

MINIREVIEW

Overview of the Marine *Roseobacter* Lineage†

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Despite the overwhelming bacterial diversity present in the world's oceans, the majority of recognized marine bacteria fall into as few as nine major clades (36), many of which have yet to be cultivated in the laboratory. Molecular-based approaches targeting 16S rRNA genes demonstrate that the *Roseobacter* clade is one of these major marine groups, typically comprising upwards of 20% of coastal and 15% of mixed-layer ocean bacterioplankton communities (see, e.g., references 36, 37, 42, 98, and 109). *Roseobacters* are well represented across diverse marine habitats, from coastal to open oceans and from sea ice to sea floor (see, e.g., references 16, 28, 37, 42, 52, and 98). Members have been found to be free living, particle associated, or in commensal relationships with marine phytoplankton, invertebrates, and vertebrates (see, e.g., references 4, 6, 7, 44, 49, 115, and 119). Furthermore, representatives of the clade stand out as representing one of the most readily cultivated of the major marine lineages (36). These isolated representatives are serving as the foundation for an improved understanding of marine bacterial ecology and physiology.

DESCRIPTION OF THE GROUP

The *Roseobacter* clade falls within the α -3 subclass of the class *Proteobacteria*, with members sharing >89% identity of the 16S rRNA gene. The first strain descriptions appeared in 1991, about the time that 16S rRNA-based approaches for cataloging microbial diversity were revealing the immensity of prokaryotic diversity in the world's oceans. Interest in the clade has risen steadily since the initial discovery of these strains; at present the clade contains 36 described species, representing 17 genera, and literally hundreds of uncharacterized isolates and clone sequences. The first described members were *Roseobacter litoralis* and *Roseobacter denitrificans*, both pink-pigmented bacteriochlorophyll *a*-producing strains isolated from marine algae (99). Subsequent cultivation of clade members, however, revealed that many strains are neither pink nor bacteriochlorophyll *a* producers (see, e.g., references 20, 41, 43, and 61). With the exceptions of the described strains of the genus *Ketogulonicigenium* (113) and several clones from a South African gold mine (GenBank accession numbers AF546906, -13, -17, -22

to -24, and -26), the *Roseobacter* clade is exclusively marine or hypersaline, with characterized isolates demonstrating either a salt requirement or tolerance (see, e.g., references 60 and 62). The described strains demonstrate a diverse range of physiological and morphological features (e.g., gas vacuoles [43], holdfasts [41], poly- β -hydroxybutyrate granules [20, 118], rosette formation [60, 86], toga-like morphologies [39], sulfur metabolism [39, 104], secondary metabolite production [61], methylotrophy [51], and mixotrophy [73]) that suggest unique adaptations to various marine environments. However, few of these traits are representative of the entire clade.

ABUNDANCE AND DISTRIBUTION IN MARINE ENVIRONMENTS

Based on culture collections, 16S rRNA clone libraries, and single-cell analyses, *roseobacters* have been identified in most marine environments sampled. The group is prevalent in 16S rRNA gene inventories of seawater (Table 1) and marine sediments (Table 2) and is noticeably absent from analogous inventories of freshwater and terrestrial soil environments. Fluorescent in situ hybridization (FISH) studies quantifying *Roseobacter* populations in coastal waters of the southeastern United States and the North Sea indicate different relative population sizes (20% of all bacterial cells versus 8%), but similar seasonal trends (populations highest in summer months and dropping off during winter) (28, 82). Quantitative 16S rRNA gene inventories (Table 1) show that 20 to 30% *Roseobacter* representation is not uncommon in bacterial communities in the upper mixed layer of the ocean, but depth profiles suggest that populations fall off with depth (Fig. 1) (1, 42, 107). *Roseobacters* are often most abundant in bacterial communities associated with marine algae, including natural phytoplankton blooms and algal cultures (see, e.g., references 4, 42, 78, 90, and 125). *Roseobacter* sequences are also abundant in communities associated with polar sea ice (16, 17), diseased corals (21, 80), sponges (111, 119), hypersaline microbial mats (54), cephalopods (cuttlefish and squid) (9, 46), scallop larvae (93), sea grasses (120), and coastal biofilms (24, 25) (Table 2).

ARE ISOLATES REPRESENTATIVE OF NATURAL POPULATIONS?

While the culturability of *roseobacters* is well established, an unresolved question is whether these isolates are truly repre-

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† Supplemental material for this article may be found at <http://aem.asm.org/>.

TABLE 1. Representation of *Roseobacter* sequences in quantitative 16S rRNA gene clone libraries from seawater

Location	Depth (m)	No. of clone sequences	% <i>Roseobacter</i> sequences	Reference
Pacific Ocean	<10	16	6	96
Sargasso Sea	10	14	0	32
Coastal Pacific Ocean (California)	10	71	0	26
Sargasso Sea	2	42	24	77
Coastal Pacific Ocean (Oregon)	10	58	7	108
Coastal North Atlantic Ocean (France)	<10	51	18	11
Mediterranean Sea (France)	<10	50	12	11
Coastal North Atlantic Ocean (North Carolina)	10	112	21	88
Coastal North Atlantic Ocean (Long Island Sound)	<10	17	18	33
Coastal Pacific Ocean (California)	<10	16	25	33
Western Mediterranean Sea; free living	Various	120	2	1
Coastal Pacific Ocean and Estuary (Washington)	10	189	0.4	23
Various	Various	660	16	36
Coastal North Sea (German Bay)	1	54	1.9	27
Coastal Pacific Ocean (Oregon)	10	51	10	89
Coastal North Atlantic Ocean (Long Island Sound)	<1	126	0	56
Antarctic Polar Front	3,000	15	0	65
Changjiang Estuary (China)	Various	241	19	97
Coastal Caribbean Sea; coral reef tracts	<10	65	6	30
Black Sea	Various	38	11	116
Coastal North Atlantic Ocean (Massachusetts)	<10	2,040	4	2
Coastal North Atlantic (Portugal)	0	198	25	48
Coastal Pacific Ocean (California) ^a	Various	27,840	27	107
Sargasso Sea; seven libraries ^b	<10	2,328	1.7	115
Coastal North Atlantic Ocean (Georgia)	<10	794	17	W. B. Whitman et al., unpublished data ^c

^a BAC libraries of coastal Pacific Ocean assemblages.

^b Shotgun clone libraries of the Sargasso Sea.

^c Sequences are available at <http://simo.marsci.uga.edu>.

sentative of the populations that are abundant in the environment. Representative strains have been isolated by Brinkmeyer et al. (16), who cultured an *Ociadecabacter*-like strain that comprised ~20% of an Arctic sea ice bacterial community; by Pinhassi et al. (83), who cultured two *Roseobacter* strains that by whole genome hybridization contributed 7 and 20% of North Sea and Baltic Sea bacterial communities; and by Fuhrman et al. (31), who cultivated the type strain of *Roseovarius nubinhibens*, which by whole genome hybridization contributed 20% of a Caribbean Sea bacterial community. In some of these cases, the criterion used to assess taxonomic similarity was not stringent, which is an important issue in light of findings that bacterioplankton with as much as 97% identity of the 16S rRNA gene can be functionally and genetically divergent (72, 91). Other studies have concluded that cultured members of the *Roseobacter* group are not representative of their environmentally abundant relatives; these studies include those of Eilers et al. (28), who found that specific *Roseobacter* strains constituted <1% of the bacterial community in the German Bight (even though the group as a whole comprised ~10% of the community), and of Selje et al. (98), who found a *Roseobacter* phylotype with wide geographic distribution in the Arctic and Southern Oceans that is not well represented in culture. Thus, there is evidence for both sides of the debate on the ecological relevance of *Roseobacter* group members that have been gathered into culture collections, with methodology and environment as two potentially important variables.

An alternative approach to addressing the question of ecological relevance of isolates is to determine whether *Roseobacter* 16S rRNA gene sequences fall into phylogenetic

clusters that contain only cultured members, only uncultured members, or both cultured and uncultured members. This approach uses the single criterion of 16S rRNA similarity to determine relatedness and integrates sequences across sites and dates into a single analysis. To this end, we compiled a data set of *Roseobacter* 16S rRNA gene sequences and identified clusters of sequences with $\geq 99\%$ identity.

CLUSTERS WITHIN THE CLADE

The *Roseobacter* 16S rRNA gene data set was established with 565 sequences from the Ribosomal Database Project II release 9.22 (RDP) that were assigned to any of the five *Roseobacter* genera used by the RDP Classifier (*Antarctobacter*, *Roseivivax*, *Roseobacter*, *Roseovarius*, and *Sulfitobacter*). An additional 1,251 RDP sequences that were listed as unclassified *Rhodobacteraceae* family members were screened for *Roseobacter* clade members based on $\geq 97\%$ identity in pairwise Smith-Waterman alignments (103) to a reference set of 391 *Roseobacter* sequences (89 clones and 302 isolates). This reference set included all described strains and all clone and isolate sequences of $\geq 1,000$ bp in length from the RDP-recognized *Roseobacter* genera. This screen identified 772 additional *Roseobacter* sequences (61% of the unclassified *Rhodobacteraceae* sequences). The 1,337 *Roseobacter* sequences obtained from the RDP accounted for 1% of all bacterial sequences and 9.5% of all α -proteobacterial sequences in RDP release 9.22. An additional 160 *Roseobacter* sequences were added to the data set because they represented described genera not in the RDP 9.22 release ($n = 3$), were identified in

TABLE 2. Representation of *Roseobacter* sequences in bacterial communities from diverse marine environments^a

Environment	Approach ^b	n ^c	% <i>Roseobacter</i> contribution ^d	Reference
North Sea coastal biofilms	Culture collection	463	1	6
Southern U.S. salt marsh biofilms	Clone library FISH	43 NA	67 ~45	24 25
Dinoflagellate (<i>Alexandrium</i> sp. and <i>Prorocentrum lima</i>) cultures	Culture collection	44	30	6
Dinoflagellate (<i>Alexandrium</i> sp. and <i>Scrippsiella trochoidea</i>) cultures	Culture collection	76	55	50
Dinoflagellate (<i>Gymnodinium catenatum</i>) cultures	Culture collection	61	31	45
Dinoflagellate (<i>Alexandrium</i> sp.) cultures	Culture collection	31	87	3
Dinoflagellate (<i>Gymnodinium catenatum</i>) cultures	Culture collection	61	30	45
Marine red algae (<i>Prionitis filiformis</i>)	Clone library FISH	3 NA ^e	100 100	8 8
Marine green algae (<i>Laminaria</i> sp.) cultures	Culture collection	17	35	81
Green algae (<i>Enteromorpha</i>)	Culture collection	99	1	81
Diatom (<i>Thalassiosira</i> sp.) cultures	Culture collection	12	8	6
North Atlantic algal (<i>Emiliania huxleyi</i>) bloom	FISH T-RFLP Clone library	NA NA 300	26 32 5	42 42 42
North Sea alga (<i>Emiliania huxleyi</i>) bloom	FISH Clone library	NA 50	29.5 24	125 125
Phytoplankton bloom off Plymouth, United Kingdom	Clone library	160	9	78
<i>Halophila stipulacea</i> (sea grass)	Clone library	59	8	120
North Sea bryozoan (<i>Flustra foliacea</i>)	Culture collection	82	10	87
Scleractinian corals with black band disease	Clone library	200	24	21
Diseased Caribbean coral (<i>Montastrea annularis</i>)	Clone library	41	17	80
<i>Loligo pealei</i> (squid) accessory nidamental gland	Clone library Culture collection	7 12	100 17	9 9
<i>Loligo pealei</i> (squid) egg capsules	Clone library Culture collection	12 6	17 0	9 9
Hong Kong soft corals (<i>Dendronephthya</i> sp.)	Culture collection	11	27	47
Diseased Eastern oyster (<i>Crassostrea virginica</i>)	Culture collection	2	100	13
<i>Sepia officinalis</i> (cuttlefish); accessory nidamental glands	Clone library	33	64	46
Antarctic hypersaline microbial mats	Culture collection	746	3	114
Hypersaline microbial mat	Culture collection	3	100	54
Sea ice, Arctic	Clone library FISH Culture collection	192 NA 115	11.5 27 32	16 16 16
Sea ice, Antarctic	Clone library FISH Culture collection	198 NA 87	5.5 11.5 24	16 16 16

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TABLE 2—Continued

Environment	Approach ^b	n ^c	% <i>Roseobacter</i> contribution ^d	Reference
Sea ice, Arctic	Culture collection	28	7	55
Nankai Trough, cold seep sediments, 0–15 cm	Clone library	57	2	63
San Francisco Bay marsh surface sediments	Clone library	100	4	110
French Guiana “mobile” mud deposits, 10–30 cm	Clone library	96	10	66
Mud volcano sediments near Mt. Etna, Italy; 20 cm	Clone library	140	3	121
Antarctic continental shelf sediments, 0–21 cm	Clone library	936	1	14
Sea of Okhotsk subfloor sediments; ash layers, 0–58 m	Clone library	322	4	52
Mid-Atlantic Ridge hydrothermal vent surface sediments	Clone library	82	1	64
Gulf of Mexico gas hydrate surface sediments	Clone library	126	4	70
Anoxic sediments under microbial mat in coastal saltern (Mediterranean Sea)	Clone library	92	3	76
Antarctic coastal surface sediments; petroleum and heavy metal impacted	Clone library	98	2	84
Southeastern US coastal sediments, 0–16 cm	Clone library	1,460	2	W. B. Whitman et al., unpublished data ^f
Decaying salt marsh grass (<i>Spartina alterniflora</i>)	Clone library	210	15	W. B. Whitman et al., unpublished data ^f

^a Excludes seawater samples, which are covered in Table 1.

^b Studies using the following approaches were included: quantitative 16S rRNA gene clone libraries (“clone library”), FISH, cultivation (“culture collection”), and terminal restriction fragment length polymorphisms (“T-RFLP”).

^c Number of total clones or isolates analyzed in 16S rRNA gene clone libraries or by cultivation, respectively.

^d Contribution of *Roseobacter* members to total bacterial community analyzed with the various approaches. Clone library and culture collection results are shown as percentage of *roseobacters* with respect to total clones or isolates analyzed. FISH results are provided as percentage of total community enumerated with a *Bacteria*-specific probe.

^e NA, not applicable.

^f Sequences are available at <http://simo.marsci.uga.edu/>.

the Sargasso Sea metagenomic library ($n = 35$) (115), or were part of the Sapelo Island Microbial Observatory 16S rRNA sequence database ($n = 122$) (<http://simo.marsci.uga.edu>). The sequence data set ($n = 1,497$) represents clones and isolates from diverse origins, with the overwhelming majority originating in coastal seawater samples (Fig. 2).

Sequences in the *Roseobacter* data set were then used to define phylogenetic clusters within the clade, based on a 1% consensus rule ($\geq 99\%$ sequence similarity). This criterion is more likely to group organisms with similar ecological niches and physiological adaptations than the 97% “species” criterion (2). Initial analyses suggested that two modifications were needed to obtain meaningful clusters from this diverse data set. First, to reduce biases in cluster identification due to differences in sampling efforts among studies, the *Roseobacter* sequence data set was culled to remove similar sequences derived from the same sample. Second, to reduce the influence of short sequences, the *Roseobacter* reference set (described above) was used to anchor clusters with nearly full-length sequences from acknowledged *Roseobacter* lineages. The non-redundant data set ($n = 974$) was subjected to pairwise Smith-Waterman alignments to sequences in the *Roseobacter*

reference set, and sequences were placed in the same cluster if they had $\geq 99\%$ identity to any member of that cluster.

Half (55%) of the *Roseobacter* sequences, representing 248 clones and 292 isolates, clustered into groups containing a reference sequence. These sequences formed 141 clusters that ranged in size from 1 to 56 members. Most of these sequences (79%) fell into 51 clusters of ≥ 3 nonredundant members (see Table S2 and Fig. S1 in the supplemental material). The majority (80%) of these 51 clusters contained both clone and isolate representatives, 8 clusters (17%) were comprised solely of isolates, and 2 clusters contained only clone representatives. The remaining sequences fell into 90 clusters of one ($n = 67$) or two ($n = 23$) members. Most of these clusters (72%) contained only isolates, 19 contained only clones, and 6 contained both a clone and an isolate.

The other half of the sequences in the nonredundant data set (323 clones and 111 isolates) did not cluster with a *Roseobacter* reference sequence. In order to determine whether these sequences would form clusters among themselves, a separate series of pairwise alignments were run on solely these sequences. Of these sequences, 358 (82%) were $< 99\%$ similar to any other sequence; most of these singleton

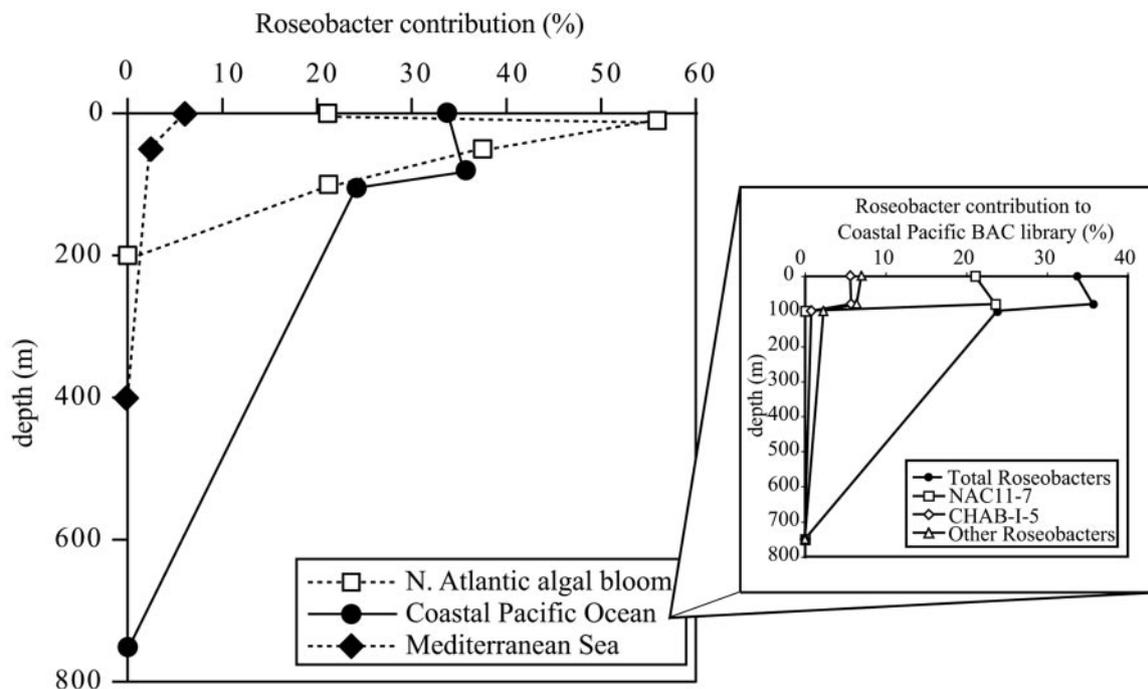


FIG. 1. Contribution of roseobacters to the total bacterial community at different depths from three sites. Roseobacters associated with a North Atlantic algal bloom community were identified by group-specific 16S rRNA gene oligonucleotide probes (42). Roseobacters associated with western Mediterranean Sea waters were identified by PCR-generated 16S rRNA gene libraries (1). Roseobacters in coastal Pacific Ocean assemblages were identified by BAC libraries (107). The coastal Pacific BAC libraries revealed significant representation of two of the major sequence clusters described in the text (inset).

sequences (80%) are partial sequences (<1,000 bp), and over half (73%) represent clones.

To specifically address the issue of whether the cultured strains are representative of environmental *Roseobacter* populations, we first focused on the large clusters containing ≥ 10 nonredundant members (Fig. 3). Together, these 13 major clusters contain 251 sequences (26% of the nonredundant *Roseobacter* data set). Three of the major clusters (DC5-80-3, NAC11-7, and ANT9093 [Fig. 3]) are comprised primarily of clones ($\geq 75\%$), and one (CHAB-I-5) is composed exclusively of clones. Two major clusters (OBULB and SPON) contain mostly isolate sequences ($\geq 68\%$), while the remaining major clusters (7 of 13) are fairly well represented by both clones and isolates (e.g., AS-26, AS-21, and TM1040 [Fig. 3]).

Because the clusters were defined in an associative fashion (i.e., membership required $\geq 99\%$ similarity to only one other member), sequences in the same cluster can have 16S rRNA sequences similarities of $< 99\%$. Therefore, we also addressed the issue of phylogenetic congruence between cultured and cloned *Roseobacter* members by determining whether individual clone sequences have $\geq 99\%$ similarity to any isolated strain. Fifty percent of all nonredundant clone sequences (288 of 571) clustered with at least one other sequence. Of these, 64% (184 sequences) were $\geq 99\%$ similar to an isolate sequence, while 36% were not (see Table S2 in the supplemental material). There were several instances of 100% identity in pairwise alignments between nonredundant sequences ($n = 121$). One-third of these identical pairs (34%) involved a clone and an isolate, one-third (30%) involved two isolates, and one-third (35%) involved two clones. When all of the nonre-

dundant *Roseobacter* clones in the data set are considered, 68% did not cluster with $\geq 99\%$ similarity to an isolate. These results are greatly influenced by sampling effort and available sequences up to the point of data set compilation. Nonetheless, this analysis estimates that for two-thirds (68%) of the *Roseobacter* diversity identified thus far, it is not yet possible to access relevant physiological information through studies of cultured organisms.

Phylogeny of the *Roseobacter* group is somewhat problematic. This is primarily due to the assignment of genus names to more than one monophyletic lineage (e.g., *Roseobacter* and *Ruegeria*) and instability in tree branching patterns. Nonetheless, it is possible to identify robust superlineages within the clade, including the *Loktanella* group, the *Antarctobacter-Sagittula* group, the *Octadecabacter-Ruegeria* group, the *Sulfitobacter-Staley-Oceanibulbus* group, the *Roseobacter* group, the *Silicibacter-Ruegeria* group, and the *Roseivivax-Salipiger* group (74). Of these superlineages, only three (the *Octadecabacter-Ruegeria* group, the *Sulfitobacter-Staley-Oceanibulbus* group, and the *Silicibacter-Ruegeria* group) are well represented by both clones and isolates (i.e., $> 30\%$ of nonredundant members are clones) (Fig. 4). Both the *Antarctobacter-Sagittula* and *Roseobacter* groups are presently comprised solely of isolates.

PATTERNS IN HABITAT AND DISTRIBUTION

We examined the source environment and geographical distribution of the nonredundant *Roseobacter* sequences in the data set to determine if characteristic habitats or ecological

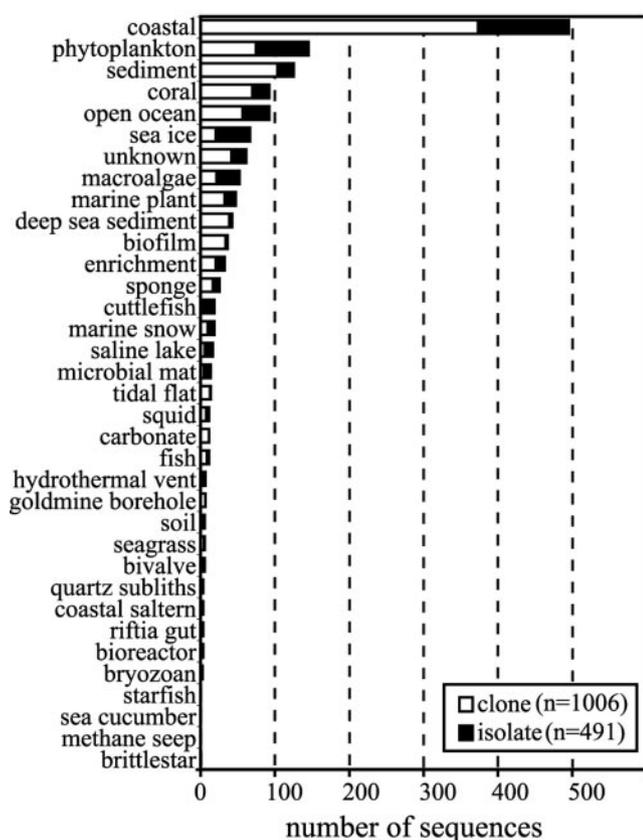


FIG. 2. Type (clone or isolate) and origin (environment sampled) of 16S rRNA gene sequences in the *Roseobacter* data set. Definitions for environments are as follows: “coastal,” seawater samples collected from intertidal regions to edges of continental shelves; “open ocean,” seawater samples taken beyond continental shelves; “deep sea sediment,” samples collected from marine sediments at water depths of >1,000 m; “phytoplankton,” diatoms, dinoflagellates, and microalgae; “marine plant,” vascular coastal plant; “unknown,” insufficient information to determine source environment.

niches could be identified for specific phylogenetic clusters within the group. Source environments were inventoried using primary literature references and unpublished RDP entries (Fig. 3). To focus on larger groups for which patterns could be studied, we analyzed only the 13 major clusters (i.e., those consisting of ≥ 10 nonredundant members and containing at least one nearly full-length reference sequence), which represented 26% of the nonredundant data set. A similar analysis using the less stringent criterion of ≥ 3 nonredundant members per cluster is provided in Fig. S1 in the supplemental material.

DC5-80-3 cluster. The DC5-80-3 group represents the largest of all the major clusters, with 56 nonredundant members that are primarily clone sequences (86%) from planktonic habitats (79%) (Fig. 3). This cluster is the only one for which a systematic global distribution has been determined. Selje et al. (98) identified members of this group in surface waters (to 40 m) of temperate to polar oceans of both hemispheres and to depths of 2,300 m and 1,000 m in the Arctic and Southern Oceans, respectively. Based on a quantitative PCR assay, this group was estimated to comprise $\sim 20\%$ of all bacteria in the Southern Ocean (98), 5% of bacterioplankton 16S rRNA

genes in a clone library from a Portuguese estuary (48), and $\geq 5\%$ of bacterioplankton 16S rRNA genes in a clone library constructed from coastal North Carolina seawater (88). DC5-80-3 cluster members have yet to be detected in samples from tropical and subtropical waters (98).

OBULB and SPON clusters. The OBULB and SPON clusters fall within the phylogenetically cohesive *Sulfitobacter-Staleyia-Oceanibulbus* superlineage (Fig. 4) and are composed largely of isolate sequences, with $\sim 70\%$ of the sequences derived from cultivated representatives. Nearly a third (32%) of the nonredundant OBULB sequences are from coastal seawater samples (Fig. 3). Roughly another third (29%) are from sea floor environments (52). The OBULB cluster contains three described strains: *Oceanibulbus indoliflex*, cultivated from coastal North Sea waters (118), and *Sulfitobacter delicatus* and *Sulfitobacter dubius*, isolated from sea grass and starfish, respectively (53).

The representative described strain of the SPON cluster, *Sulfitobacter pontiacus*, was retrieved from the oxic/anoxic interface in the Black Sea (105). Six additional sequences are derived from geographically distinct coastal environments. In addition, five open ocean isolates belong to this major cluster. The remaining nonredundant sequences are derived from diverse environments, ranging from deep-sea vents to marine sponges (see Table S1 in the supplemental material).

OCT cluster. The OCT cluster is well represented by both clone (55%) and isolate (45%) sequences, including two described strains isolated from sea ice, *Octadecabacter antarcticus* and *Octadecabacter arcticus* (43). All but two of the 20 nonredundant representatives were obtained from polar environments, suggesting that members may be adapted to cold environments and to sea ice in particular. In fact, members of the OCT cluster have been found to comprise over 20% of sea ice microbial communities (16). The other two OCT cluster members are clone sequences derived from temperate coastal waters and deep-sea sediments (see Table S1 in the supplemental material).

RGALL cluster. The RGALL cluster (Fig. 3) is well represented by cultivated strains (68% of all sequences), many of which are found in association with eukaryotic marine organisms. This includes the described strain *Roseobacter gallaeciensis* isolated from larval cultures of the scallop *Pecten maximus* (92), an isolate recovered from larval cultures of the oyster *Ostrea edulis*, an isolate from larval cultures of the marine fish *Scophthalmus maximus* (49), and a clone from the egg capsule of the squid *Loligo pealei* (9). Two additional strains were isolated from dinoflagellates, and two clones were obtained from marine phytoplankton. The remaining members derive from coastal seawater (see Table S1 in the supplemental material) (Fig. 4).

CHAB-I-5 cluster. The CHAB-I-5 cluster is currently represented only by clone sequences, more than half of which (56%) derive from coastal seawater (Fig. 3). Members are represented in shotgun clone libraries from coastal Pacific Ocean waters and Sargasso Sea surface waters (107, 115), two datasets that are largely free of the biases associated with PCR-based studies. Nearly 6% of all 16S rRNA gene-containing clones from libraries constructed from surface and 80-m-depth waters of Monterey Bay, Calif., were traced to this major *Roseobacter* cluster (107), but none were identified in libraries from greater

Major *Roseobacter* sequence clusters

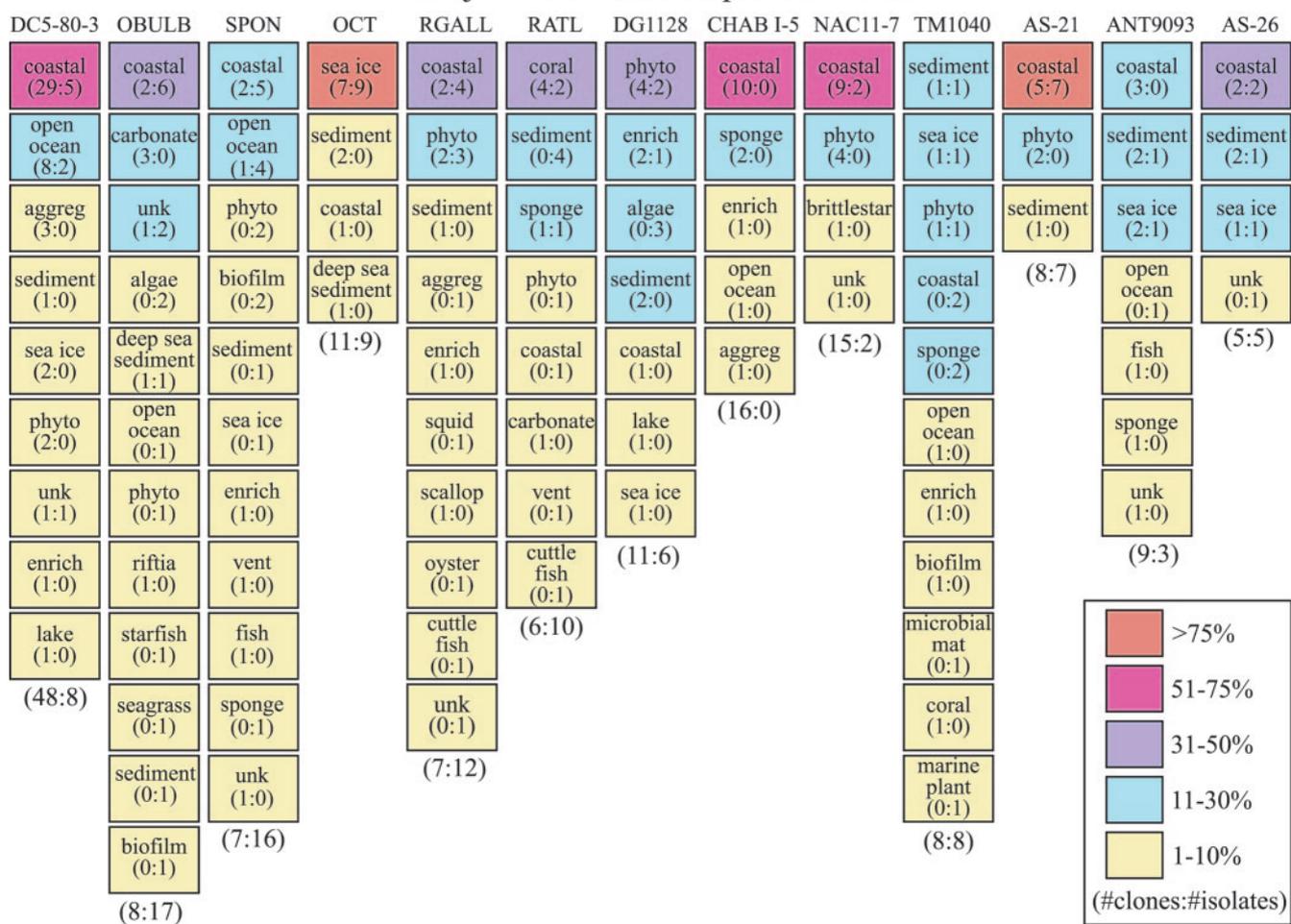


FIG. 3. Type (clone or isolate) and origin (environment sampled) of 16S rRNA sequences in each of the major *Roseobacter* sequence clusters. The percentage of sequences from a given habitat for each sequence cluster is shown by color-coded boxes. The numbers of nonredundant clones and/or isolate sequences for a given habitat are shown in parenthesis (number of clones:number of isolates); at the bottom of each column are the total numbers of nonredundant sequences for each cluster. Abbreviations: aggreg, marine aggregates; phyto, phytoplankton; unk, unknown; enrich, seawater enrichments; vent, hydrothermal vents; and carbonate, deep-sea carbonate crusts. See the Fig. 2 legend for environment definitions.

depths (Fig. 1). In both the coastal California and Sargasso Sea metagenomic libraries, this cluster constituted ~20% of the *Roseobacter* 16S rRNA gene-containing clones (73, 107, 115).

NAC11-7 cluster. The NAC11-7 cluster (Fig. 3) is represented primarily by clone sequences (88%), several of which are associated with algae and algal blooms. The two isolated representatives were cultured from coastal seawater by using oligotrophic media (102). Four clones derive from bacterial communities associated with North Atlantic algal blooms (42, 78, 125). Several studies suggest that NAC11-7 representatives are often prevalent in such assemblages, making up nearly a quarter (12 of 50) of the bacterioplankton clones sequenced from a North Sea *Emiliana huxleyi* bloom (125) and 15 of 160 clones sequenced from a bloom-associated community off Plymouth, United Kingdom (78). Suzuki et al. (107) reported that this cluster comprises 22% of all 16S rRNA gene-containing bacterial artificial chromosome (BAC) clones (and ~65% of *Roseobacter* 16S rRNA gene-containing BACs) from surface and 80-m-depth libraries of coastal California waters that

are typically characterized by phytoplankton blooms (107). Nine of the 15 nonredundant clone members were not specifically associated with algal cells or blooms but were obtained from near-shore seawater (see Table S1 in the supplemental material).

Other major clusters. The DG1128 cluster is well represented by sequences derived from macroalgae and phytoplankton (95). Many members of the RATL cluster were obtained from corals. The ANT9093 cluster is comprised of members from diverse environments, including polar sea ice, sediments, and sponges. Members of the TM1040, AS-21, and AS-26 clusters are typically derived from coastal seawater or sediment (Fig. 3 and 4; see Table S1 in the supplemental material).

In short, a few of the major clusters show fairly predictable patterns in habitat (e.g., OCT cluster members are often found in cold environments, and AS-21 members are often coastal), while several more exhibit predominance of a single habitat type (e.g., DG1128 members are frequently associated with marine phytoplankton and RATL members with corals). How-

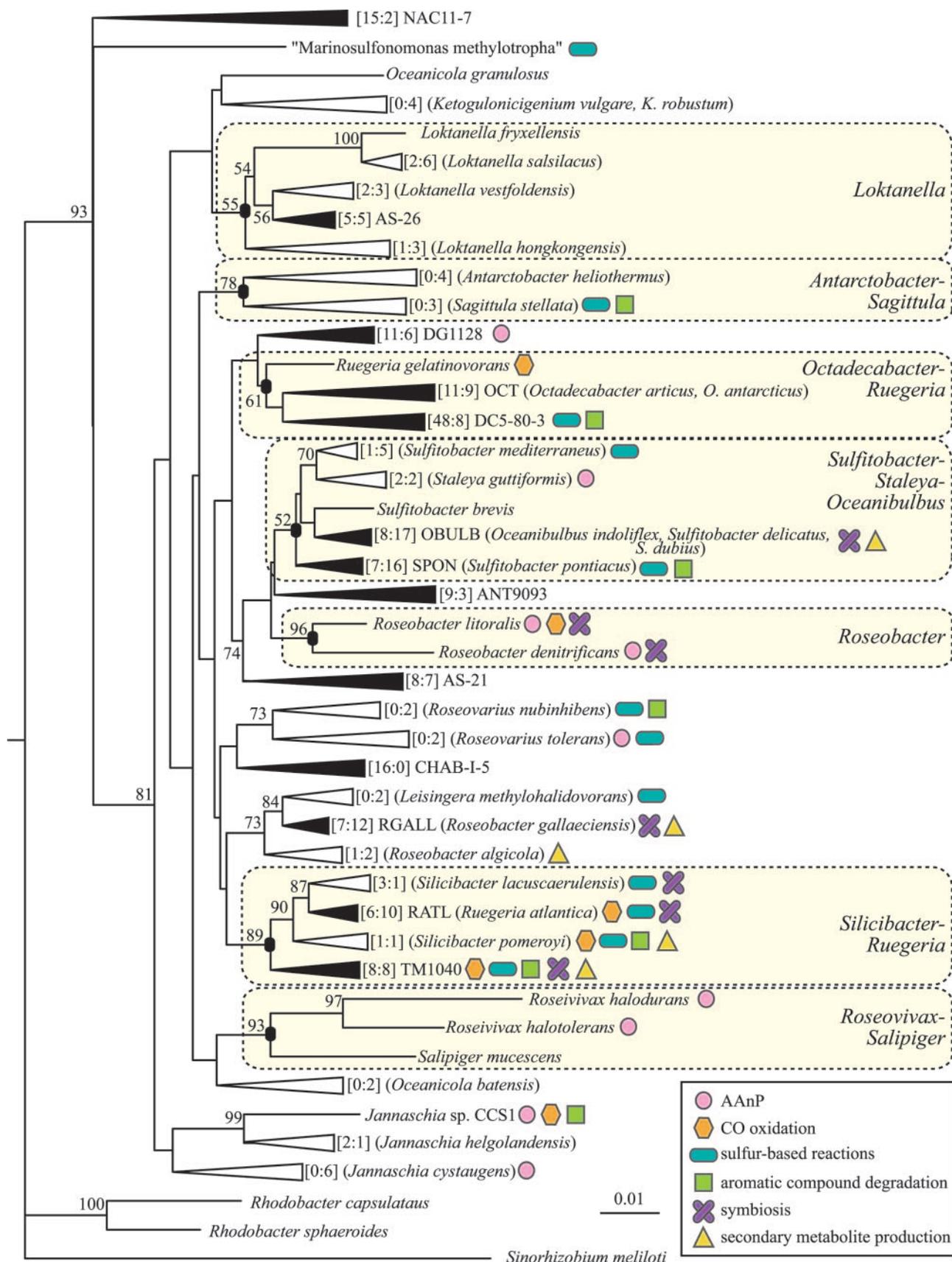


FIG. 4. The 41 major lineages of the *Roseobacter* clade. The tree includes all currently described genera and the 13 major clusters defined in the text. Filled triangles represent clusters of ≥ 10 nonredundant members, and unfilled triangles represent clusters with < 10 members. Described

ever, the variability evident within these clusters suggests that 16S rRNA gene sequence data alone are not a reliable predictor of ecological niche.

EMERGING PHYSIOLOGIES

Despite the metabolic diversity harbored within the *Roseobacter* clade, several physiologies appear to be characteristic of the lineage. To look for patterns within the *Roseobacter* clusters of phenotypes of ecological interest, we examined the distribution of known physiological attributes among group members. For this analysis, we focused on clusters for which physiological information was available, including (i) major clusters that contained ≥ 10 nonredundant members ($n = 13$), (ii) clusters that contained a described strain ($n = 33$), and/or (iii) clusters that contained a strain for which a genome sequence is available ($n = 3$). Forty-one clusters representing 337 nonredundant sequences (174 clones and 163 isolates) met at least one of these criteria (Fig. 4).

Aerobic anoxygenic phototrophy. The first described members of the *Roseobacter* clade, and the inspiration for the name, were among the earliest recognized aerobic anoxygenic phototrophs (AAnPs). These bacteriochlorophyll *a*-containing strains are able to derive energy from light without the generation of oxygen. *R. denitrificans* and *R. litoralis* (99, 101) are physiologically similar to their anaerobic relatives in the purple sulfur bacteria. However, in contrast to the case for purple sulfur bacteria, there is currently little evidence for CO₂ fixation beyond what might be attributable to anaplerotic reactions (100). This suggests that *Roseobacter* AAnPs are photoheterotrophic, although this issue has not yet been conclusively resolved. Seven of the 41 *Roseobacter* lineages contain phototrophic members (Fig. 4). However, there is little indication that the trait segregates into distinct clusters within the clade (6).

Although AAnPs were initially considered atypical marine bacteria restricted to unusual habitats, the discovery of bacteriochlorophyll *a* in ocean surface waters (59) along with the subsequent retrieval of both photosynthetic reaction center (*pufLM*) and bacteriochlorophyll biosynthesis (*bch*) genes from bacterioplankton (10, 79) established the ecological relevance of AAnPs in the ocean. One biogeochemical implication of *Roseobacter*-mediated phototrophy in surface seawater is an enhanced growth yield on available organic matter, which could provide an advantage to the organism in carbon-limited

environments as well as affect the magnitude and dynamics of the organic carbon reservoir in the ocean.

Sulfur transformations. Key transformations for the biogeochemical cycling of sulfur that involve both organic and inorganic compounds have been identified in *Roseobacter* clade members and recently reviewed by Moran et al. (74). Isolates of the clade were the first marine strains found to simultaneously possess two key pathways for the degradation of the sulfur-based algal osmolyte dimethylsulfoniopropionate (40). These competing pathways may play a role in determining the balance between the incorporation of sulfur into the marine microbial food web (the demethylation/demethiolation pathway) and the release of sulfur in the form of the climate-influencing gas dimethyl sulfide (the cleavage pathway) (57, 122). Field studies show that clade members are prevalent and active members of dimethylsulfoniopropionate-assimilating communities in the surface ocean (42, 67, 117). In addition, many *Roseobacter* strains are capable of transforming other organic sulfur compounds, including dimethyl sulfide, methanethiol, methanesulfonate, and dimethyl sulfoxide (39, 40, 51, 94).

Clade members also harbor abilities to transform inorganic forms of sulfur, including elemental sulfur, sulfide, sulfite, and thiosulfate (see, e.g., references 39, 73, and 104–106). These pathways facilitate sulfur-based lithoheterotrophy, which has been demonstrated in several *Roseobacter* strains (53, 73, 104). Inorganic sulfur oxidation is an important process in many coastal and benthic marine environments (e.g., sediments and sulfide-rich habitats), and the recent discovery of genes encoding sulfur oxidation enzymes (*sax* genes) in open ocean bacterioplankton (73, 115) suggests a previously unrecognized role for sulfur oxidation in these systems as well. Reactions involving sulfur (organic and inorganic) have been found in 12 of the 41 major *Roseobacter* lineages (Fig. 4).

Carbon monoxide oxidation. Members of the *Roseobacter* clade have been implicated in the consumption of carbon monoxide (CO), an important greenhouse gas that forms in seawater when sunlight oxidizes marine dissolved organic matter (123). Evidence that clade members are participating in biological CO oxidation in the ocean includes the demonstration that strains can oxidize CO in culture (58, 112) and that the *Roseobacter* *Silicibacter pomeroyi* harbors two CO oxidation (*cox*) operons in its genome (73). *S. pomeroyi* has been demonstrated to oxidize CO at concentrations typically measured

strains within each cluster are shown in parentheses. Robust phylogenetic lineages are indicated with filled ovals at branch nodes and vertical black lines. Numbers of clone and isolate sequences representing each cluster are provided in brackets ([number of clones:number of isolates]). Colored symbols represent evidence for the indicated physiologies. The tree is based on the following sequences: NAC11-7 (GenBank accession number AF245635), "*M. methylophila*" (U62894), *O. granulosus* (AY424896), *K. robustum* (AF136850), *L. fryxellensis* (AJ582225), *L. salsilacus* (AJ582228), *L. vestfoldensis* (AJ582226), *L. hongkongensis* (AY600301), AS-26 (AJ391187), *S. mediterraneus* (Y17387), *S. guttiformis* (Y16427), *S. pontiacus* (Y13155), *S. brevis* (Y16425), *O. indoliflex* (AY550939), *R. litoralis* (X78312), *R. denitrificans* (M96746), ANT9093 (AY167254), AS-21 (AJ391182), *O. batensis* (AY424898), DG1128 (AY258100), *R. gelatinovorans* (D88523), *O. antarcticus* (U14583), DC5-80-3 (AY145589), *R. nubinihibens* (AF098495), *R. tolerans* (Y11551), CHAB-I-5 (AJ240910), *S. lacuscaerulensis* (U77644), *R. atlantica* (D88526), *S. pomeroyi* (AF098491), TM1040 (AY332662), *L. methylohalidovorans* (AY005463), *R. gallaeciensis* (Y13244), *R. algicola* (X78313), *R. halodurans* (D85829), *R. halotolerans* (D85831), *S. mucescens* (AY527274), "*C. thiooxidans*" (AY639887), *A. heliothermus* (Y11552), *S. stellata* (U58356), *Jannaschia* sp. strain CCS1 (www.jgi.doe.gov), *J. helgolandensis* (AJ438157), *J. cystaogens* (AB121782), *R. capsulatus* (D16427), and *R. sphaeroides* (D16418). *S. meliloti* (D14509) served as the outgroup. The tree is based on positions 92 to 1443 of the 16S rRNA gene (*E. coli* numbering system). Prior to analysis, a filter was applied to the aligned sequences to exclude positions with <50% conservation. The tree was constructed using Phylip (29) and the neighbor-joining method. The bar represents Jukes-Cantor evolutionary distances. Bootstrap values of >50% are shown at branch nodes (100 iterations).

in coastal and open ocean surface waters (10 nM and 2 nM, respectively). However, it differs from previously characterized CO oxidizers in that it does not grow autotrophically and instead uses CO as a supplementary energy source during heterotrophic growth (73). Evidence for CO oxidation has been found in six of the major *Roseobacter* lineages thus far (Fig. 4), and CO oxidation may prove to be a successful ecological strategy for planktonic roseobacters in sunlit surface waters.

Aromatic compound degradation. Vascular plant-derived aromatic compounds are often a significant component of the carbon pool in coastal environments where roseobacters are abundant (75). Based on evidence that clade members might play a role in the transformation of lignin (38), a gene encoding a key ring-cleaving enzyme of the β -ketoacid pathway (*pcaH*) was identified in 16 of 19 *Roseobacter* strains by a PCR assay (18, 19). Enrichments of a salt marsh bacterial community with fused ring and hydroxy-, methyl-, and amino-substituted ring structures showed that over half of the 120 *pcaH* genes sequenced could be traced to the *Roseobacter* clade (19). Those findings complemented phenotypic assays carried out on cultivated organisms and indicated that many roseobacters are capable of using aromatic compounds as primary growth substrates (18, 19). Evidence for aromatic compound degradation has been identified in 7 of the 41 major *Roseobacter* lineages (Fig. 4).

The genome sequence of *S. pomeroyi* has revealed that in addition to the widely distributed *pca* pathway, other catabolic routes for phenolics may be represented in the clade (73). These include the gentisate pathway, which is widespread in phylogenetically diverse soil bacteria (124), and a novel pathway for the aerobic degradation of benzoate (35) that may also be present in a limited number of α - and β -*Proteobacteria* from soil.

Symbiotic relationships. *Roseobacter* strains form symbiotic relationships with diverse eukaryotic marine organisms. Ashen and Goff (8) identified *Roseobacter* phylotypes in three gall-bearing species of the marine red alga *Prionitis*. Clade members are also dominant components of bacterial assemblages associated with the reproductive accessory nidamental glands in the cephalopods *Loligo pealei* (squid) and *Sepia officinalis* (cuttlefish) (9, 46). Roseobacters have developed close associations with *Pfiesteria* and *Pfiesteria*-like species, where they are found within the nutrient-rich phycosphere of, or polarly attached to, these dinoflagellates (4). Alavi (5) recently identified a complex interaction between one such isolate (MA03), the dinoflagellate *Pfiesteria piscicida*, and the green alga *Rhodomonas*, in which MA03 positively affects the predation rate of the dinoflagellate on the alga. In addition, the *Roseobacter* strain *Silicibacter* strain TM1040 has been shown to exhibit chemotaxis toward compounds typically released from *Pfiesteria* (69).

Although not as commonly reported, pathogenic activities have also been attributed to clade members. *Roseobacter* strains and phylotypes have been implicated as causative agents of juvenile oyster disease in the Eastern oyster (12) and of black band disease in scleractinian corals (21, 80). While symbiotic interactions involving roseobacters are prevalent, the extents and bases of most of these relationships are not yet fully understood.

Secondary metabolite production. In bacteria, secondary metabolite production is often the basis for chemical signaling and defense, as well as host-microbe interactions. Evidence suggests that many roseobacters, particularly those within the RGALL lineage, produce bioactive compounds. Hjelm et al. (49) identified RGALL lineage members that were antagonistic against fish larval bacterial pathogens. *R. galleaeciensis* was demonstrated to have similar probiotic effects on scallop larvae (92). Another RGALL isolate produces a novel antibiotic, tropodithietic acid, which is effective against marine bacteria and algae (15). Finally, a strain isolated from the toxic dinoflagellate *Alexandrium affine* produces a suite of paralytic shellfish toxins (34).

Other *Roseobacter* lineages also harbor secondary metabolite producers (Fig. 4). *Roseobacter algicola*, isolated from the toxin-producing dinoflagellate *Prorocentrum lima*, produces the shellfish poison okadaic acid (61). *Oceanibulbus indoliflex* produces indole, indole derivatives, cyclic dipeptides, and the antimicrobial compound tryptanthrin (118).

Cell-density-dependent regulation via the LuxIR system is mediated by a specific class of secondary metabolites that have been identified in *Roseobacter* strains. Gram et al. (44) found that three of five *Roseobacter* isolates from marine snow produced LuxR-activating acylated homoserine lactones (AHLs). Mitova et al. (71) identified a sponge isolate (within the TM1040 cluster) capable of producing 10 distinct cyclic dipeptides structurally similar to the bioactive AHLs. Finally, evidence of the Lux system has also been found in *S. pomeroyi*, which has two *luxI* homologs that generate functional AHLs when expressed in *Escherichia coli* (73). Density-dependent signaling systems have been implicated in biofilm formation, exoenzyme production, and antibiotic production, all of which are activities exhibited by clade members (15, 22, 24, 25, 62, 118).

GENOMIC FEATURES

The roseobacters analyzed thus far have large genomes (averaging 4.4 Mb) and rRNA operon copy numbers ranging from 1 to 4 (average, 2.7) (73, 85; www.jgi.doe.gov). These traits are consistent with the metabolic diversity and ease of cultivation that are characteristic of the group. Plasmids are common among roseobacters and can exhibit a linear conformation (68, 73, 85, 113). In some strains, a significant amount of the genome content is plasmid borne (e.g., 5% in *R. littoralis* and 10% in *S. pomeroyi*), and ecologically relevant gene sets have been traced to plasmids in several strains (e.g., *pca*, *puf*, and *nir* [nitrite reduction] genes) (73, 85). While plasmid mobility has yet to be examined in *Roseobacter* strains, these extrachromosomal genetic elements may contribute to the physiological diversity evident within the clade.

The first genome sequence of *Roseobacter* clade member *S. pomeroyi* provided insight into the ecology and physiology of this successful marine clade (73). As the sequences of 2 additional isolates are near completion (www.jgi.doe.gov) and 13 more isolates are in the early stages of sequencing (www.moore.org/microgenome/), evidence for additional physiological features previously unsuspected in this lineage may emerge.

CONCLUDING REMARKS

As the physiology and ecology of cultured *Roseobacter* group members continue to be revealed, extrapolation of this information to uncultured relatives remains a central challenge. The extent of this challenge is best illustrated by the two-thirds of clade members that harbor a significant fraction of the group's phylogenetic diversity but presently have no close relatives in culture. Yet the opposite perspective is that with one-third of the known diversity represented by cultivated strains already in hand, this clade is one of the most accessible of the major marine taxa. For those major clusters that are currently well represented by cultured strains, considerable diversity is emerging with respect to habitat (Fig. 3) and physiology (Fig. 4). This makes extrapolation of ecological roles based on 16S rRNA gene sequences alone unlikely, at least given current levels of resolution of both physiology and phylogenetic diversity within the clade. Insights gained from cultured relatives will undoubtedly continue to serve as the basis of testable hypotheses for illuminating the ecological roles of this fundamentally important group of marine bacteria.

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