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Phylogenetic Diversity and Specificity of Bacteria Closely Associated with *Alexandrium* spp. and Other Phytoplankton

Suresh Jasti,[1] Michael E. Sieracki,[2] Nicole J. Poulton,[2] Michael W. Giewat,[1] and Juliette N. Rooney-Varga[1]*

University of Massachusetts Lowell, Lowell, Massachusetts,[1] and Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine[2]

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While several studies have suggested that bacterium-phytoplankton interactions have the potential to dramatically influence harmful algal bloom dynamics, little is known about how bacteria and phytoplankton communities interact at the species composition level. The objective of the current study was to determine whether there are specific associations between diverse phytoplankton and the bacteria that co-occur with them. We determined the phylogenetic diversity of bacterial assemblages associated with 10 *Alexandrium* strains and representatives of the major taxonomic groups of phytoplankton in the Gulf of Maine. For this analysis we chose xenic phytoplankton cultures that (i) represented a broad taxonomic range, (ii) represented a broad geographic range for *Alexandrium* spp. isolates, (iii) grew under similar cultivation conditions, (iv) had a minimal length of time since the original isolation, and (v) had been isolated from a vegetative phytoplankton cell. 16S rRNA gene fragments of most *Bacteria* were amplified from DNA extracted from cultures and were analyzed by denaturing gradient gel electrophoresis and sequencing. A greater number of bacterial species were shared by different *Alexandrium* cultures, regardless of the geographic origin, than by *Alexandrium* species and nontoxic phytoplankton from the Gulf of Maine. In particular, members of the *Roseobacter* clade showed a higher degree of association with *Alexandrium* than with other bacterial groups, and many sequences matched sequences reported to be associated with other toxic dinoflagellates. These results provide evidence for specificity in bacterium-phytoplankton associations.

Bacteria and phytoplankton dynamics are thought to be closely linked, and the "phycosphere," or region immediately surrounding and influenced by phytoplankton cells, is an important bacterial habitat that is distinct from the surrounding water. Phytoplankton excrete organic compounds that represent an important fraction of primary production, form the base of the marine microbial food web (36), and stimulate bacterial growth (51). Bacteria can be free living in the phycosphere (7), can be attached to the surface of the algal cells (34, 57), or can occur as intracellular algal symbionts (38). Bacterium-phytoplankton interactions play a key role in processes ranging from biogeochemical cycling within the microbial loop (6) to biochemically mediated interactions that influence phytoplankton growth, reproduction, cyst formation, and mortality (13). The influence of bacteria on toxic algae has been of particular interest, and bacteria have been implicated in the production and/or modification of algal toxins (29, 30, 33).

Several studies have reported evidence for species-specific interactions between bacteria and phytoplankton, which has led to the conclusion that bacteria can play a major role in controlling phytoplankton dynamics. For example, Fukami et al. (18) found that natural bacterial communities collected during a *Gymnodinium nagasakiense* bloom inhibited *Skeletonema costatum* but stimulated *G. nagasakiense*. Later, Fukami et al. (19) isolated *Flavobacterium* sp. strain 5N-3, which was found to have algicidal activity against *G. nagasakiense* but to have no effect on *Chattonella antiqua*, *Heterosigma akashiwo*, or *S. costatum*. These findings have important implications, especially if they hold true for interactions between bacteria and members of the general phytoplankton community that form the basis for carbon cycling in coastal marine environments (32).

A number of studies have shown that bacteria attached to particles are phylogenetically distinct from free-living bacteria (9, 11, 49). This finding indicates that there are selective forces that drive the community succession on particles toward a phylogenetic composition that differs from the composition in the surrounding water. Rooney-Varga et al. (49) recently reported a link between the community dynamics of phytoplankton and particle-associated bacteria in the Bay of Fundy, suggesting that species-specific associations occur in the phycosphere. However, given the complexity of coastal temperate phytoplankton communities, it has not yet been possible to determine whether links between dynamics at the community level are indeed the result of specific interactions between particular bacteria and phytoplankton and, if so, which bacterial species are associated with which phytoplankton. For example, Savin et al. (50) observed as many as 42 phytoplankton species in a surface seawater sample from the Bay of Fundy, which made it impossible to use direct molecular analysis of the attached bacterial community to determine which bacteria were associated with a particular phytoplankton species. Clearly, the process by which an associated bacterial community might be "selected" by an algal species is complex and not well understood (12).

Given the complexity of natural samples, another approach

* Corresponding author. Mailing address: Center for Complex Environmental Systems, University of Massachusetts Lowell, 1 University Avenue, Lowell, MA 01854. Phone: (978) 934-4715. Fax: (978) 934-3044. E-mail: Juliette_RooneyVarga@uml.edu.

TABLE 1. Phytoplankton cultures analyzed to determine the phylogenetic identities of their bacterial associates

Species	Source	Taxonomic class	Medium	Strain or CCMP no.[a]	Toxicity	Year isolated	Location of isolation	
							Latitude	Longitude
Alexandrium fundyense	Gulf of Maine	*Dinophyceae*	f/2	38.3	Yes	1993	43°30′N	70°6′W
Alexandrium fundyense	Gulf of Maine	*Dinophyceae*	f/2	48.1	Yes	1993	43°2′N	70°39′W
Alexandrium fundyense	Gulf of Maine	*Dinophyceae*	f/2	48.2	Yes	1993	43°2′N	70°39′W
Alexandrium fundyense	Gulf of Maine	*Dinophyćeae*	f/2	5.6	Yes	1993	43°44′N	69°22′W
Alexandrium fundyense	Gulf of Maine	*Dinophyceae*	f/2	CB301	Yes	1998	43°30′N	69°53′W
Alexandrium tamarense	France	*Dinophyceae*	f/2	ATTL01	Yes	1998	43°23′N	3°36′E
Alexandrium tamarense	Bering Sea	*Dinophyceae*	f/2	ATRU 10/1	Yes	2000	61°7′N	172°16′E
Alexandrium tamarense	Bering Sea	*Dinophyceae*	f/2	ATRU 4/1	Yes	2000	61°21′N	170°38′E
Alexandrium minutum	Portugal	*Dinophyceae*	f/2	GTPORT	Yes	NA[b]	39°24′N	9°13′W
Alexandrium tamarense	Japan	*Dinophyceae*	f/2	OK905-7	Yes	1990	39°5′N	141°51′E
Chaetoceros cf. *tortissimus*	Gulf of Maine	*Coscinodiscophyceae*	f/2	1601	No	1993	43°51′N	69°38′W
Chlorarchnion cf. sp.	Gulf of Maine	*Chlorarchniophyceae*	K	1258	No	1980	43°N	69°W
Emiliana huxleyi	Gulf of Maine	*Prymnesiophyceae*	L1	377	No	1988	43°N	68°W
Micromonas pusilla	Gulf of Maine	*Prasinophyceae*	L1	485	No	1989	43°51′N	69°38′W
Nanochloropsis granulata	Gulf of Maine	*Eustigmatophyceae*	f/2-Si	534	No	1986	43°51′N	69°38′W
Phaeocystis cf. *globosa*	Gulf of Maine	*Prymnesiophyceae*	L1	1805	No	1997	42°30′N	68°W
Prorocentrum minimum	Gulf of Maine	*Dinophyceae*	f/2-Si	699	Yes	1988	43°51′N	69°38′W
Pseudopedinella elastica	Gulf of Maine	*Dictyochophyceae*	Prov	716	No	1988	43°N	68°W
Rhodomonas sp.	Gulf of Maine	*Cryptophyceae*	K	763	No	1986	44°27′N	67°52′W
Scrippsiella sp.	Gulf of Maine	Dinophyceae	f/2-Si	772	No	1959	33°11′N	65°15′W
Skeletonema costatum	Woods Hole	*Coscinodiscophyceae*	f/2	780	No	1974	41°32′N	70°40′W
Tetraselmis sp.	Gulf of Maine	*Prasinophyceae*	K	1267	No	1982	43°49′N	69°40′W
Thalassiosira cf. *gravida*	Gulf of Maine	*Coscinodiscophyceae*	f/2	1543	No	1992	43°51′N	69°38′W

[a] CCMP, Provasoli-Guillard National Center for Culture of Marine Phytoplankton.
[b] NA, not available.

is to characterize bacteria associated with phytoplankton in culture. Many algal isolates are obtained by micropipetting a single algal cell from an environmental sample, thereby producing a clonal culture. In the absence of treatment to render the culture axenic, bacteria that were initially present in the phycosphere and are capable of growing in association with the algae are selected for with successive transfers. In the current study, we investigated the possibility of specific bacterium-phytoplankton associations by analyzing the phylogenetic diversity of bacteria associated with cultures of diverse nontoxic phytoplankton, as well as members of the toxic dinoflagellate genus *Alexandrium*, isolated from several regions of the world. Our analysis included a survey of bacterial associates of the major phylogenetic groups of phytoplankton common in the Gulf of Maine and similar temperate coastal environments, as well as *Alexandrium* species from the Gulf of Maine, Japan, Portugal, France, Russia, and the Bering Sea. The results reveal a pattern of specific bacterial associations with toxic *Alexandrium* species, regardless of the location of isolation.

MATERIALS AND METHODS

Cultivation of phytoplankton. The phytoplankton cultures used in this study are listed in Table 1 and included 10 *Alexandrium* cultures, as well as 13 cultures that represent dominant taxa in the Gulf of Maine phytoplankton community, including members of the *Dinophyceae*, *Coscinodiscophyceae*, *Chlorarchniophyceae*, *Prymnesiophyceae*, *Prasinophyceae*, *Eustigmatophyceae*, *Dictyochophyceae*, and *Cryptophyceae*. *Alexandrium* spp. cultures were maintained in modified f/2 medium. The f/2 medium was prepared as described by Guillard and Ryther (27), except that it contained 10^{-8} M Na_2SeO_3 and 10^{-8} M $CuSO_4 \cdot 5H_2O$. All other cultures were grown in f/2 medium (25, 27), K medium (31), L1 medium (26), Prov medium (48), or f/2-Si medium (f/2 medium with $Na_2SiO_3 \cdot 9H_2O$ omitted), as shown in Table 1. Phytoplankton cultures were grown at 15°C with cycles consisting of 14 of light and 10 h of darkness (for *Alexandrium* spp.) or of 13 h of light and 11 h of darkness (for all other cultures); the light intensity was ca. 200 μmol photons m^{-2} s^{-1}. *Alexandrium* cultures were kindly provided by D. M. Anderson (Woods Hole Oceanographic Institute, Woods Hole, MA), and all other cultures were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton at Bigelow Laboratory, Boothbay Harbor, ME. All Provasoli-Guillard National Center for Culture of Marine Phytoplankton and *Alexandrium fundyense* cultures were isolated from the Gulf of Maine, while *Alexandrium tamarense* and *Alexandrium minutum* cultures were isolated from France, the Bering Sea, Portugal, and Japan (Table 1). All phytoplankton cultures were maintained as unialgal, xenic cultures from the time of original isolation and, with the exception of *A. fundyense* CB301, were isolated from vegetative cells (not cysts or resting stages).

Analysis of attached and free-living bacteria. In order to compare the phylogenetic composition of bacteria attached to *A. fundyense* cells with the phylogenetic composition of free-living bacteria, a 20-μm-pore-size sieve was used to separate *A. fundyense* strain CB301 cells and attached bacteria from free-living bacteria in the culture. A sample of a mid-exponential-phase *A. fundyense* strain CB301 culture was aseptically washed over a 20-μm-pore-size sieve. *A. fundyense* cells collected on the 20-μm-pore-size sieve were rinsed carefully with sterile seawater in order to remove free-living bacteria. Cells collected on the sieve were then backwashed with sterile f/2 medium and collected by centrifugation at 5,000 × *g* for 5 min. Bacterial cells that passed through the sieve (considered free living) were collected by centrifugation at 10,000 × *g* for 10 min.

PCR and DGGE analysis of 16S rRNA gene fragments. A PCR-denaturing gradient gel electrophoresis (DGGE) approach was used to analyze the 16S rRNA gene phylogeny of bacteria associated with phytoplankton. Phytoplankton cells and their associated bacteria were subjected to centrifugation at 10,000 × *g* for 10 min, washed with sterile seawater, and collected by centrifugation at 10,000 × *g* for 10 min. Genomic DNA extraction, amplification of 16S rRNA gene fragments, DGGE, and sequence analysis were conducted as previously described (49). The DGGE conditions included a 55 to 70% denaturant gradient gel (where 100% was equivalent to 7 M urea and 40% formamide) (46) that was electrophoresed for 16 h at 70 V. For each sample, approximately 1.0 μg PCR product was analyzed by DGGE. After DGGE, isolated bands were excised and pulverized with a sterile mortar and pestle, and DNA was eluted overnight in 50 μl 0.1 M Tris, pH 8.0, at 4°C. Partial 16S rRNA genes were then reamplified from excised bands and analyzed by a second DGGE in order ensure that heteroduplexes were resolved. In addition, for these second DGGEs, bands from different samples that appeared to migrate to the same or similar positions in the original analysis were placed in adjacent lanes to confirm their relative positions. DGGE profiles were analyzed using the Quantity One gel documentation software (Bio-Rad, Hercules, CA) in order to determine the positions of individual bands.

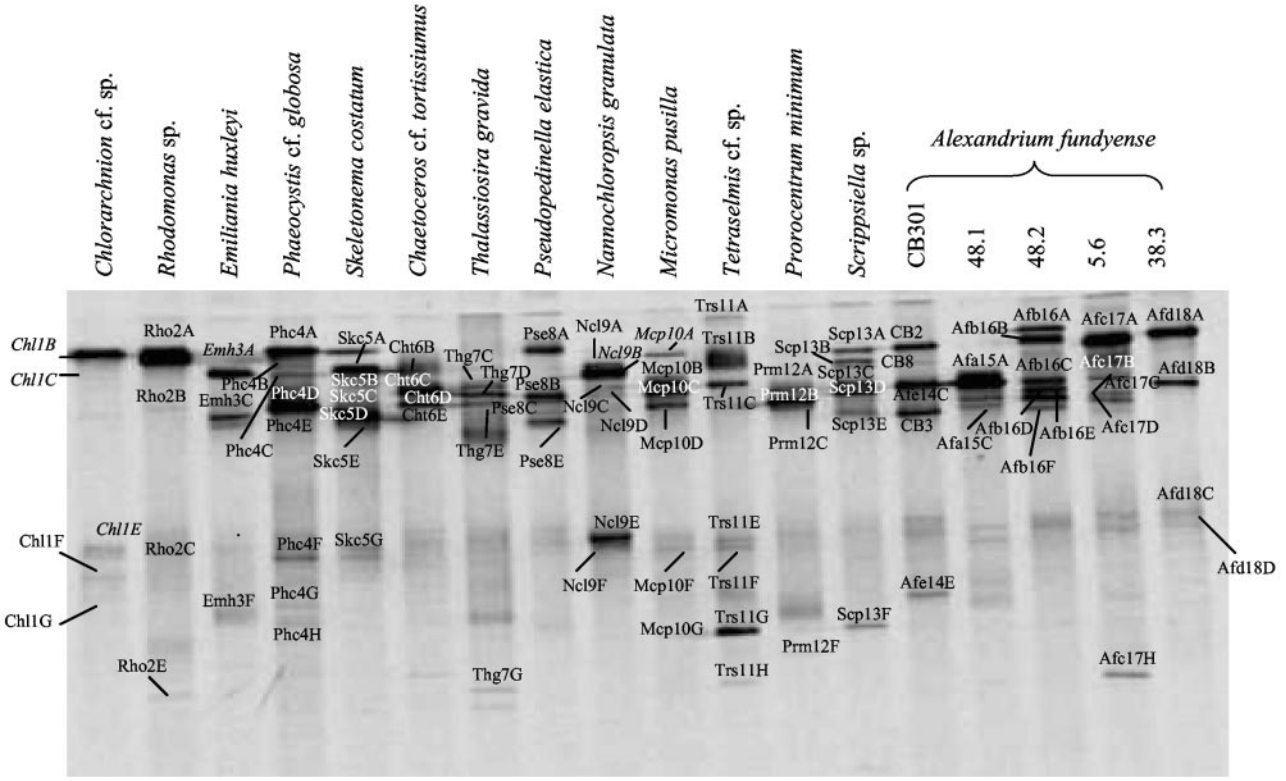

FIG. 1. DGGE profiles of 16S rRNA gene fragments of bacteria in cultures of diverse phytoplankton isolated from the Gulf of Maine. All labeled bands were analyzed by multiple DGGEs and/or sequence analysis. Chimeric sequences were not included in the analysis. Sequences related to chloroplast 16S rRNA genes are indicated by italics.

Cloning and sequencing. Direct sequencing of some of the DGGE bands was unsuccessful, and these bands were therefore reamplified and cloned prior to sequencing. Clones were constructed using a TOPO TA cloning kit (Invitrogen Corp., Carlsbad, CA) with the pCR 2.1-TOPO vector and TOP10 One Shot chemically competent cells as described by the manufacturer. Sequencing reactions were performed using primer 338F according to the instructions of the manufacturer of the sequencing kit (Beckman-Coulter, Fullerton, CA), and sequences were analyzed using a Beckman-Coulter CEQ 2000 automated DNA sequencer in the University of Massachusetts Lowell Biological Sciences Department.

16S rRNA gene sequences were checked for potential chimeras with the Ribosomal Database Project II (RDP II) Chimera Check program (39). Sequences were then aligned with closely related 16S rRNA sequences from GenBank and the RDP II, and alignments were edited manually using the program SeqPup. Unambiguously aligned base positions were used to construct phylogenetic trees with maximum-likelihood and maximum-parsimony methods using PAUP* (Sinauer Associates, Sunderland, MA) and the Weighbor-Joining method using RDP II (39).

Analysis of DGGE results. Once the DGGE analysis and sequencing were complete, a schematic diagram representing each unique DGGE band was constructed. Any bands found to be related to chloroplast rRNA sequences were assumed to originate from the phytoplankton themselves and were not considered in further analyses. In many cases, multiple bands were found to represent the same sequence and were therefore represented as a single DGGE band position. Using the compiled DGGE information, pairwise similarity values for DGGE profiles were calculated as follows: $S_{AB} = M_{AB}/N$, where M_{AB} is the number of matches (i.e., the number of bands present in both lane A and lane B for each possible band position) and N is the number of band positions (i.e., the total number of bands in the composite lane) (42). DGGE band intensities were not taken into account.

Nucleotide sequence accession numbers. The sequences determined have been deposited in the GenBank database under accession numbers AY744706 to AY744773.

RESULTS

DGGE profiles and phylogenetic diversity. DGGE profiles revealed a high degree of variability among bacterial associates of different phytoplankton cultures, and there were relatively few common bands for multiple cultures (Fig. 1 and 2). Because of this complexity in the DGGE profiles, further analysis by multiple DGGEs and sequencing was necessary in order to obtain robust intersample comparisons. A compilation of the DGGE and sequencing data is shown in Fig. 3, as described above. There was also substantial variability in the number of bands associated with each culture; as few as one nonchloroplast DGGE band was associated with the *Chlorarchnion* sp. culture, and as many as seven nonchloroplast DGGE bands were associated with the *Tetraselmis* sp. culture (Fig. 3).

Comparison of the DGGE profiles of free-living, attached, and bulk bacterial samples of the *A. fundyense* strain CB301 culture revealed similar profiles for all three sample types, and all dominant bands were present in all sample types (Fig. 2). Similarly, DGGE analysis of bacteria associated with strain CB301 on multiple occasions and during different growth stages showed the same major bands (data not shown), although the band intensities sometimes varied.

Phylogenetic analysis of DGGE band sequences revealed the dominance of two major groups of bacteria, the *Roseobacter* group in the alpha-*Proteobacteria* and the *Cytophaga-Flavobacterium-Bacteroides* (CFB) phylum. Together, these

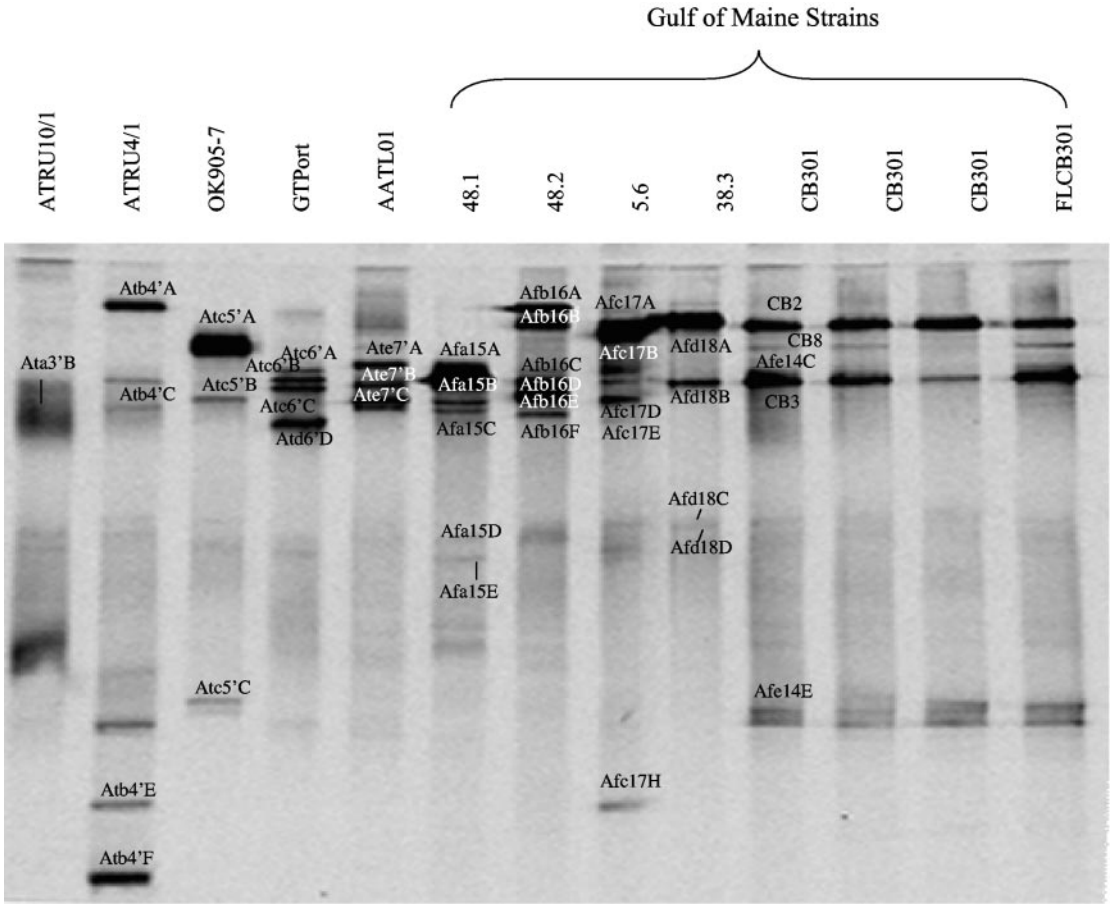

FIG. 2. DGGE profiles of 16S rRNA gene fragments of bacteria in *Alexandrium* spp. cultures. All labeled bands were analyzed by multiple DGGEs and/or sequence analysis. Chimeric sequences were not included in the analysis. Bands for attached (ATTL01) and free-living (FLCB301) bacteria associated with CB301 are indicated.

two groups accounted for 78% of the DGGE bands analyzed. Other groups that were represented in multiple samples included members of the family *Alteromonadaceae*, the *Sphingomonas* group, and the *Rhizobium-Agrobacterium* group and *Marinobacter* sp. strain PCOB-2 (Table 2 and Fig. 3). Interestingly, most (62%) of the sequences analyzed were more than 99% similar to their closest relatives in the databases, while only 15% exhibited similarity values of less than 97%.

Sequences were considered to show evidence of association with toxic dinoflagellates if they exhibited more than 99% similarity with another sequence retrieved from a dinoflagellate culture or bloom (Table 2). This similarity value was chosen as the operational limit for defining members of the same phylotype, given PCR and sequencing errors and interoperon sequence variability (1). Sequences found in multiple *Alexandrium* spp. cultures in the current study were also marked as showing evidence of association with toxic dinoflagellates (Fig. 3). Interestingly, the majority of the bands (68%) in *Alexandrium* spp. cultures matched other bacteria associated with toxic dinoflagellates, including other *Alexandrium* species and *Gymnodinium* species. In contrast, only 19% of the bands from nondinoflagellate cultures matched sequences associated with toxic dinoflagellates. Nondinoflagellate cultures also had a much lower incidence of sequences that matched (i.e., were >99% similar to) sequences in the databases that were found in other algal cultures. For example, for nondinoflagellate cultures, 66% of the sequences with database matches were most closely related to sequences obtained directly from environmental samples and were not specifically associated with algae. This result was in contrast with the fact that 77% of the sequences with database matches from *Alexandrium* cultures had closest relatives that were directly associated with dinoflagel-

FIG. 3. Schematic representation of DGGE bands of bacteria associated with diverse phytoplankton from the Gulf of Maine and *Alexandrium* species from around the world. DGGE bands that were sequenced are indicated by "seq." Sequences that matched sequences from other toxic dinoflagellate cultures (including those analyzed here, which are indicated by an asterisk) were considered to be associated with toxic dinoflagellates. Data are sorted by whether bands are associated with toxic dinoflagellates and by the closest relative. Alt, *Alteromonadaceae*; Gam, gamma-*Proteobacteria*; Rhiz, *Rhizobium-Agrobacterium* group; Ros, *Roseobacter* group; Sphi, *Sphingomonas* group; Mar, *Marinobacter* sp.

DGGE Band	Closest relative of sequence	Percent similarity	Phylogenetic group[a]	Associated with toxic dinoflagellates?[b]	*Chlorarchnion* cf. sp.	*Rhodomonas* sp.	*Emiliania huxleyi*	*Phaeocystis* cf. *globosa*	*Skeletonema costatum*	*Chaetocercos* cf. *tortissimus*	*Thalassiosira* cf. *gravida*	*Pseudopedinella elastica*	*Nannochloropsis granulata*	*Micromonas pusilla*	*Tetraselmis* cf. sp.	*Prorocentrum minimum*	*Scrippsiella* sp.	*Alexandrium fundyense* 48.1	*Alexandrium fundyense* 48.2	*Alexandrium fundyense* 5.6	*Alexandrium fundyense* 38.3	*Alexandrium fundyense* CB301	*Alexandrium fundyense* CB301 Att	*Alexandrium fundyense* CB301 FL	*Alexandrium tamarense* ATRU10/1	*Alexandrium tamarense* ATRU4/1	*Alexandrium tamarense* OK905-7	*Alexandrium minutum* GTPORT	*Alexandrium tamarense* AATL01
Cht6C	*Pseudoalteromonas* sp. RE10F/2	100.0%	Alt	No						seq																			
Emh3F	*Alteromonas* sp. str. JLN-A587	100.0%	Alt	No			seq																						
Prm12B	Uncultured bacterium CTD70B	96.8%	Alt	No												seq													
Pse8C	Uncultured Gamma proteobacterium	97.9%	Alt	No								seq																	
Afb16B	Uncultured *Bacteroidetes* bacterium	90.7%	CFB	No															seq										
Afc17A	*Bacteroidetes* KMM 3912	97.5%	CFB	No																seq									
Afd18A	Bacterium OTU_B	98.6%	CFB	No																	seq								
Afd18C, 18D	*Antarcticum aff. vesiculatum*	99.1%	CFB	No																	seq								
Afe14A, CB2	Arctic sea ice bacterium ARK10007	93.0%	CFB	No																		seq							
Ate7'A	Uncultured bacterium SB-29-AG	99.7%	CFB	No																									seq
Cht6B	Uncultured bacterium ARKIA-137	96.9%	CFB	No						seq																			
Mcp10C,Thg7D	Bacterium DG890	90.9%	CFB	No										seq															
Mcp10G,Trs11G	*Caulobacter* sp. E4		CFB	No										seq															
Phc4a, Skc5A	Marine bacterium OTU_A	99.4%	CFB	No				seq	seq																				
Pse8A	Bay of Fundy bacterium BA10-1C	98.6%	CFB	No								seq																	
Rho2A	*Bacteroidetes* bacterium D21	97.8%	CFB	No		seq																							seq
Rho2C	*Flexibacter* sp. D8	98.5%	CFB	No		seq																							
Scp13A	*Cryomorphaceae* str. UST20020801	92.9%	CFB	No													seq												
Skc5B,Skc5C	*Bacteroidetes* bacterium	98.5%	CFB	No					seq																				
Thg7C	Bacterium clone s34	95.3%	CFB	No							seq																		
Ncl9A	*Cytophagales* clone SL-6a	96.5%	CFB	No													seq												
Afb16E	Gamma proteobacterium SIMO-358	100.0%	Gam	No															seq										
Scp13F	Marine proteobacterium HTCC2188	100.0%	Gam	No													seq												
Atb4'F	Bacterium clone K2-S-5	87.3%	None	No																							seq		
Rho2E, Thg7G, Trs11H	Str. GWS-BW-H54M	99.7%	Rhiz	No							seq																		
Afe14E	GMDJE10F1	98.8%	Ros	No																		seq							
Atc5'C	*Ruegeria* sp. TCg-9	99.4%	Ros	No																								seq	
Mcp10B	*Roseobacter* sp. KT0202a	98.7%	Ros	No										seq															
Phc4D, Prm12A, Phc4E, Phc4F,	Bacterium SB-8/30-AG	100.0%	Ros	No				seq																					
Prm12D	Proteobacterium BMS8	98.3%	Ros	No												seq													
Pse8B	Alpha proteobacterium AS-26	99.2%	Ros	No								seq																	
Thg7E, Cht6D	*Sulfitobacter* sp. HEL-77	100.0%	Ros	No							seq																		
Trs11A	*Roseobacter* sp. ANT9276a	99.2%	Ros	No											seq														
Trs11C	Bacterium OHKB4.31	98.1%	Ros	No											seq														
Trs11E	*Rhodobacter* group clone D103	100.0%	Ros	No											seq														
Trs11F	*Rhodobacter* group clone D103	99.0%	Ros	No											seq														
Cht6E	*Erythrobacter aquimaris* SW-140	98.4%	Sphi	No						seq																			
Mcp10D, Mcp10F	Alpha proteobacterium MBIC3952	99.5%	Sphi	No										seq															
Ncl9F, Skc5G	*Erythrobacter aquimaris*	99.4%	Sphi	No					seq																				
Skc5D, Skc5F	*Erythrobacter aquimaris*	100.0%	Sphi	No					seq																				
Atc5'A	Bacterium DG945	100.0%	CFB	Yes																								seq	
Skc5E	Bacterium DG1030	100.0%	CFB	Yes			seq																						
Ata3'B, Ata3'C,	*Marinobacter* sp. PCOB-2	100.0%	Mar	Yes								seq											seq	seq					
Atb4'E, Afc17H	*Mesorhizobium* sp. DG1023	99.7%	Rhiz	Yes																seq							seq		
Afb16C	Alpha proteobacterium	99.7%	Ros	Yes															seq										
Afb16D	Marine bacterium ATAM173a_17	99.3%	Ros	Yes															seq										
Afb16F	*Sulfitobacter* sp. H24	99.7%	Ros	Yes															seq										
Afc17C	Marine bacterium ATAM173a_16	99.2%	Ros	Yes																seq									
Atd6'A, Atd6'D	*Roseobacter* 253-16	99.5%	Ros	Yes																								seq	
Atd6'B, Atd6'D	*Roseobacter* 253-16	100.0%	Ros	Yes																								seq	
Ate7'C	Marine bacterium ATAM407_56	99.7%	Ros	Yes																									
Ncl9C	Alpha proteobacterium C20 1	100.0%	Ros	Yes									seq																
Ncl9D	*Roseobacter* sp. DG869	99.2%	Ros	Yes									seq																
Ncl9E	*Roseobacter* sp. DG869	100.0%	Ros	Yes									seq																
Prm12C	*Roseobacter* sp. DG942	99.3%	Ros	Yes												seq													
Prm12F	*Antarctobacter* sp. 667-12	100.0%	Ros	Yes												seq													
Scp13C, Scp13D, Rho2B	Marine bacterium ATAM173a_17	100.0%	Ros	Yes		seq											seq												
Afb16A, Atb4'A	*Tenacibaculum mesophilum*	96.3%	CFB	Yes*															seq										
Afd18B, Ate7'B	Alpha proteobacterium A115-20	98.1%	Rhiz	Yes*																	seq								
Afa15B	Marine bacterium SCRIPPS_732	100.0%	Ros	Yes*														seq											
Atc5'B, Atb4'C	*Ruegeria atlantica*	99.7%	Ros	Yes*																								seq	
CB8	Marine bacterium SCRIPPS_732	99.8%	Ros	Yes*																					seq				

TABLE 2. Similarity values for the closest relatives of 16S rRNA gene sequences retrieved from phytoplankton cultures[a]

Source culture	DGGE band	Phylogenetic group	Closest relative	% Similarity	No. of base positions compared	GenBank accession no.	Association with toxic dinoflagellate[b]
Rhodomonas sp.	Rho2A	CFB	*Bacteroidetes* bacterium D21	97.8	267	AY030100	No
Rhodomonas sp.	Rho2B	*Roseobacter*	Marine bacterium ATAM173a_17	100.0	247	AF359532	Yes
Rhodomonas sp.	Rho2C	CFB	*Flexibacter* sp. strain D8	98.5	269	AF125323	No
Emiliana huxleyi	Emh3C	CFB	Bacterium DG1030	100.0	294	M62799	Yes
Emiliana huxleyi	Emh3F	*Alteromonadaceae*	*Alteromonas* sp. strain JLN-A587	100.0	484	X86461	No
Phaeocystis cf. *globosa*	Phc4a	CFB	Uncultured marine bacterium OTU_A	98.3	292	AF207850	No
Phaeocystis cf. *globosa*	Phc4C	*Roseobacter*	Uncultured bacterium SB-8/30-AG	100.0	302	AJ319843	No
Skeletonema costatum	Skc5A	CFB	Uncultured marine bacterium OTU_A	99.4	311	AF207850	No
Skeletonema costatum	Skc5B	CFB	Uncultured *Bacteroidetes* bacterium	98.5	407	AF466714	No
Skeletonema costatum	Skc5D	*Sphingomonas*	*Erythrobacter aquimaris*	100.0	329	AY461443	No
Skeletonema costatum	Skc5G	*Sphingomonas*	*Erythrobacter aquimaris*	99.4	477	AY461443	No
Chaetoceros cf. *tortissimus*	Cht6B	CFB	Uncultured bacterium clone ARKIA-137	96.9	288	AF468277	No
Chaetoceros cf. *tortissimus*	Cht6C	*Alteromonadaceae*	*Pseudoalteromonas* sp. strain RE10F/2	100.0	329	AF118018	No
Chaetoceros cf. *tortissimus*	Cht6E	*Sphingomonas*	*Erythrobacter aquimaris* strain SW-140	98.4	246	AY461443	No
Thalassiosira gravida	Thg7C	CFB	Uncultured bacterium clone s34	95.3	428	AY171371	No
Thalassiosira gravida	Thg7E	*Roseobacter*	*Sulfitobacter* sp. strain HEL-77	100.0	301	AJ534229	No
Thalassiosira gravida	Thg7G	*Rhizobium*	Uncultured alpha-proteobacterium strain GWS-BW-H54M	99.7	288	AY515417	No
Pseudopedinella elastica	Pse8A	CFB	Uncultured Bay of Fundy bacterium BA10-1C	98.6	212	AF323553	No
Pseudopedinella elastica	Pse8B	*Roseobacter*	Marine alpha-proteobacterium AS-26	99.2	425	AJ391187	No
Pseudopedinella elastica	Pse8C	*Alteromonadaceae*	Uncultured gamma-proteobacterium	97.9	329	AF466930	No
Pseudopedinella elastica	Pse8E	*Marinobacter*	*Marinobacter* sp. strain PCOB-2	100.0	338	AJ000647	Yes
Nannochloropsis granulata	Nc19C	*Roseobacter*	Alpha-proteobacterium DGGE band C201	100.0	267	AF466928	Yes
Nannochloropsis granulata	Nc19D	*Roseobacter*	*Roseobacter* sp. strain DG869	99.2	242	AY258074	Yes
Nannochloropsis granulata	Nc19E	*Roseobacter*	*Roseobacter* sp. strain DG869	100.0	248	AY258074	Yes
Micromonas pusilla	Mcp10B	*Roseobacter*	*Roseobacter* sp. strain KT0202a	98.7	253	AF305498	No
Micromonas pusilla	Mcp10C	CFB	Bacterium DG890	90.9	452	AY258122	No
Micromonas pusilla	Mcp10D	*Sphingomonas*	Alpha-proteobacterium MBIC3952	99.5	380		No
Tetraselmis sp.	Trs11A	*Roseobacter*	*Roseobacter* sp. strain ANT9276a	99.2	257	AY167262	No
Tetraselmis sp.	Trs11C	*Roseobacter*	Uncultured bacterium clone OHKB4.31	98.1	209	AB094841	No
Tetraselmis sp.	Trs11E	*Roseobacter*	Uncultured *Rhodobacter* group clone D103	100.0	265	AF367399	No
Tetraselmis sp.	Trs11F	*Roseobacter*	Uncultured *Rhodobacter* group clone D103	99.0	391	AF367399	No
Prorocentrum minimum	Prm12B	*Alteromonadaceae*	Uncultured bacterium CTD70B	96.8	282	AF469317	No
Prorocentrum minimum	Prm12c	*Roseobacter*	*Roseobacter* sp. strain DG942	99.3	281	AY258088	Yes
Prorocentrum minimum	Prm12D	*Roseobacter*	Uncultured proteobacterium clone BMS8	98.3	289	AY193221	No
Prorocentrum minimum	Prm12F	*Roseobacter*	*Antarctobacter* sp. strain 667-12	100.0	261	AJ294356	Yes
Scrippsiella sp.	Scp13A	CFB	*Cryomorphaceae* bacterium UST20020801	92.9	297	AB125062	No
Scrippsiella sp.	Scp13B	CFB	Uncultured *Cytophagales* bacterium clone SL-6a	96.5	462	AY337037	No
Scrippsiella sp.	Scp13D	*Roseobacter*	Marine bacterium ATAM173a_17	100.0	276	AF359532	Yes
Scrippsiella sp.	Scp13F	Gamma-*Proteobacteria*	Marine gamma-proteobacterium HTCC2188	100.0	279	AY386344	No
A. fundyense CB301	Isolate CB2	CFB	Arctic sea ice-associated bacterium ARK10007	93.0	299	AF468407	No
A. fundyense CB301	Isolate CB8	*Roseobacter*	Marine bacterium SCRIPPS_732	99.8	492	AF359534	No
A. fundyense CB301	Isolate CB3	*Marinobacter*	*Marinobacter* sp. strain PCOB-2	100.0	520	AJ000647	Yes
A. fundyense CB301	Afe14E	*Roseobacter*	Alpha-proteobacterium GMDJE10F1	98.8	397	AY162092	No
A. fundyense 48.1	Afa15A	*Roseobacter*	Marine bacterium SCRIPPS_732	100.0	269	AF359534	No
A. fundyense 48.1	Afa15B	*Roseobacter*	Marine bacterium SCRIPPS_732	99.8	492	AF359534	No
A. fundyense 48.1	Afa15D	*Roseobacter*	Marine bacterium SCRIPPS_732	99.0	409	AF359534	No
A. fundyense 48.1	Afa15D	*Roseobacter*	Marine bacterium SCRIPPS_732	99.6	515	AF359534	No
A. fundyense 48.2	Afb16A	CFB	*Tenacibaculum mesophilum*	96.3	299	AB032502	No
A. fundyense 48.2	Afb16B	CFB	Uncultured *Bacteroidetes* bacterium	90.7	389	AY274863	No
A. fundyense 48.2	Afb16C	*Roseobacter*	Uncultured alpha-proteobacterium	99.7	350	AF466897	Yes
A. fundyense 48.2	Afb16D	*Roseobacter*	Marine bacterium ATAM173a_17	99.3	300	AF359532	Yes
A. fundyense 48.2	Afb16E	Gamma-*Proteobacteria*	Uncultured gamma-proteobacterium clone SIMO-358	100.0	46	AF022407	No
A. fundyense 48.2	Afb16F	*Roseobacter*	*Sulfitobacter* sp. strain H24	99.7	334	AY277266	Yes
A. fundyense 5.6	Afc17A	CFB	*Bacteroidetes* bacterium KMM 3912	97.5	360	AY243096	No
A. fundyense 5.6	Afc17B	CFB	*Bacteroidetes* DGGE band AB-1	100.0	475	AY353553	No

Continued on facing page

TABLE 2—*Continued*

Source culture	DGGE band	Phylogenetic group	Closest relative	% Similarity	No. of base positions compared	GenBank accession no.	Association with toxic dinoflagellate[b]
A. fundyense 5.6	Afc17C	*Roseobacter*	Marine bacterium ATAM173a_16	99.2	264	AF359531	Yes
A. fundyense 5.6	Afc17H	*Rhizobium-Agrobacterium*	*Mesorhizobium* sp. strain DG1023	99.7	393	AY258096	Yes
A. fundyense 38.3	Afd18A	CFB	Uncultured marine eubacterium OTU B	98.6	347	AF207851	No
A. fundyense 38.3	Afd18B	*Rhizobium-Agrobacterium*	Uncultured alpha-proteobacterium clone A115-20	98.1	312	AY323139	No
A. fundyense 38.3	Afd18C	CFB	*Antarcticum* aff. *vesiculatum*	99.1	220	Y17133	No
A. fundyense 38.3	Afd18D	CFB	Uncultured *Bacteroidetes*	100.0	307	AY353555	No
A. tamarense ATRU4/1	Atb4′F	No close relatives	Unidentified bacterial clone K2-S-5	87.3	401	AY344413	No
A. tamarense OK905-7	Atc5′A	CFB	Bacterium DG945	100.0	427	AY258123	Yes
A. tamarense OK905-7	Atc5′B	*Roseobacter*	*Ruegeria atlantica*	99.7	400	AF124521	No
A. tamarense OK905-7	Atc5′C	*Roseobacter*	*Ruegeria* sp. strain TCg-9	99.4	345	AJ515042	No
A. minutum GT-Port	Atd6′A	*Roseobacter*	Uncultured *Roseobacter* 253-16	99.5	206	AJ294352	Yes
A. minutum GT-Port	Atd6′B	*Roseobacter*	Uncultured *Roseobacter* 253-16	100.0	306	AJ294352	Yes
A. minutum GT-Port	Atd6′D	*Roseobacter*	Uncultured *Roseobacter* 253-16	100.0	398	AJ294352	Yes
A. tamarense AATL01	Ate7′A	CFB	Uncultured bacterium SB-29-AG	99.7	341	AJ319850	No
A. tamarense AATL01	Ate7′C	Roseobacter	Marine bacterium ATAM407_56	100.0	300	AF359535	Yes

[a] Sequences that were related to chloroplast 16S rRNA genes are not included.
[b] Cases in which there was >99% similarity between our sequence and a sequence that originated from a toxic dinoflagellate culture or bloom were considered to have positive evidence for association with toxic dinoflagellates. In cases where a reference is not listed, evidence for an association with toxic dinoflagellates was based on information available from GenBank entries.

lates. These results provide further evidence that there is selection of specific bacterial species associated with dinoflagellates.

The *Roseobacter* clade, in particular, was prevalent among sequences that we considered to be associated with toxic dinoflagellates. For example, for the *Alexandrium* spp. cultures analyzed here, 11 of 19 bands that were considered bands from associates of toxic dinoflagellates were *Roseobacter* sequences. Other phylogenetic groups that were represented among the associates of toxic dinoflagellates, but were less prevalent, included *Marinobacter* sp. strain PCOB-2, the *Rhizobium-Agrobacterium* group, and the CFB phylum. Sequences that fell within the *Altermonadaceae* family and the *Sphingomonas* group were associated only with nontoxic phytoplankton cultures.

A phylogenetic tree of the *Roseobacter* group 16S rRNA gene fragments (Fig. 4) contained a particular cluster of closely related sequences that are associated with the *A. fundyense* cultures analyzed here and an *A. tamarense* culture analyzed by Hold et al. (30), and similar topologies were obtained by all three methods used for tree construction (data not shown). While sequences of our DGGE bands did not provide sufficient phylogenetic information for a robust bootstrap analysis of this group, members of this cluster are clearly very closely related. Also evident from Fig. 4 is the divergence between sequences affiliated with *Alexandrium* spp. and several sequences from the *Tetraselmis* sp. culture (sequences Trs11C, Trs11E, and Trs11F), the *Micromonas pusilla* culture (Mcp10B), and the *Nannochloropsis granulata* culture (Ncl9E and Ncl9D).

In contrast to the *Roseobacter* group sequences, which were at least 98% similar to their closest GenBank relatives (Table 2), sequences that fell within the CFB group exhibited greater phylogenetic diversity and were frequently not closely related to any reported sequences, and the levels of similarity with their closest GenBank relatives were as low as 90.7% (Table 2). CFB group sequences that were considered to be from associates of toxic dinoflagellates (Emh3C and Atc5′A) were found to be identical to sequences retrieved from toxic *Gymnodinium catenatum* cultures (24).

Similarity values for DGGE profiles of bacteria associated with phytoplankton. While the complexity of the DGGE profiles made it difficult to visually discern patterns across samples, analysis of pairwise similarity values for DGGE profiles revealed several interesting results. Pairwise coefficients (Fig. 5) were calculated using DGGE data compiled in Fig. 3, which took multiple DGGEs and sequence analysis into consideration. Interestingly, two areas where there were higher similarity values are evident in Fig. 5; these areas are the comparisons among nontoxic phytoplankton from the Gulf of Maine and the comparisons among *Alexandrium* spp. from the Gulf of Maine and other regions of the world. In fact, if one sequence (which was identical to the *Marinobacter* sp. strain PCOB-2 sequence and was present in *Pseudopedinella elastica*, *Scrippsiella*, *A. fundyense* strain CB301, and *A. tamarense* strain ATRU10/1) was excluded, all similarity values for *Alexandrium* spp. and non-*Alexandrium* spp. bacterial assemblages were zero. With all of the data considered, a two-tailed *t* test showed that the similarity values among non-*Alexandrium* samples were significantly higher than the similarity values among non-*Alexandrium* samples and *Alexandrium* samples at a P value of <0.001. Similarly, the similarity values among *Alexandrium* samples were significantly higher than the similarity values for comparisons between *Alexandrium* samples and non-*Alexandrium* samples at a P value of 0.014. There was no significant difference ($P = 0.655$), however, between *Alexandrium* cultures from the Gulf of Maine and *Alexandrium* cultures from around the world (Japan, Portugal, Russia, France, and the Bering Sea).

DISCUSSION

While bacteria are known to be closely associated with phytoplankton and are thought to influence phytoplankton population dynamics and toxicity, the phylogenetic identity and specificity of bacterium-phytoplankton interactions are only beginning to be explored. In the current study, we conducted an extensive survey of bacteria associated with toxic and nontoxic phytoplankton. Our approach was to use 16S rRNA gene DGGE to analyze bacterial associates of at least one representative of each major phylogenetic group of phytoplankton from the Gulf of Maine, as well as multiple toxic *Alexandrium* spp. cultures originating from the Gulf of Maine, Japan, coastal Portugal, France, Russia, and the Bering Sea, in order to determine the phylogenetic diversity of the bacterial associates and whether there were specific associations between bacteria and phytoplankton. While the DGGE profiles were complex and highly variable, we found higher levels of similarity for bacterial assemblages from *Alexandrium* spp. cultures from around the world than for *Alexandrium* spp. cultures and nontoxic cultures from the same region, indicating that there was selection for certain bacterial phylotypes in *Alexandrium* cultures. In particular, a cluster in the *Roseobacter* group was associated with toxic dinoflagellate cultures analyzed here and in other studies (3, 4, 24, 30), as were several CFB strains.

Limitations of the experimental approach. Given the complexity of natural phytoplankton communities (50) and the fact that *Alexandrium* spp. can cause paralytic shellfish poisoning at levels as low as 200 cells liter^{-1}, we relied on unialgal cultures to study bacterium-phytoplankton interactions. While a similar approach has been used in other studies (24, 30, 51), this experimental system introduced several potential biases into our analyses. For example, the environment of a unialgal culture growing in nutrient-enriched medium under laboratory conditions is quite different from the environment experienced by a member of a complex community subjected to variable natural conditions. Only bacteria capable of growing with that alga under laboratory conditions persist after successive transfers. Perhaps equally important, only the bacteria associated with the single algal cell used to establish the culture have the opportunity to be present as the culture is grown and transferred. It is unlikely that the bacterial species associated with a single cell accurately represent all bacteria associated with a particular algal population, and it is even more unlikely that they are representative of the bacteria associated with a given alga over a range of ecological and environmental conditions. Nonetheless, analyzing the bacteria in unialgal cultures provides a means to separate individual algal species from their complex natural matrix and is a first step toward understanding bacterium-phytoplankton interactions.

We attempted to minimize potential biases of this experimental system by using several criteria to select cultures for analysis. For our survey of diverse phytoplankton taxa, we focused on cultures from one geographic area (the Gulf of Maine) and included at least one representative of each major taxonomic group of phytoplankton. We felt that the Gulf of Maine provided an ideal geographic area as it is a highly productive, temperate, coastal environment in which diverse eukaryotic phytoplankton are the dominant primary producers and in which *Alexandrium* blooms occur annually (5, 50, 53). For *Alexandrium* species cultures, we included five cultures from the Gulf of Maine and then chose other cultures that represented a broad geographic range of this cosmopolitan genus. In order to minimize biases associated with cultivation, we chose cultures with a minimal time since the original isolation and similar growth conditions (Table 1). Because we were interested in bacteria associated with actively growing phytoplankton, we focused on cultures that originated from vegetative cells from the water column, not dormant stages, such as *Alexandrium* spp. cysts, found in the sediment. In contrast with other studies of bacterium-phytoplankton associations in cultures (24, 30), we analyzed a relatively large number of diverse phytoplankton cultures in order to obtain a robust comparison of bacterial associates and more accurately determine the specificity of the associations.

Our comparison of DGGE profiles of free-living and attached bacteria associated with *A. fundyense* strain CB301 (Fig. 2), as well as our analysis of the DGGE profiles of samples taken during different phases of *A. fundyense* batch culture (results not shown), indicated that there was no qualitative difference in the dominant DGGE bands. These results are similar to those reported by Hold et al. (30) and suggest that it was not necessary to analyze multiple phytoplankton growth stages or free-living and attached bacterial fractions in order to determine the phylogenetic identities of dominant bacteria associated with phytoplankton cultures.

Evidence for specific interactions. Our results revealed patterns of specific associations between *Alexandrium* and bacteria. Pairwise similarity coefficients for bacterial species composition in phytoplankton cultures (Fig. 5) revealed significantly higher levels of similarity among *Alexandrium* cultures than between nontoxic phytoplankton and *Alexandrium* cultures. This relationship was true regardless of the geographic location from which *Alexandrium* species cultures were obtained. In addition, compared to diverse nontoxic phytoplankton, the *Alexandrium* spp. cultures analyzed here contained a higher percentage of bacterial sequences that matched database sequences from other dinoflagellate cultures (Table 2 and Fig. 3), including cultures of *G. catenatum* isolated from New Zealand and Japan (24), *Alexandrium* species from British Columbia, Canada, and Plymouth, United Kingdom, and *Scrippsiella trochoidea* from British Columbia, Canada (30). While cross-contamination between cultures is always a concern, the fact that these dinoflagellate cultures were isolated from and grown in different regions of the world makes this an unlikely explanation of our results. Similarly, while we cannot rule out selection by artificial laboratory growth conditions, the fact that diverse phytoplankton were cultivated under similar conditions (Table 1) suggests that factors other than cultivation conditions contributed to the observed similarities. For example, *S. costatum*, *Thalassiosira gravida*, *N. granulata*, *Prorocentrum minimum*, *Scrippsiella* sp., and all *Alexandrium* cultures were cultivated in f/2 medium, yet *S. costatum*, *T. gravida*, *N. granulata*, and *P. minimum* shared no bacterial associates with *Alexandrium* cultures. Lastly, phylogenetic analysis of members of the *Roseobacter* clade, which was the most predominant group among the *Alexandrium*-associated bacteria, indicated that many sequences from *Alexandrium* spp. cultures were closely related to each other and divergent from several *Roseobacter* sequences obtained from cultures of *Micromonas pu-*

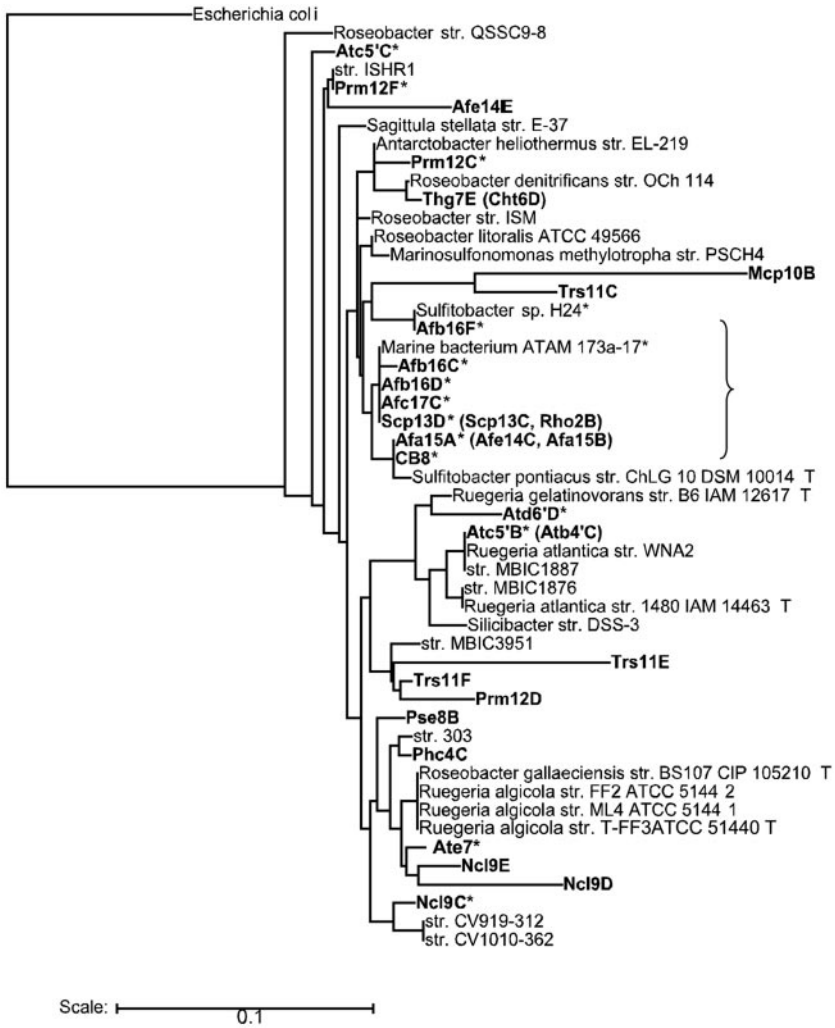

FIG. 4. Phylogenetic tree of 16S rRNA gene sequences belonging to the *Roseobacter* group. Sequences obtained in the current study are indicated by boldface type. Sequences considered to show evidence of association with toxic dinoflagellates are indicated by asterisks. Distances were calculated using the maximum-likelihood method, and the tree was constructed using the neighbor-joining method.

silla (e.g., Mcp10B), *Tetraselmis* sp. (Trs11C and Trs11E), and *N. granulata* (Ncl9D) (Fig. 4). Not surprisingly, these results did not reveal a single bacterium that was consistently found in all toxic or nontoxic dinoflagellate cultures. Instead, they revealed a pattern in which certain bacterial phylotypes were more commonly observed in dinoflagellate cultures than in other cultures. However, given the potential limitations of our approach (i.e., the bacterial associates of each culture were a subset of the bacteria associated with a single phytoplankton cell at the time of isolation), the emergence of such a pattern is especially interesting.

The predominant phylogenetic groups of bacteria found in this study were very similar to those previously found to be associated with algal cultures (3, 4, 24, 30, 41, 51). In fact, more than 60% of all the sequences found were more than 99% similar to their closest database relative, and only three sequences (Afb16B, Atb4′F, and Mcp10C) had no close relatives. Several studies have found a prevalence of *Roseobacter* spp. associated with algal cultures, including cultures of dinoflagellates (3, 4, 24, 30) and diatoms (51). Similarly, members of the family *Alteromonadaceae*, the CFB group, and the sphingomonads have been found to be associated with algae (16, 17, 24, 30). While these bacterial families and broader phylogenetic groups appear to be important associates of algae in general, a pattern of specific bacterium-alga associations is evident from the prevalence of closely related phylogenetic clusters within these groups that are associated with closely related phytoplankton.

In the current study, only one nontoxic dinoflagellate (*Scrippsiella* sp.) was analyzed. Other studies of bacteria associated with dinoflagellates have also focused on toxic species (4, 24, 30). Therefore, it is difficult to determine whether the observed selection of specific bacterial phylotypes in the presence of dinoflagellates was due to the toxicity of *Alexandrium* species or to some other feature of *Alexandrium* physiology. Our finding that there were two groups with higher levels of similarity for the bacterial profiles, bacteria from diverse nontoxic phytoplankton and bacteria from *Alexandrium* (Fig. 5), suggests that toxicity may play a role in selecting for specific bacteria. Saxitoxin has recently been shown to influence so-

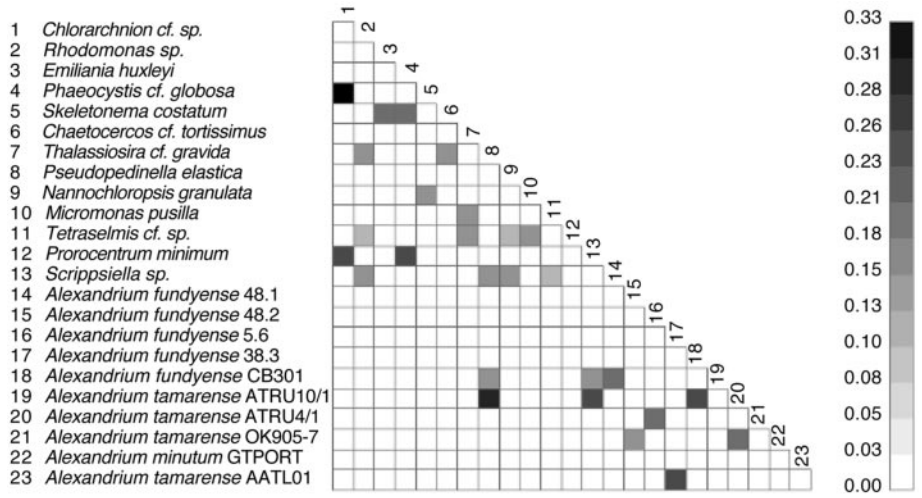

FIG. 5. Plot of pairwise similarity values for DGGE profiles of bacterial assemblages associated with phytoplankton cultures.

dium and potassium channel activity in prokaryotes and, in saxitoxin-producing cyanobacteria, may maintain homeostasis under sodium stress conditions (47). It has also been shown to mitigate the effects of a sodium channel activator, veratridine, in *Vibrio fischeri* (47). It is therefore conceivable that the production of saxitoxin by dinoflagellates selects for specific bacteria that are better able to maintain sodium and/or potassium homeostasis in its presence. Alternatively, cell wall components, exudates, production of osmolytes such as dimethyl sulfoniopropionate (DMSP), pigment composition, and life cycle features characteristic of dinoflagellates may select for specific bacterial phylotypes. For example, in some thecate dinoflagellates ecdysis occurs as part of the life cycle (45). During this process the outermost membranes, thecal plates, and flagella are shed during cell division. In natural actively growing populations, these cell coverings could provide a substrate for specific bacterial consortia. Also, hypnocysts formed during the life cycle of *Alexandrium* have recently been shown to harbor intracellular bacteria that could maintain a long-term connection between the vegetative and dormant stages of *Alexandrium*. These cyst-associated bacteria could seed germinating populations of vegetative *Alexandrium* cells, maintaining a bacterial association even without the need for endocytobiosis after each dormancy period (52).

Dominant bacterial associates of other phytoplankton. The phytoplankton cultures analyzed here represent the full taxonomic breadth of the Gulf of Maine eukaryotic phytoplankton community (Table 1). While all of the phytoplankton are photosynthetic, they vary greatly in terms of size, life history, motility, cell wall constituents, pigment composition, and cellular storage products. For example, the cell walls of the dinoflagellates analyzed (*Alexandrium* spp., *Scrippsiella* sp., and *Prorocentrum lima*) are comprised of cellulose theca, while those of the diatoms (*Chaetoceros tortissimus*, *S. costatum*, and *T. gravida*) are comprised of silica frustules and those of the cryptophyte *Rhodomonas* sp. are comprised of an organic periplast (37). Storage products range from starch (dinoflagellates, cryptophytes) to chrysolaminarin and lipids (diatoms, prymnesiophytes) and mannitol (prasinophytes) (37). These differences in the biochemical makeup of divergent phytoplankton species are likely to select for different associated bacterial species that may rely on organic compounds available in the phycosphere. In addition, some phytoplankton are known to produce antibiotics that can exert strong selective pressures (54, 55).

Indeed, several differences were evident in the bacterial assemblages found in diverse nontoxic phytoplankton cultures compared with those found in *Alexandrium* species cultures. Compared to *Alexandrium* species associates, these bacteria included a greater prevalence of members of the CFB phylum, the family *Alteromonodaceae*, and the *Sphingomonas* group; the latter two were absent from *Alexandrium* cultures (Fig. 3). While members of the *Roseobacter* clade were present in diverse nontoxic phytoplankton cultures analyzed here, they were not observed as frequently as they were in dinoflagellate cultures (Fig. 3). In addition, bacteria from nontoxic cultures were more likely to match database sequences retrieved directly from the environment or to have no close relatives in the databases (Table 2). These results are not surprising given the dearth of studies of (and therefore 16S rRNA gene sequence information for) bacteria associated with phytoplankton other than dinoflagellates.

Potential function of bacterial associates of phytoplankton. Bacteria have been shown to have many effects on phytoplankton, including algicidal activity (44, 58), stimulation of phytoplankton growth (16), production or modulation of toxicity (20, 21, 35), and inhibition or promotion of cyst formation (2, 18). Bacteria may stimulate phytoplankton growth via the production of vitamins (28), iron chelators (siderophores) (56), and cytokinins (40). Our findings and those of other workers underscore the importance of the *Roseobacter* clade in associations with phytoplankton and, especially, dinoflagellates. For example, members of the *Roseobacter* clade were found to be prevalent in cultures of *A. tamarense* (2, 30), *Alexandrium ostenfeldii* (4), *S. trochoidea* (30), *G. catenatum* (24), and *Pfiesteria*-like species (3). This clade is known to be a dominant member of coastal bacterial communities (10, 14, 15, 23), and previously, we found that it is ubiquitous across seasonal (February, May, July, and September) and spatial gradients in the

northern Gulf of Maine (49). Members of this group possess diverse metabolic capabilities. *Roseobacter* species have been shown to be capable of degrading lignins and other aromatic ring compounds, including vanillate, coumarate, cinnamate, ferulate, benzoate, and *p*-hydroxybenzoate (8), which may be present in dinoflagellate phycospheres. Many *Roseobacter* isolates have been shown to utilize DMSP as both a carbon source and a sulfur source, and it is likely DMSP metabolism is important in *Roseobacter*-phytoplankton interactions (22, 23, 43). However, because the abilities to produce and consume DMSP are so widespread among phytoplankton and roseobacters, respectively, it seems unlikely that DMSP metabolism resulted in the prevalence of a narrow phylogenetic cluster of roseobacters in the *Alexandrium* spp. cultures observed here.

Conclusions. Interactions between bacteria and phytoplankton are thought to be important in controlling the dynamics of both communities and yet are only beginning to be understood at the species composition level. Our results provide evidence for specific bacterium-phytoplankton associations, especially between toxic dinoflagellates and members of the *Roseobacter* clade. This result was supported even though toxic dinoflagellates were isolated from different regions of the world and were grown under conditions that were very similar to those used for diverse phytoplankton taxa. Phylogenetic analysis of bacteria associated with a wide diversity of phytoplankton revealed the prevalence of members of the *Roseobacter* clade, the CFB phylum, and the *Alteromonadaceae* family, indicating that members of these groups are well adapted to living in close association with phytoplankton and that specific clusters within these groups are selected for in association with different phytoplankton.

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