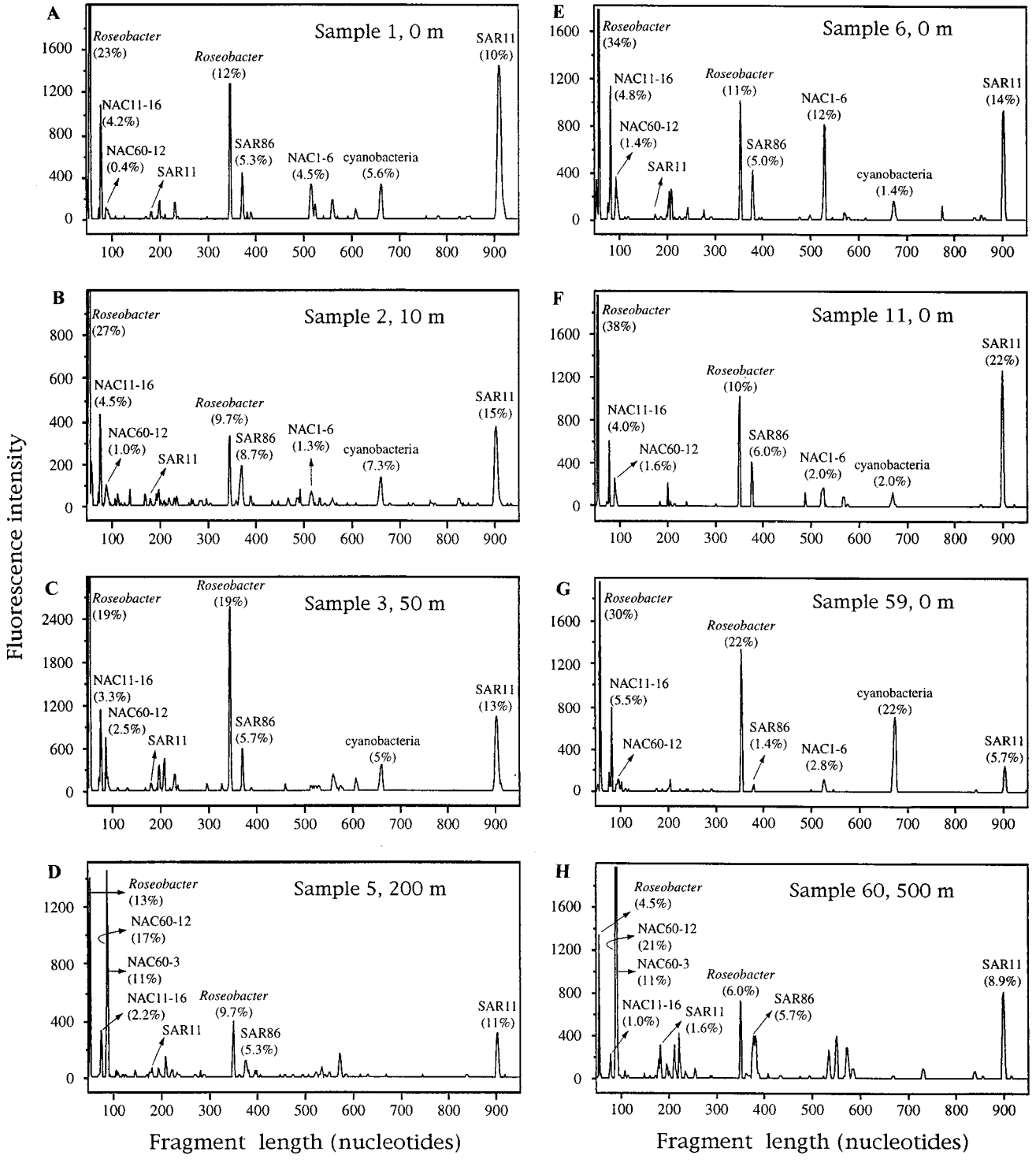

FIG. 1. Bacterial community structure as determined by T-RFLP analysis using the restriction enzyme *Hha*I. Chromatograms A to D show a depth profile of the amplified 16S rDNA sequences at a single station. Chromatograms A, F, and H are from the samples used to construct the 16S rDNA clone libraries (two surface, one 500 m). Chromatograms A, E, F, and G represent four surface samples located inside (E, F) and outside (A, G) the eddy. Peak identification was based on expected fragment sizes of clones from sample 1, 11, and 60 libraries and was confirmed by T-RFLP analyses of clones using the restriction enzymes *Alu*I, *Hae*III, *Hha*I, and *Sau*3AI. The percentages shown are based on the total area under the chromatogram. Percentages are given only for identified peaks with values above 0.4%.

nobacterial clones, and NAC1-6 (α *Proteobacteria*). Two taxa were more abundant in deeper water, including NAC60-12 (SAR324 clade) and NAC60-3 (*Cytophagales*). In the 17 samples for which T-RFLP fragments were analyzed, *Roseobacter* abundance was positively correlated with chlorophyll *a* concentration, as were those of the cyanobacterial clones and NAC11-16 (Pearson correlation: *Roseobacter*, $r = 0.52$, $P < 0.05$; cyanobacteria, $r = 0.58$, $P < 0.01$; NAC11-16, $r = 0.85$, $P < 0.001$; $n = 17$). NAC60-3 and NAC60-12 percent abundance was negatively correlated with chlorophyll *a* concentra-

TABLE 2. Abundance of amplified bacterial 16S rDNA in a North Atlantic algal bloom as measured by T-RFLP analysis

Sample no.	Depth (m)	Chl *a*[a] (μg liter^{-1})	% Contribution of[b]:								
			Roseobacter[c]	SAR86	SAR11	Cyano[c]	Cytoph[d]	NAC1-6	NAC11-16[c]	NAC60-12[d]	Σ Identified peaks
1	0	1.09	35.0	5.3	10.0	5.6	0	4.5	4.2	0.4	65.0
2	10	1.11	37.0	8.7	15.0	7.3	0	1.3	4.5	1.0	74.8
3	50	0.83	38.0	5.7	13.0	5.0	0	0	3.3	2.5	67.5
5	200	0.11	22.7	5.3	11.0	0	11	0	2.2	17	69.2
6	0	0.59	45.0	5.0	14.0	1.4	0	12	4.8	1.4	83.6
11	0	0.85	48.0	6.0	22.0	2.0	0	2	4.0	1.6	85.6
16	0	0.74	49.0	4.4	18.2	3.7	0	5.2	4.1	0	84.6
21	0	0.70	56.7	2.7	18.2	3.1	0	12.0	4.0	0	96.7
26	0	0.53	50.7	5.1	23.1	1.4	0	5.5	4.2	0	90.0
31	0	0.60	52.3	1.9	8.8	4.0	0	5.6	3.9	0	76.5
47	0	1.57	42.0	5.5	19.0	6.8	0	2.3	6.9	1.2	83.7
48	10	1.52	39.0	5.0	23.0	5.5	0	5.0	7.5	1.3	86.3
50	100	0.07	27.0	8.4	16.0	0	15	0	3.5	16	85.9
51	200	0.06	24.0	2.8	4.2	0	16	0	2.1	24	73.1
57	0	1.04	48.7	1.7	16.2	8.4	0	5.2	3.6	0.6	84.4
59	0	1.03	52.0	1.4	5.7	22.0	0	2.8	5.5	0.4	89.8
60	500	0.05	8.8	4.7	7.4	0	11	0	1.0	21.0	53.9

[a] Chl, chlorophyll.
[b] Cyano, cyanobacteria; Cytoph, *Cytophagales* clone NAC60-3. Sample location information is given in Table 3.
[c] Percent abundance of T-RFLP fragment positively correlated with chlorophyll *a* concentration.
[d] Percent abundance of T-RFLP fragment negatively correlated with chlorophyll *a* concentration.

tion (*Cytophagales*, $r = -0.78$, $P < 0.001$; NAC60-12, $r = -0.77$, $P < 0.001$; $n = 17$) (Table 2).

Quantitative dot blot hybridizations. *Roseobacter* group members accounted for a significant percentage of the 16S rRNA genes in many samples from the algal bloom, with values ranging from undetectable to 56% of the 16S rDNA pool (Table 3). Analysis of five depth profiles with the *Roseobacter* group-specific probe indicated that the maximum abundance of these bacteria occurred at approximately 10 m, that they generally were not detected in samples near 200 m, and that they were again present in all samples from 500 m (Table 3). *Roseobacter* abundance was not affected by location within the bloom, with surface samples both inside and outside the eddy having similar values for percent contribution (32.1% inside versus 31.7% outside; Mann-Whitney rank sum test, $P = 0.60$).

The distribution of *Roseobacter* rDNA generally followed the depth profiles of chlorophyll *a* concentration, and there was a significant correlation between percent *Roseobacter* rDNA and chlorophyll *a* for all samples (Pearson correlation: $r = 0.55$; $P < 0.01$; $n = 41$). Among the surface water samples (for which measures of organic sulfur concentrations and turnover were available), a correlation was found between percent *Roseobacter* rDNA and DMSP concentrations for both total ($r = 0.61$; $P < 0.05$; $n = 12$) (Fig. 3) and particulate ($r = 0.61$; $P < 0.05$; $n = 12$) forms of DMSP, but not dissolved forms. Significant positive correlations were also evident between percent *Roseobacter* rDNA and DMSO concentrations for both total ($r = 0.58$; $P < 0.05$; $n = 12$) and particulate ($r = 0.60$; $P < 0.05$; $n = 12$) forms and for total dimethylated sulfur compounds (sum of DMS, DMSP, and DMSO [Fig. 3]) ($r = 0.62$; $P < 0.05$; $n = 12$).

Hybridizations with group-specific probes for the SAR86 and SAR11 groups were conducted for a smaller subset of samples ($n = 11$ and 9). The group-specific probe hybridizations indicated percent contributions to the 16S rDNA pool of <1 to 16% for SAR86 and <1 to 32% for SAR11 (Table 3). No significant correlation between abundance and chlorophyll *a* concentrations or between abundance and organic sulfur concentrations were found for these groups.

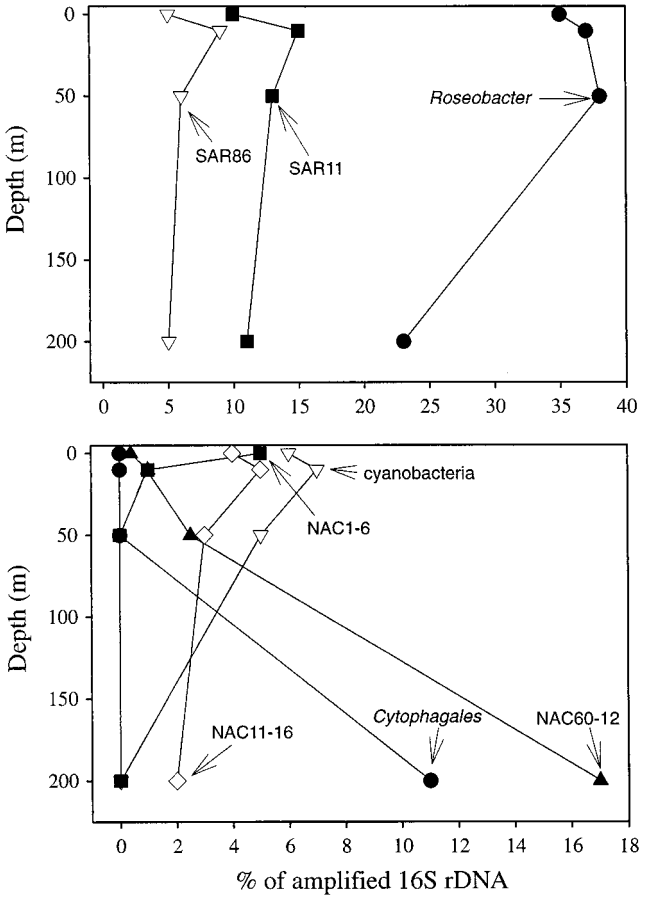

FIG. 2. Depth profiles (samples 1, 2, 3, and 5) of percent abundance of common bacterial phylotypes as determined by T-RFLP analysis of amplified 16S rRNA genes.

TABLE 3. Contributions of three dominant groups of bacterioplankton to 16S rDNA in North Atlantic algal-bloom samples based on hybridizations with group-specific probes[a]

Sample no.	North latitude	West longitude	Depth (m)	Location	% Contribution (±SD) of:		
					Roseobacter	SAR86	SAR11
1	59°20′	19°00′	0	O	21.5 ± 0.2	15.9 ± 2.2	19.0 ± 1.4
2	59°20′	19°00′	10	O	55.9 ± 0.0	17.0 ± 1.4	
3	59°20′	19°00′	50	O	37.6 ± 2.4	11.1 ± 2.3	16.4 ± 9.3
4	59°20′	19°00′	100	O	21.2 ± 1.1	9.4 ± 2.4	11.6 ± 0.4
5	59°20′	19°00′	200	O	0	10.9 ± 1.1	21.4 ± 3.7
6	59°30′	21°07′	0	I	16.5 ± 0.9		
7	59°30′	21°07′	35	I	0		
8	59°30′	21°07′	50	I	0		
9	59°30′	21°07′	100	I	0		
10	59°30′	21°07′	200	I	0		
11	59°30′	21°07′	0	I	27.4 ± 2.8	8.7 ± 1.4	32.0 ± 4.2
12	59°30′	21°07′	10	I	31.2 ± 0.1		
13	59°30′	21°07′	40	I	17.5 ± 3.9		
14	59°30′	21°07′	100	I	0		
15	59°30′	21°07′	200	I	0		
16	59°28′	21°05′	0	I	34.3 ± 11.3		
21	59°34′	21°07′	0	I	28.7 ± 12.6		
26	59°34′	20°46′	0	I	31.5 ± 10.9		
31	59°31′	21°00′	0	I	41.2 ± 12.2		
36	59°31′	21°00′	0	I	24.1 ± 9.5		
37	59°33′	21°02′	0	I	39.7 ± 10.8		
47	60°26′	20°39′	0	I	48.5 ± 10.1	2.4 ± 0.2	3.4 ± 2.8
48	60°26′	20°39′	10	I	24.9 ± 1.1	0.8 ± 0	2.1 ± 2.0
49	60°26′	20°39′	50	I		0.3 ± 0	0
50	60°26′	20°39′	100	I	16.8 ± 8.3	0.5 ± 0.3	
51	60°26′	20°39′	200	I	0	0.5 ± 0.1	0.6 ± 0.5
52	50°48′	20°57′	0	I	25.3 ± 10.7		
53	50°48′	20°57′	10	I	31.1 ± 2.1		
54	50°48′	20°57′	50	I	25.1 ± 1.5		
55	50°48′	20°57′	100	I	0		
56	50°48′	20°57′	200	I	0		
57	60°00′	20°04′	0	O	35.8 ± 7.0		
58	60°00′	20°04′	500	O	17.6 ± 0.8		
59	59°53′	20°22′	0	O	43.0 ± 1.1		
60	59°53′	20°22′	500	O	30.1 ± 2.2		
61	59°46′	20°39′	0	O	40.2 ± 1.5		
67	59°19′	21°48′	0	O	17.4 ± 0.6		
68	59°19′	21°48′	500	O	37.0 ± 6.1		
69	59°12′	22°05′	0	O	45.0 ± 0.6		
70	59°12′	22°05′	500	O	12.4 ± 6.7		
71	59°04′	22°22′	0	O	21.5 ± 5.1		
72	59°04′	22°22′	500	O	22.9 ± 2.6		

[a] A location designation of I or O indicates the sample was collected inside or outside the *E. huxleyi*-dominated eddy. Five depth profiles are included. Standard deviations are calculated based on duplicate hybridization series.

DISCUSSION

Information on the phylogenetic affiliations of bacteria associated with marine algal blooms is currently quite limited (19, 20, 38), despite the likelihood that algal-bacterial interactions have important effects on many bloom-related biogeochemical processes. This study of bacteria associated with a bloom of DMSP-producing algae in the North Atlantic was motivated first by an interest in determining the identities and distributions of the major taxa of bacterioplankton associated with DMSP-producing algal blooms and second by the hypothesis that one of those taxa would be the *Roseobacter* group, a major clade of marine bacteria recently found to have widespread abilities to degrade DMSP and related organic sulfur compounds (18, 23, 28).

Abundance and distribution of the *Roseobacter*, SAR86, and SAR11 groups. Although estimates of the percentage of *Roseobacter* 16S rDNA sequences in the microbial rDNA pool varied among methods, all approaches indicated significant *Roseobacter* contributions to the bacterial community of this North Atlantic algal bloom. Hybridizations of the group-specific oligonucleotide probe to algal-bloom microbial DNA (Table 3) and T-RFLP analysis (Table 2) showed there was little horizontal variation, with surface samples throughout the bloom having similarly high contributions by *Roseobacter* sequences despite clear differences in the composition of the algal community. There was evidence of vertical structure within the bloom, however. Both the group-specific probe data (Table 3) and the T-RFLP data (Fig. 3) showed peaks in relative abundance in near-surface samples, and both measures of *Roseobacter* abundance were positively correlated with chlorophyll *a* concentrations.

The clone libraries showed no evidence of depth-related ecological partitioning of *Roseobacter* phylotypes. Several clones with identical sequences were retrieved from both the surface libraries and the deep library (clones NAC1-19, NAC11-10, NAC11-18, and NAC60-4 were identical; clones NAC1-2 and NAC60-16 were identical). Likewise, phylogenetic analysis of *Roseobacter* clones showed no evidence of clustering based on sample depth.

Bacteria representing both the SAR86 and SAR11 groups were also found throughout the depth profiles (0 to 200 m) and at 500 m, based on hybridizations with group-specific probes (Table 3) and unambiguous T-RFLP fragments (Fig. 1), with a slight subsurface maximum suggested by the T-RFLP data (Fig. 2). These groups showed little horizontal variation, being equally abundant inside and outside the bloom eddy.

Other bloom-associated bacteria. Other bacterial groups exhibited pronounced vertical structure within the bloom region. T-RFLP analysis indicated that the cyanobacterium clones were only present in samples collected at ≤50 m, as expected for autotrophic prokaryotes. Two α *Proteobacteria* phylotypes were also associated with surface samples (NAC1-6 and NAC11-16) (Fig. 1). The δ *Proteobacteria* clone NAC60-12 was characteristic of T-RFLP chromatograms from deeper water, in agreement with a previous study examining the depth distribution of the SAR324 clade (48). The *Cytophagales* clone NAC60-3 was also more abundant in deeper water, although other phylotypes from this division have previously been retrieved from surface waters (9), including those associated with algal blooms (20, 35). In assigning identities to T-RFLP fragments based on clone library sequences, we note that the fragments may derive from one or more related phylotypes

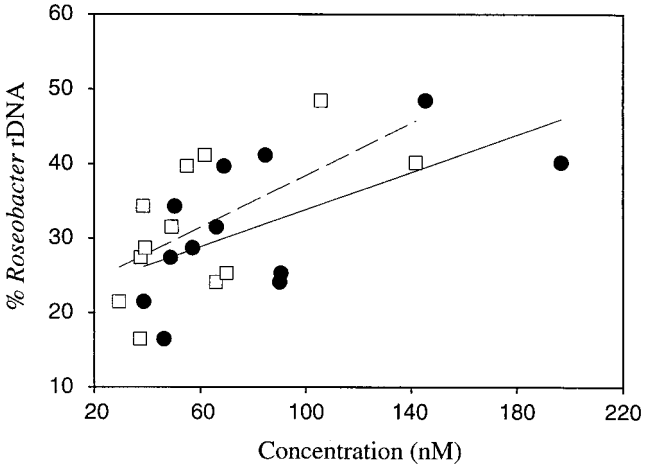

FIG. 3. Correlation of percent contribution of *Roseobacter* group rDNA in surface samples with DMSP concentrations (open squares) and total dimethylated sulfur compound concentrations (sum of DMS, DMSP, and DMSO) (solid circles).

TABLE 4. Contributions of the three major bacterial lineages to algal-bloom DNA as determined by various 16S rRNA-based techniques

Sample no.	Phylogenetic group	% Contribution to algal-bloom DNA based on:			
		Group-specific probe	Screened 16S rDNA clones	Sequenced 16S rDNA clones[a]	T-RFLP analysis[b]
1	*Roseobacter*	21.5	5	12	35
	SAR86	15.9	16	13	5.3
	SAR11	19.0	16	18	10.0
	Σ[f]	56.4	37	43	50.3
11	*Roseobacter*	27.4	7	14	48.0
	SAR86	8.7	18	35	6.0
	SAR11	32.0	2	4	22.0
	Σ	68.1	27	53	76.0
60	*Roseobacter*	30.1	2	1	10.5
	SAR86	ND[d]	0	0	5.7
	SAR11	ND	12	34[c]	10.5
	Σ		14	35	26.7
All samples[e]	*Roseobacter*	22.5 ± 15.9	5	9	39.8 ± 12.9
	SAR86	7.0 ± 6.4	11	16	4.7 ± 2.1
	SAR11	11.8 ± 11.2	10	19	14.4 ± 5.9

[a] Percent contribution to sequenced clones is adjusted to account for bias toward *Roseobacter* sequences in original selection of the clones and underrepresentation of sequences with multiple mismatches to the group-specific probe.
[b] Based on identification of unambiguous peaks as a percentage of the total area under the chromatogram.
[c] The two chimeric sequences are not included.
[d] ND, not determined.
[e] Group-specific probe data are based on 41 (*Roseobacter*), 11 (SAR86), or 9 (SAR11) samples; 16S rDNA data are based on 3 samples; and T-RFLP data are based on 17 samples. Values are averaged across stations and depths.
[f] Σ, total bacterial rRNA genes or rDNA amplicons accounted for by the three major lineages.

with identical locations of the restriction site (for all of the restriction enzymes used to verify peak identity). Thus, NAC1-6 and NAC11-16, or their close relatives, have distributions that suggest a biogeochemical linkage to actively growing phytoplankton, while NAC60-3 and NAC60-12, or their close relatives, are associated with deeper waters.

Other than the clones in the *Roseobacter* group, only two clones representing heterotrophic bacteria were closely related to previously cultured bacteria: NAC60-9 (99% similar to the α proteobacterium *H. jannaschiana*) and NAC60-6 (99.8% similar to the γ proteobacterium *Pseudoalteromonas haloplanktis*). The rest of the clones were affiliated with other environmental phylotypes but not closely related to cultured bacteria.

Comparisons with nonbloom 16S rDNA clone libraries. We compared the compositions of our two surface clone libraries (40 total clones) to the results of seven previous studies in which surface ocean clone abundance was reported in a quantitative fashion (1, 9, 12, 32, 36, 39, 44). The three groups of heterotrophic bacteria that dominated our samples from the North Atlantic algal bloom were also found to be major components of the seven previous nonbloom surface samples. *Roseobacter* phylotypes accounted for 13% of the clones in our algal-bloom library (mean of samples 1 and 11 [Table 4]), compared to an average of 10.7% (±9.5%) for previous studies of surface ocean waters. SAR86 phylotypes accounted for 24% in our bloom library and 21.0% (±4.9%) in the previous studies, while SAR11 phylotypes accounted for 11% in our library and 26.1% (±13.3%) previously. In a survey of all available seawater clones, pooled across depth and regardless of selection criterion, Giovannoni and Rappé (15) found *Roseobacter*, SAR11, and SAR86 sequences to account for 16, 13, and 26% of retrieved phylotypes. Thus, despite differences in the methodologies, these three groups of heterotrophic bacteria have been consistently found to dominate the surface ocean bacterial communities under both bloom and nonbloom conditions.

Method intercomparison. While the current view of marine bacterial community composition is becoming increasingly shaped by 16S rRNA-based methods, it is not yet known whether the commonly used molecular approaches yield similar descriptions of community structure. We compared the results of the three 16S rDNA-based methods used in this study, two of which involve PCR amplification of 16S rDNA prior to quantitative analysis (16S rDNA clone library construction and T-RFLP analysis) and one of which does not (group-specific oligonucleotide probes). We have the most complete comparative information for the *Roseobacter* lineage because of our initial interest in this group, particularly for the three samples used to construct 16S rDNA clone libraries. Both group-specific probe hybridizations and T-RFLP analysis indicate large contributions of *Roseobacter* 16S rDNA to the algal-bloom community (10 to 48% [Table 4]). In contrast, the 16S rDNA clone libraries show a smaller contribution (always <14% [Table 4]). Replicate T-RFLP analyses of the same sample (including independent PCR amplification and digestion) consistently produced chromatograms that were virtually identical, even when differing concentrations of DNA were used (data not shown). Thus, the differences between the two PCR-based methods (T-RFLP analysis and clone library construction) must be attributable to relatively minor differences in the conditions of PCR amplification or to cloning bias. A comparison of a larger subset of samples ($n = 17$) that were analyzed by both group-specific probe hybridization and T-RFLP analysis showed a positive correlation between the two methods in estimates of *Roseobacter* rDNA relative abundance ($r = 0.48$; $P < 0.05$; $n = 17$), although the values obtained by T-RFLP analysis (mean = 39.8) were significantly higher then those obtained with the group-specific probe (mean = 29.3) (Mann-Whitney test; $P < 0.05$).

The three molecular methods also agree on the importance of the SAR11 and SAR86 groups in the algal-bloom community, although abundance estimates vary by as much as fivefold (Table 4). We suspect that the group-specific probe data provide the most accurate quantification of bacterial community composition, since no PCR amplification step is involved, although problems may arise from poor probe complementarity or nonspecific probe binding. We have evidence that the former may be occurring here, since a subset of *Roseobacter* phylotypes gave hybridization signals that were 50-fold lower than those of phylotypes with better complementarity. The latter appears not to be a problem, however, since checking the sequence of clones giving positive signals with group-specific probes invariably yielded a correct sequence. Group-specific 16S rRNA-targeted probes have the disadvantage of being limited to only those groups already suspected of being present in the community and abundant enough to be detected without prior amplification, and they depend on a sequence database that is not necessarily representative of environmental phylotypes. T-RFLP analysis, although dependent on a PCR amplification step, can broadly inventory the bacterial community and, in conjunction with 16S rDNA clone libraries, provide information on the identity and distribution of specific phylotypes over time and space scales. Despite differences among the three molecular approaches, however, they all indicate that the *Roseobacter*, SAR11, and SAR86 clades account for approximately 50% of the 16S rDNA pool in surface waters of the algal bloom and 20 to 30% below the mixed layer (Table 4).

Links to biogeochemical roles. Physiological studies of *Roseobacter* isolates show that many cultured members of this group can degrade DMSP; this metabolic ability is evident even in isolates that have been cultured by methods that involve no selection for DMSP utilization, suggesting it may be a fundamental trait of the group (18). Field studies have demonstrated that the two competing pathways for DMSP degradation, producing either DMS or MeSH, operate simultaneously in oceanic surface waters (21, 27, 46) and that the relative balance between the two has important implications for DMS emission from the sea surface (22, 40). *Roseobacter* isolates are thus far the only known cultured bacteria that possess both pathways for DMSP degradation and potentially play a critical role in DMS regulation.

The group-specific probe data indicated a significant positive correlation between the percent *Roseobacter* rDNA and concentrations of chlorophyll *a* and in surface waters between percent *Roseobacter* rDNA and concentrations of DMSP and total dimethylated sulfur compounds (Fig. 3). The passage of cyanobacterial cells though the 2.0-μm-pore-size filters used to collect microbial DNA complicates correlation analysis with the group-specific probe data, since the cyanobacterial DNA can dilute (to varying extents) the heterotrophic bacterial DNA. However, *Roseobacter* abundance was also positively correlated with the amount of chlorophyll *a* passing through a 2-μm-pore-size filter ($r = 0.51$; $P < 0.002$; $n = 36$), suggesting a robust positive correlation with autotrophic biomass that is not masked by variations in DNA contributions from small autotrophs. T-RFLP analysis confirmed the significant correlation between percent *Roseobacter* 16S rDNA fragments and chlorophyll *a* concentrations. Together these data argue for a spatial linkage between *Roseobacter* cells and living algal cells, which we hypothesize may be related to organic sulfur cycling within the bloom. DMSP turnover rates in surface waters were not correlated with *Roseobacter* 16S rDNA (data not shown), although the analytical approach we used to measure DMSP turnover did not separate activities of heterotrophic bacteria from those of algae and algal grazers (41, 42).

In contrast to the *Roseobacter* group, the SAR86 and SAR11 lineages have no representatives in culture, and thus there are few available hints as to their biogeochemical roles. The SAR86 group is related to a cluster of autotrophic sulfur-oxidizing symbionts of marine animals (34), but the percent similarity between the 16S rRNA genes of the symbionts and those of the SAR86 clones is relatively low (approximately 84%). Giovannoni and Rappé (15) hypothesized that ecologically successful heterotrophs in the surface ocean are most likely utilizing phytoplankton-derived dissolved organic matter and that their dominance may be the result of a competitive advantage in procuring limited inorganic nutrients. Utilization of phytoplankton-derived dissolved organic sulfur by *Roseobacter* isolates would support this hypothesis, and SAR11 and SAR86 bacteria may likewise be growing at the expense of alga-related dissolved compounds.

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