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Numerical Dominance of a Group of Marine Bacteria in the α-Subclass of the Class *Proteobacteria* in Coastal Seawater

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A cluster of marine bacteria within the α-3 subclass of the class *Proteobacteria* accounted for up to 28% of the 16S ribosomal DNA (rDNA) sequences in seawater samples from the coast of the southeastern United States. Two independent oligonucleotide probes targeting 16S rDNA of this "marine alpha" cluster indicate that the group dominates bacterioplankton communities in estuarine and nearshore regions of the southeastern U.S. coast. Marine alpha bacteria decline predictably in abundance with decreasing salinity along estuarine transects and are not detectable in low-salinity (5‰) or freshwater samples. Sequences of 16S rDNA obtained from seawater by PCR with one group-specific oligonucleotide as a primer confirm that the oligonucleotide targets only members of this phylogenetic cluster. Likewise, sequences of 16S rDNA obtained from seawater by PCR with several different pairs of nonspecific primers show an unusually high abundance of marine alpha sequences (52 to 84%) among the clones, which possibly indicates a PCR bias toward the group. Members of the marine alpha group were readily cultured from coastal seawater, accounting for 40% of the colonies isolated on low-nutrient marine agar, based on hybridizations with the group-specific 16S rDNA probe and on sequence analysis. This is the first description of a numerically dominant cluster of coastal bacteria, identified by molecular techniques, that can be readily cultured and studied in the laboratory.

The composition of bacterial communities of estuaries and coastal regions of oceans is largely unknown, despite the substantial roles many coastal bacteria play in critical biogeochemical cycles and the potential utility of such bacteria for bioremediation and other biotechnological applications. The lack of knowledge of this important group of microorganisms can be attributed directly to their low culturability by standard microbiological techniques and to a reluctance on the part of marine microbiologists to study the small, potentially unrepresentative group of bacterioplankton that can be readily cultured from coastal seawater. Recent research on bacterial clones from marine environments has presented a further dilemma: most of the major lineages of oceanic bacteria identified by 16S rRNA gene sequences do not include previously cultured organisms (4, 7, 10, 23, 25, 34). Thus, although several of these lineages have proven to be ubiquitous in the ocean (23), little or nothing is known about the functional and ecological roles of their members. Successful culturing of representative bacteria from numerically dominant coastal lineages is therefore of enormous interest for studying the microbiology, biogeochemistry, and ecology of the land-sea interface.

We recently obtained from a sample of coastal Georgia seawater a number of culturable bacteria and bacterial ribosomal DNA (rDNA) clones that appeared to be close phylogenetic relatives based on 16S rDNA sequence analysis (12, 13). These isolates and clones are affiliated with a phylogenetically distinct group of exclusively marine bacteria in the α-3 subclass of the class *Proteobacteria* which we refer to as the "marine alpha group" (Fig. 1). The marine alpha group includes isolates from a number of marine environments (e.g., Mediterranean coastal lagoon, Atlantic Ocean central gyre, Black Sea, and Antarctic sea ice) with diverse metabolic capabilities (e.g., phototrophy, aerobic sulfite oxidation, organic sulfur compound degradation, and lignin degradation) (1, 2, 12, 14, 18, 28, 31). It also includes several oceanic (e.g., SAR83 and SAR102/122) and coastal ocean region (e.g., OCS19 and OCS84) bacterial clones from important lineages of marine bacteria whose metabolic capabilities are yet to be determined (23, 34). In every case investigated, isolates from the marine alpha group have been found to have a salt requirement for growth (2, 12, 15, 16, 18, 30).

Based on the large number of marine alpha isolates and clones retrieved from the coastal seawater sample and indications that this group is exclusively marine, we designed oligonucleotide probes to 16S rDNA to quantify the marine alpha cluster in a number of estuarine and coastal environments of the southeastern United States. We report here that the marine alpha group comprises a surprisingly large fraction of these bacterial communities, and we describe the retrieval of new isolates and 16S rDNA clones from the group, including a clone and isolate that have identical 16S rDNA sequences.

MATERIALS AND METHODS

Design of 16S rDNA oligonucleotide probes. A group-specific probe for members of the marine alpha cluster was designed based on 16S rRNA sequences from GenBank. The probe, 5′-GCCGGGGTTTCTTTACCA (positions 488 to 507 [*Escherichia coli* numbering system]), designated MALF-1 (13), is common to members of the marine alpha group but contains several mismatches with closely related groups (Table 1). The exception is the dimethylsulfoniopropionate (DMSP)-degrading bacterium isolated by Ledyard et al. (18), which falls within the marine alpha group but is not targeted by MALF-1. Based on sequence information from isolates and clones within the marine alpha group (Fig. 1), the MALF-1 probe targets a group of organisms with >90% sequence similarity. The marine alpha probe was checked for specificity in dot blot hybridizations with members of the group as positive controls (*Roseobacter algicola*, *Roseobacter litoralis*, *Roseobacter denitrificans*, *Sagittula stellata*, and isolate EE-36 [see below]) and organisms outside the group as negative controls (*Paracoccus denitrificans* and *Rhodobacter sphaeroides*) (Table 1). A second probe was designed to target a subgroup (but possibly not a monophyletic subgroup) of sequences inside the marine alpha cluster. The probe, 5′-GGAATAGCCWCT GGAAACGGWGAGTAATACC (positions 145 to 177 in *E. coli*), designated MALF-1b, is common to the coastal clones obtained in this study and to *R. algicola* (Table 1). *R. denitrificans* and *R. litoralis* served as negative controls in dot blot hybridizations. Probe labeling and hybridization conditions were as described in González et al. (13).

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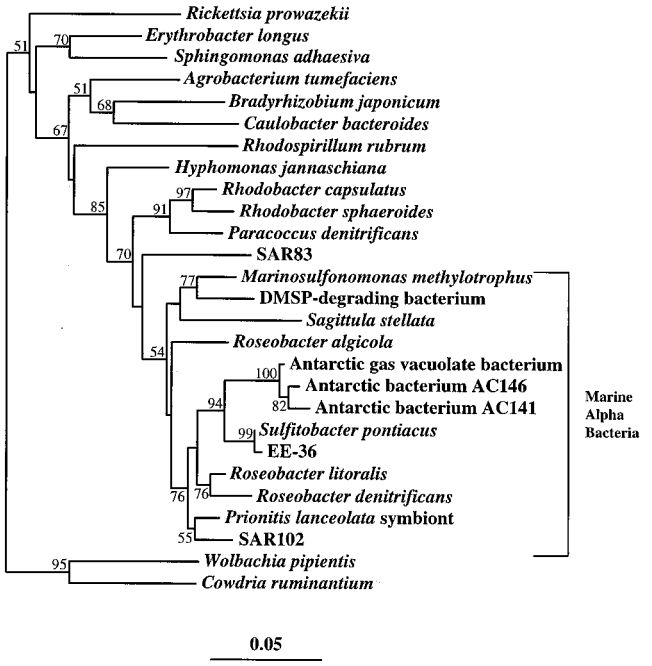

FIG. 1. Phylogenetic tree with representative members of the α-subclass of *Proteobacteria*. The marine alpha bacteria, indicated by the bracket, all have sequence similarities of >90%. The most closely related bacteria outside the group are in the genera *Rhodobacter* and *Paracoccus*. The tree is based on positions 40 to 430 (*E. coli* numbering system) of the 16S rRNA gene and is unrooted, with *E. coli* as the outgroup. Bootstrap values greater than 50% are indicated. The bar represents the Jukes-Cantor distance.

Community DNA samples. Water samples were collected from four sites in coastal Georgia: Sapelo Island (January 1994), the Altamaha River estuary (May 1996), and the Satilla and Savannah River estuaries (October 1996) (Fig. 2). For DNA extraction, approximately 15 liters of water was prefiltered through 1-μm (Sapelo Island and Altamaha River samples)- or 3-μm (Satilla and Savannah River samples)-pore-size polycarbonate filters. Bacterial cells in the filtrate were collected on 0.2-μm-pore-size filters with a 293-mm-diameter plexiglass filter holder and stored at −20°C until DNA extraction (13). Extracted DNA was treated with 50 μg of RNase ml^{-1} for 15 min. This procedure yielded approximately 0.1 mg of bacterial DNA per filter.

Community DNA hybridization. The numerical importance of members of the marine alpha group in DNA samples from coastal seawater was determined by dot blot hybridizations with the oligonucleotide probes. DNA from representatives of the group (*R. denitrificans* and *R. litoralis* for hybridizations with MALF-1 and *R. algicola* for hybridizations with MALF-1b) was extracted by the same protocol as for community DNA, with the exception that cell pellets rather than membrane filters were used as the source of DNA. Membranes were prepared by spotting multiple dots of increasing amounts of DNA from the standards, ranging from 50 to 500 ng, and duplicate dots of 1,000 ng of community DNA. DNA was quantified with Hoechst dye 33258 (Sigma, St. Louis, Mo.) immediately before use, and the volume used for membrane preparation was adjusted to 100 μl with Tris-EDTA buffer. DNA was denatured by the addition of 300 μl of 0.4 M NaOH and incubation for 30 min at room temperature, spotted on the membrane with a dot blot apparatus, and then neutralized with 1 M ammonium acetate for 1 min. Finally, the DNA was UV cross-linked to the membrane. Oligonucleotide labeling and hybridization conditions were as described previously (13). The hybridization signal was quantified by using a laser densitometer (Molecular Dynamics, Sunnyvale, Calif.). The probe 19F (19) was also used in dot blot hybridizations to quantify the α-subclass of *Proteobacteria* in the coastal DNA samples, and the universal probe 1406R (17) was used to quantify total rDNA genes. Contributions from the marine alpha group and α-*Proteobacteria* to community DNA were determined by comparisons of the hybridization signals from the group-specific probes to the signal from the universal probe (11).

PCR amplification and cloning of PCR products. A primer pair consisting of MALF-1 and 19F was used to amplify 16S rRNA genes belonging to members of the marine alpha group from the Sapelo Island sample. The PCR reaction mixture contained 25 ng of DNA, 0.1 mM (each) deoxynucleoside triphosphate, 100 nM (each) primer, 5 mM $MgCl_2$, and 1 U of Thermalase rec-Tbr (Amresco, Solon, Ohio) polymerase in a final volume of 100 μl. After a hot start at 82°C for 10 min, the PCR temperature parameters were as follows: preheating at 94°C for 3 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min. After 45 cycles, PCR products were analyzed by electrophoresis in 3% (wt/vol) agarose gels to confirm size. Appropriate negative controls without community DNA were included. PCR products were cleaned with Wizard DNA cleanup minicolumns (Promega Biological Research Products, Madison, Wis.), and the purified product was ligated into pT7Blue T-vector and cloned into NovaBlue *E. coli* cells (Novagen Inc., Madison, Wis.) as described by the manufacturer. From the transformed colonies, nine clones were picked at random, and the plasmid DNA was extracted and purified with Wizard minicolumns. The sequence of each PCR product was determined on an automated sequencer (University of Georgia Molecular Genetics Instrumentation Facility) with MALF-1 as the sequencing primer.

Several other collections of 16S rDNA PCR products for which the regions targeted by MALF-1 and MALF-1b were internal were generated from the Sapelo Island sample. To generate PCR products containing the MALF-1 target region, a bacterial (338F [33])-universal (926R [17]) primer pair or the α-subclass-specific (19F)-926R primer pair was used. DNA from isolate EE-36 (see below) served as the positive control. To generate products containing the MALF-1b target region, the 19F-926R primer pair or the 19F-338R primer pair was used. In this case, DNA from *R. algicola* served as the positive control. The PCR products were spotted in triplicate (200 ng per dot) on nylon membranes, and hybridizations were performed with the MALF-1 or MALF-1b probes as described above. The signal was quantified as before, and the relative contributions of the sequences targeted by the marine alpha group probes in the sets of PCR products were calculated by setting the positive control signal equal to 100%.

Bacterial isolation. Bacteria were isolated from a coastal seawater sample collected in the Skidaway River in January 1997 (Fig. 2). Ten-microliter volumes of water were plated on low-nutrient agar plates containing 10 mg of peptone

TABLE 1. Specificity of probes MALF-1 and MALF-1b based on sequence analysis

Sequence	Accession number	Reference	MALF-1[a]	MALF-1b[a]
R. algicola	X78313	16	++	++
Isolates GAI-23 and GAI-36	AF007259	This study	++	+
GAC-1	AF007249	This study	*	+
GAC-2, GAC-6, and GAC-7	AF007250	This study	*	+
GAC-3	AF007251	This study	*	+
GAC-4 and GAC-9	AF007259	This study	*	+
GAC-5	AF007252	This study	*	+
GAC-8	AF007253	This study	*	+
R. denitrificans	M96746	29	++	−−
R. litoralis	X78312	29	++	−−
Antarctic gas vacuolate bacterium	U14583	14	+	−
Antarctic bacterium AC141	U85840	2	+	−
Antarctic bacterium AC146	AF001377	2	+	−
P. lanceolata gall symbiont	U37762	GenBank	+	−
Marinosulfonomonas methylotrophus	U62894	15	+	−
S. pontiacus	Y13155	GenBank	+	−
Isolate EE-36	AF007254	This study	++	−
Isolates GAI-3b and GAI-5b	AF007255	This study	++	−
Isolates GAI-5 and GAI-6	AF007256	This study	++	−
Isolate GAI-15	AF010285	This study	++	−
Isolates GAI-16 and GAI-21	AF007257	This study	++	−
Isolates GAI-26 and GAI-42	AF007258	This study	++	−
Isolates GAI-33 and GAI-47	AF010286	This study	++	−
Isolate GAI-37	AF007260	This study	++	−
S. stellata	U58356	12	++	−
SAR102	L35460	23	+	−
OCS84	U78943	34	+	−
OCS122	U78945	34	+	−
DMSP-degrading bacterium	L15345	18	−	−
SAR83	M63810	3	−	−
Rhodobacter spp.[b]			−−	−
Paracoccus spp.[b]			−−	−

[a] +, no internal mismatches with the probe; −, two or more mismatches. An additional + or − indicates that specificity was confirmed with dot blot hybridizations. An asterisk indicates that the probe was used as a PCR primer to obtain the clone.

[b] Only *R. sphaeroides* and *P. denitrificans* were used in the hybridizations.

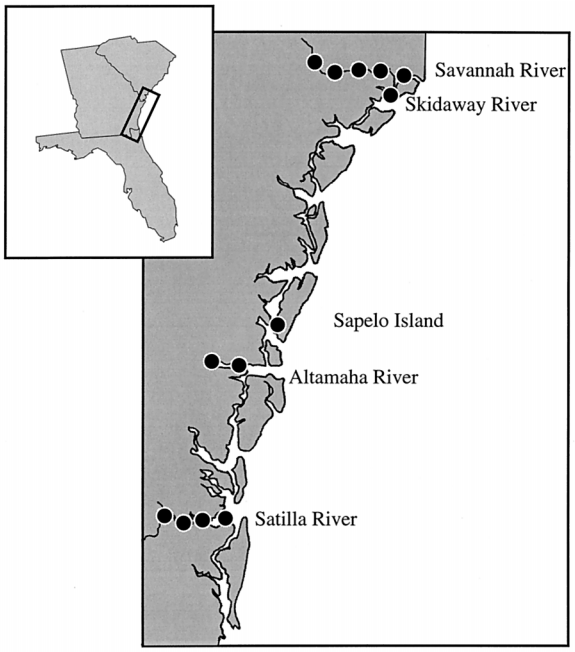

FIG. 2. Map of the coast of the southeastern United States showing sampling locations. All samples were collected between January 1994 and October 1996.

(Difco Laboratories, Detroit, Mich.)/liter, 5 mg of yeast extract (Difco)/liter, and 1.5% purified agar (Difco) in filter-sterilized Sargasso Sea water (24‰ final salinity) that had been aged for more than 1 year in the dark. Plates were incubated at 15°C for 35 days, after which 48 colonies were randomly selected for further study. Colonies were transferred twice on low-nutrient medium and then transferred to medium with a higher substrate concentration (1 g of peptone/liter and 0.5 g of yeast extract/liter). Finally, colonies were transferred to 10% YTSS medium (0.4 g of yeast extract [Difco]/liter, 0.25 g of tryptone [Difco]/liter, 20 g of sea salts [Sigma], 18 g of agar/liter in distilled water) and then stored at −70°C in freezing medium (20 g of sea salts/liter, 12% dimethyl sulfoxide, and 12% glycerol).

Isolates belonging to the marine alpha group (see below) were tested for pigment production by using methanol extractions as in Ledyard et al. (18). Cells were grown in the dark on 1 g of peptone/liter–0.5 g of yeast extract/liter medium. *R. denitrificans* served as a positive control. The production of bacteriochlorophyll *a* in the dark is a characteristic of the genus *Roseobacter* (30).

Screening of coastal isolates. DNA was extracted from the 48 colonies by an NaOH procedure (20). To screen for members of the marine alpha group, a sample containing 1 μg of extracted DNA was spotted onto a nylon membrane and hybridizations were carried out with the MALF-1 probe. Ten of the colonies giving positive hybridization signals were sequenced. For sequencing, colonies were washed twice in distilled water, resuspended in 50 μl of 10 mM Tris–1 mM EDTA–0.01% Nonidet P-40, and boiled for 5 min. A 1-μl aliquot of this solution was used directly in PCR reactions with the MALF-1–19F primer pair. PCR conditions were the same as those described for cloning, and the resulting products were cleaned with Wizard minicolumns and sequenced with the MALF-1 oligonucleotide as primer. Isolates GAI-5 and GAI-36 were also sequenced in the opposite direction after amplification with the 19F-926R primer pair, with the 19F primer being used for sequencing.

Phylogenetic analyses. For phylogenetic analyses of clones and isolates, only unambiguous positions were considered for the region spanning positions 40 to 430 of the 16S rRNA gene (*E. coli* numbering system). Sequences were aligned by using the Genetics Computer Group Inc. package (9). Phylogenetic trees and bootstrap analysis (100 replicates) were performed with the PHYLIP package (6) by using evolutionary distances (Jukes-Cantor distances) and the neighbor-joining method.

RESULTS

16S rDNA-based quantification of marine alpha bacteria. The MALF-1 oligonucleotide probe was used in dot blot hybridizations with community DNA to approximately measure the contribution of the marine alpha group to the total rDNA genes in coastal seawater samples. Hybridizations indicated

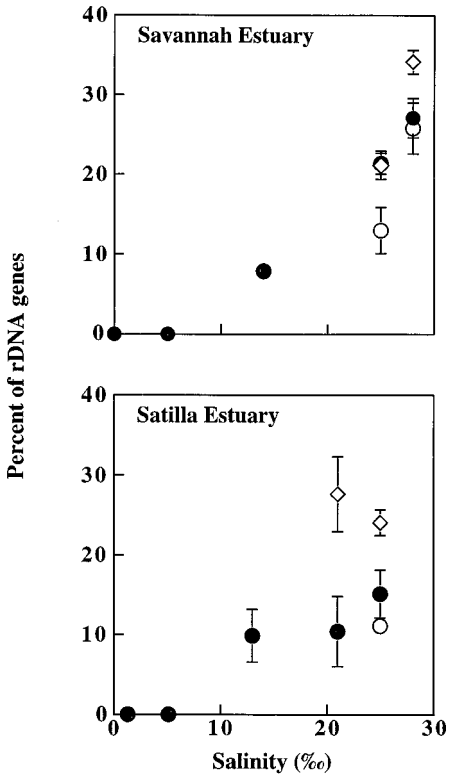

FIG. 3. Results of the hybridizations of MALF-1 (●)-, MALF-1b (○)-, and α-subclass (◇)-specific probes with samples from the Savannah River and Satilla River estuaries. Error bars (±1 standard deviation) fall within symbols if they are not shown.

that the marine alpha group accounted for 28% (±2%) of the bacterial DNA from the seawater sample collected at Sapelo Island. Within the Altamaha River estuary, the marine alpha signal at midestuary (14‰ salinity) was relatively strong (13% ± 0.2%), but the group was not detected in the upper, freshwater portion of the Altamaha River.

Subsequent seawater collections in two adjacent Georgia estuaries were carried out to confirm the high signal obtained with the MALF-1 probe and to better explore its relationship with salinity. Hybridization signals with the MALF-1 probe were likewise strongest for the high-salinity seawater in the Savannah (27% ± 4%) and Satilla (15% ± 3.0%) estuaries, and signals decreased predictably with decreasing salinity along each estuary (Fig. 3). The group was not detected in any sample with a salinity below 5‰.

Hybridizations of the oligonucleotide probe 19F to the two highest-salinity samples in the Savannah and Satilla estuaries indicated that the contribution from the α-subclass of *Proteobacteria* was always equal to or greater than that of the marine alpha bacteria. In the Savannah River samples, the marine alpha bacteria accounted for most of the α-subclass signal, while in the Satilla River samples, they accounted for about half of the signal (Fig. 3).

Cloning of marine alpha group sequences. Given the very high abundance of marine alpha bacteria indicated in the dot blot hybridizations, we used MALF-1 as one member of a primer pair to retrieve 16S rDNA sequences of this group from the Sapelo Island sample. All nine clones that were randomly selected and sequenced fell within the marine alpha cluster (clones GAC-1 through GAC-9 [Fig. 4]). Two clone sequences

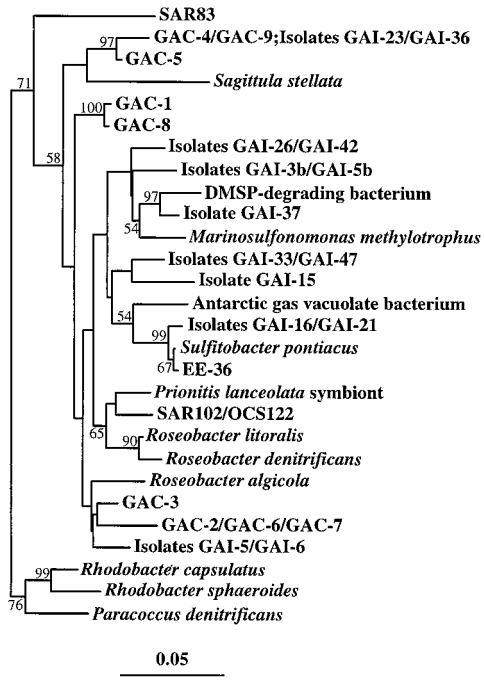

FIG. 4. Phylogenetic tree showing positions of clones and isolates with affinities to the marine alpha group based on positions 40 to 430 of the 16S rRNA gene. The tree is unrooted, with *E. coli* as the outgroup, and bootstrap values greater than 50% are indicated. The bar indicates the Jukes-Cantor distance. Clones and isolates with a "GA" prefix are from the southeastern U.S. coast (this study). Clones with an "OSC" prefix are from Suzuki et al. (34), clone SAR83 is from Britschgi and Giovannoni (3), and clone SAR102 is from Mullins et al. (23).

were retrieved more than once: GAC-4 and GAC-9 were identical and GAC-2, GAC-6, and GAC-7 were identical. None of the clone sequences was an exact match to any previously sequenced clone or organism represented in GenBank; the highest similarity was with the sequence of *R. algicola* (93 to 94% similarity).

16S rDNA-based quantification of marine alpha clones. The second marine alpha probe, MALF-1b, was designed to target only the sequences that were obtained by PCR amplification and cloning (GAC-1 through GAC-9) plus *R. algicola*. Dot blot hybridizations were carried out with a subset of the coastal DNA samples representing only the highest salinity. In the Altamaha River sample at 14‰ salinity, the MALF-1b group accounted for only 2% ± 0.1% of the rDNA, and in the Sapelo Island sample, it accounted for only 1% ± 0.3%. In the two highest-salinity samples from the Savannah River, the MALF-1b group accounted for 26% (± 3%) and 13% (± 3%) of the rDNA. In the highest-salinity sample from the Satilla River, the MALF-1b group accounted for 11% (± 0.4%).

Quantification of marine alpha 16S rDNA sequences in random PCR products. To further investigate the apparent dominance of the marine alpha group in coastal seawater DNA, we amplified 16S rDNA sequences from Sapelo Island DNA samples with nonspecific primers and then carried out dot blot hybridizations with the mixture of PCR products as the target and the MALF-1 and MALF-1b oligonucleotides as the probes. Hybridizations indicated that 52% (± 3%) of the sequences retrieved with the primer pair 19F-926R belonged to the marine alpha group based on hybridizations with the MALF-1 probe. From a separate PCR reaction with these same primers, hybridization indicated that 76% (± 8%) of the sequences belonged to the subset of the marine alpha group targeted by the MALF-1b probe. Similarly, 84% (± 12%) of the sequences retrieved with the primer pair 338F-926R hybridized to the MALF-1 probe and 86% (± 6%) of the sequences retrieved with the primer pair 19F-338R hybridized to the MALF-1b probe.

Culturable members of the marine alpha group. We also quantified the relative importance of the marine alpha group within the random subset of 48 bacteria isolated from Skidaway River seawater. The low-nutrient medium used in the isolation contained 0.1% of the substrate content commonly used in marine agar (i.e., Zobell's medium) and more closely matched the natural substrate concentration in coastal seawater. Previously, use of this medium with coastal Georgia seawater samples was found to increase the number and diversity of colonies obtained (27). Dot blot hybridizations of the MALF-1 probe against DNA from the 48 isolates indicated that 19 (40%) were members of the marine alpha group. The 16S rDNA of 14 of these isolates randomly chosen for sequencing (positions 40 to 430) indicated strong phylogenetic affinity to the clones obtained by PCR. Two of the isolates (GAI-23 and GAI-36) had sequences identical to each other and to GAC-4 and GAC-9 obtained by PCR with the MALF-1 probe (Fig. 4). Five other sets of duplicates were found: GAI-3b and GAI-5b, GAI-5 and GAI-6, GAI-16 and GAI-21, GAI-26 and GAI-42, and GAI-33 and GAI-47. All these pairs had identical 16S rDNA sequences but different colonial morphologies. In two cases (GAI-5 and GAI-36) the accuracy of the sequences was confirmed by repeating the sequencing in the opposite direction. None of the isolates' 16S rDNA sequences were an exact match with any of the previously sequenced bacteria in GenBank.

Isolates that were designated as marine alpha bacteria based on their 16S rRNA sequences were assayed for bacteriochlorophyll *a* production after growth in the dark. Isolates GAI-37 and GAI-5 showed an absorption spectrum of metabolic extracts typical of carotenoids (absorbance maxima between 450 and 500 nm), but bacteriochlorophyll production was not detected.

DISCUSSION

Evidence that marine alpha bacteria dominate bacterioplankton communities of coastal regions of the southeastern United States comes from three sources: hybridizations with two independent group-specific 16S rDNA oligonucleotide probes, sequences of 16S rDNA clones retrieved by PCR with both specific and nonspecific primers, and successful culturing of marine alpha bacteria in low-nutrient seawater agar. Hybridizations with the MALF-1 and MALF-1b probes indicated contributions by the marine alpha group ranging up to 28% of the bacterial rDNA genes in coastal seawater samples collected over a 3-year period. In estuarine water, results of hybridizations showed a predictable decline in the percent contribution from marine alpha bacteria to the rDNA pool, correlated with distance up the estuaries and decreasing salinity. 16S rDNA clones retrieved from coastal seawater with the MALF-1 primer were all found to cluster with the marine alpha bacteria (nine of nine), providing evidence that this oligonucleotide was specifically targeting only members of this phylogenetic group.

Four collections of random 16S rDNA clones retrieved from the Sapelo Island sample by PCR with nonspecific primers were also used to quantify the abundance of sequences from the marine alpha group. Hybridizations of the MALF-1 and MALF-1b probes to the random PCR products indicated that marine alpha sequences made up a large percentage of the

products (52 to 86%). These values are significantly higher than those obtained from hybridizations of the oligonucleotide probes to community DNA from this same sample (28% for MALF-1 and 1% for MALF-1b), suggesting a possible bias during PCR amplification toward 16S rDNA genes from marine alpha bacteria relative to other bacteria in this sample. Since the MALF-1b probe was designed to target clones retrieved earlier by PCR with group-specific primers, this probe may specifically target that subset of the marine alpha group whose 16S rDNA sequences are most easily amplified by PCR.

We readily cultured members of the marine alpha group from a Skidaway River seawater sample (24‰ salinity) by using low-nutrient, nonselective solid medium. Of the 48 isolates randomly selected from the low-nutrient plates, 19 hybridized with the MALF-1 probe; the results of the hybridizations were confirmed by sequencing the 16S rDNA genes of 10 of the 19 positive isolates. Although the composition of bacterial culture collections, particularly those obtained by using high-nutrient medium, are not expected to reflect natural bacterial communities due to well-recognized culturing biases (11, 34, 35), the fact that marine alpha bacteria had been isolated previously by a number of research groups (1, 34) suggested to us that some species might be readily cultured. Given the minimal effort required to culture marine alpha bacteria from southeastern U.S. seawater, they have likely been isolated previously but escaped the interest of taxonomists. Alternatively, the group may require low-nutrient culture medium for isolation (26). In addition to providing further evidence for the abundance of marine alpha bacteria in coastal seawater of the southeastern United States, successful culturing of bacteria from this group furnishes organisms for studies of the physiology and ecology of this important cluster.

Marine alpha bacteria previously isolated from marine environments show a diversity of metabolic strategies. Two members of the group are phototrophic (bacteriochlorophyll *a*-containing) bacteria of the genus *Roseobacter* (*R. denitrificans* and *R. litoralis*) (28), while a third member is a nonphototrophic *Roseobacter* (*R. algicola*) (16). The group also contains an aerobic sulfite oxidizer (*Sulfitobacter pontiacus*) isolated from the Black Sea (31, 32), a lignin-transforming bacterium isolated from the Georgia coast (*S. stellata*) (12), a lignin-transforming sulfite-oxidizing bacterium isolated from the Georgia coast (isolate EE-36) (13), an open-ocean DMSP-degrading bacterium (18), a methanesulfonate-degrading bacterium (15), Antarctic sea ice bacteria (2, 14), and a gall symbiont from the marine red alga *Prionitis lanceolata.*

16S rDNA clones clustering close to or inside the marine alpha group have been obtained previously from other marine environments, including the Sargasso Sea (3, 8, 11, 23), coastal Oregon seawater (34), and coastal lagoons (1). Marine alpha bacteria are thus widely distributed among marine environments, although possibly not as numerically dominant in other locations as in our southeastern U.S. samples. However, Suzuki et al. (34) found a large percentage of the clones and isolates obtained from coastal Oregon seawater to be affiliated with the genus *Roseobacter*, and these clones cluster with the bacteria we refer to as the marine alpha group (Fig. 4). The clone OCS84 of Suzuki et al. (34) grouped with our GAC-4–GAC-9 subcluster, suggesting that marine alpha bacteria from geographically distant coastal environments may nonetheless have close phylogenetic affinities. Differences between the regions of the 16S rDNA gene sequenced in the present study and those sequenced in previous ones make more detailed phylogenetic analyses difficult, but evidence suggests a wide geographic distribution of marine alpha bacteria.

A marine alpha clone (GAC-4 and/or GAC-9) and isolate (GAI-23 and/or GAI-36) were determined to have identical 16S rDNA sequences. Thus, a single species accounted for 2 of 9 sequenced clones and 2 of 10 sequenced isolates from southeastern U.S. seawater. The cultivation of a marine bacterium that is also represented among 16S rDNA clones from the same environment has not been reported previously and provides further evidence for the high culturability of the marine alpha group compared to other marine lineages (23, 34). Since the seawater sample used to obtain isolates was collected 3 years after the sample used to obtain clones, marine alpha bacteria and isolate GAI-23 and/or GAI-36 (clone GAC-4 and/or GAC-9) in particular may be permanent components of coastal bacterial communities in the southeastern United States.

The ease with which members of this group are cultured provides a unique opportunity to characterize the functional and ecological roles of important marine bacterioplankton in estuaries and nearshore systems of the southeastern United States. Some of our marine alpha isolates are capable of degrading lignin (12, 13) and humic substances (5), two types of naturally-occurring aromatic compounds that are exported in high concentrations from salt marshes and estuaries of the southeastern United States (21, 22). Sulfur-based metabolisms are also common among the marine alpha bacteria (12, 15, 18, 31, 32). Given the high diversity of metabolic strategies among cultured bacteria from this group, however, it is not yet possible to predict the ecological niches likely to be occupied by marine alpha bacteria.

Recent applications of 16S rDNA methodologies have led to important advances in understanding the biodiversity of marine bacteria (24), including the identification of several major lineages of oceanic bacterioplankton (4, 7, 23, 25, 34). Most or all of these lineages, however, are represented only by 16S rDNA clones and do not include known, culturable species (23, 34). The marine alpha group appears to be the exception to this rule, being the first phylogenetic group of marine bacteria identified by molecular techniques that is both numerically dominant and readily cultured.

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