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Characterization of Bacterial Communities Associated with Deep-Sea Corals on Gulf of Alaska Seamounts†

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Although microbes associated with shallow-water corals have been reported, deepwater coral microbes are poorly characterized. A cultivation-independent analysis of Alaskan seamount octocoral microflora showed that *Proteobacteria* (classes *Alphaproteobacteria* and *Gammaproteobacteria*), *Firmicutes*, *Bacteroidetes*, and *Acidobacteria* dominate and vary in abundance. More sampling is needed to understand the basis and significance of this variation.

The most abundant corals on Gulf of Alaska seamounts are octocorals (9), which create a habitat structure for mobile fauna (4). Concerns about the benthic impacts of commercial fishing have renewed interest in habitat-forming deep-sea corals (4). Studies of shallow-water scleractinian corals (12) have revealed a diverse microflora and evidence of host-microbe interactions. Although studies of the deep-sea octocoral microflora are under way (10), there have been no published reports describing the microbial community composition.

Three Gulf of Alaska seamounts were visited during research cruise AT7-15/16 aboard the R/V *Atlantis*. The biological objectives of the cruise included sampling of deep-sea octocorals for studies of their dispersal and reproductive strategies, with a particular focus on the abundant bamboo corals (Isididae). We took advantage of available coral specimens to examine their associated microflora.

Coral, rock, and water column samples (Table 1) were collected from the Warwick, Murray, and Chirikof seamounts using the deep-submergence vehicle *Alvin*. Corals and rocks were harvested using the submersible's manipulators and stored in a closed box during ascent to minimize physical disturbance by surface waters. The water adjacent to coral colonies was sampled using a Niskin bottle fired at depth. After submersible recovery, freshly extruded coral exopolysaccharide and scrapings of coral and rock surfaces were transferred to sterile cryovials. Water samples were prefiltered through 20-μm-pore-size Nitex, concentrated using a TFF apparatus (Millipore), and vacuum filtered (1.0-μm and 0.2-μm pore size). The 0.2-μm filter retentate was resuspended in sterile saline solution. Samples were frozen immediately at −70°C and shipped frozen for subsequent processing.

Genomic DNAs were extracted using Ultra Clean soil DNA kits (MoBio), and 16S rRNA genes were PCR amplified using primers 27F and 1525R (11) and Plat*Taq* PCR supermix (Invitrogen). Amplifications were performed with an initial denaturation of 2 min at 94°C, followed by 29 cycles of 30 s at 94°C, 30 s at 55°C, and 2 min at 72°C, with a final extension of 5 min at 72°C. PCR products were cloned using a TOPO TA cloning kit (Invitrogen), and primers M13F and M13R were used to sequence positions 9 to 1545 of the 16S rRNA gene.

BLASTN (1) was used to compare our query sequences with reference sequences from the RDP2 (3) database. Representative sequences from the BLASTN output were aligned with our query sequences, using an RDP2-provided profile alignment. Neighbor-joining trees were created using PHYLIP (6) and used to assign putative taxonomy down to the family level. Detailed phylogenetic trees were constructed using the relevant sequences from each clone library, two reference sequences most closely related to the query sequence, and additional reference sequences. Alignments were generated using the RDP2 profile alignment, and bootstrapped neighbor-joining trees were reconstructed using PHYLIP (6).

The clones sequenced comprised 19 phyla (see Table S1 in the supplemental material), dominated by *Proteobacteria* (classes *Alphaproteobacteria* and *Gammaproteobacteria*), *Firmicutes*, *Bacteroidetes*, and *Acidobacteria* (Fig. 1). The relative proportions of these groups varied widely across the five coral samples, as did the degree to which a given library was dominated by a single group (Fig. 1; see Table S1 in the supplemental material). At the subphylum level, families occurring in major proportions included *Rhizobiaceae*, *Rhodobacteraceae*, and *Sphingomonadaceae* (*Alphaproteobacteria*); *Pseudomonadaceae*, *Alteromonadaceae*, and *Halomonadaceae* (*Gammaproteobacteria*); *Bacillaceae*, *Clostridiaceae*, and *Mycoplasmataceae* (*Firmicutes*); and *Flexibacteraceae* and *Flavobacteraceae* (*Bacteroidetes*).

Members of the family *Rhodobacteraceae* and the family *Pseudomonadaceae* were selected for further analysis, based on their relative abundance and on the previous finding (13) that shallow-water corals contain significantly larger numbers of these bacteria than the surrounding water. Members of the family *Rhodobacteraceae* comprised 23 to 100% of the alphaproteobacterial sequences in those libraries containing at least 10% alphaproteobacteria. The majority of *Rhodobacteraceae*

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TABLE 1. Summary of Gulf of Alaska samples from which 16S rRNA gene sequences were obtained

Sample	Origin	Seamount	*Alvin* dive no.	Dive depth (m) for sample collection	No. of sequences	Taxonomy (no. of members)				FastGroup result (no. of sequences)[b]			
						Phyla	Classes	Orders	Families	Sing	Doub	Trip	Cluster
CGOA	Bamboo coral	Murray	3805	1,100–1,950[a]	99	11	13	21	23	30	3	1	7
CGOD	Black coral	Murray	3805	1,100–1,950[a]	47	1	2	2	2	0	0	0	2
NISA	Water column	Murray	3798	760	236	7	5	20	22	24	2	1	5
BRRA	Rock biofilm	Murray	3798	670–1,094[a]	58	6	7	10	13	27	5	1	1
CGOC	Bamboo coral	Chirikof	3803	2,660–3,300[a]	57	6	8	10	10	12	5	1	2
CGOF	Bamboo coral	Warwick	3808	758	343	13	15	22	28	46	13	3	12
CGOG	Bamboo coral	Warwick	3806	634	45	5	8	10	11	27	5	2	0

[a] Depths covered during entire dive (specific sample collection depth not available).
[b] Results of sorting sequences using FastGroup. Sing, unique sequences; Doub, doubletons (two identical sequences); Trip, tripletons (three identical sequences); Cluster, cluster of more than three identical sequences.

sequences obtained in this and previous (12, 13) studies fall within the marine roseobacters (see Fig. S1 in the supplemental material), a major clade of culturable marine heterotrophs (7), many of which play a role in sulfur cycles (e.g., see reference 8). One clade of six CGOF sequences is most closely related to NAC11-6 from a dimethylsulfoniopropionate-producing algal bloom (8), while CGOCA38 groups closely with NAC11-7 (from the same algal bloom study [8]) and an uncultivated marine bacterium, ZD0207, associated with dimethylsulfoniopropionate uptake (15). CGOAB33 is most similar to one (slope strain EI1*) of a group of thiosulfate-oxidizing bacteria from marine sediments and hydrothermal vents (14).

Members of the family *Pseudomonadaceae* comprised 23 to 69% of the gammaproteobacterial sequences in those samples

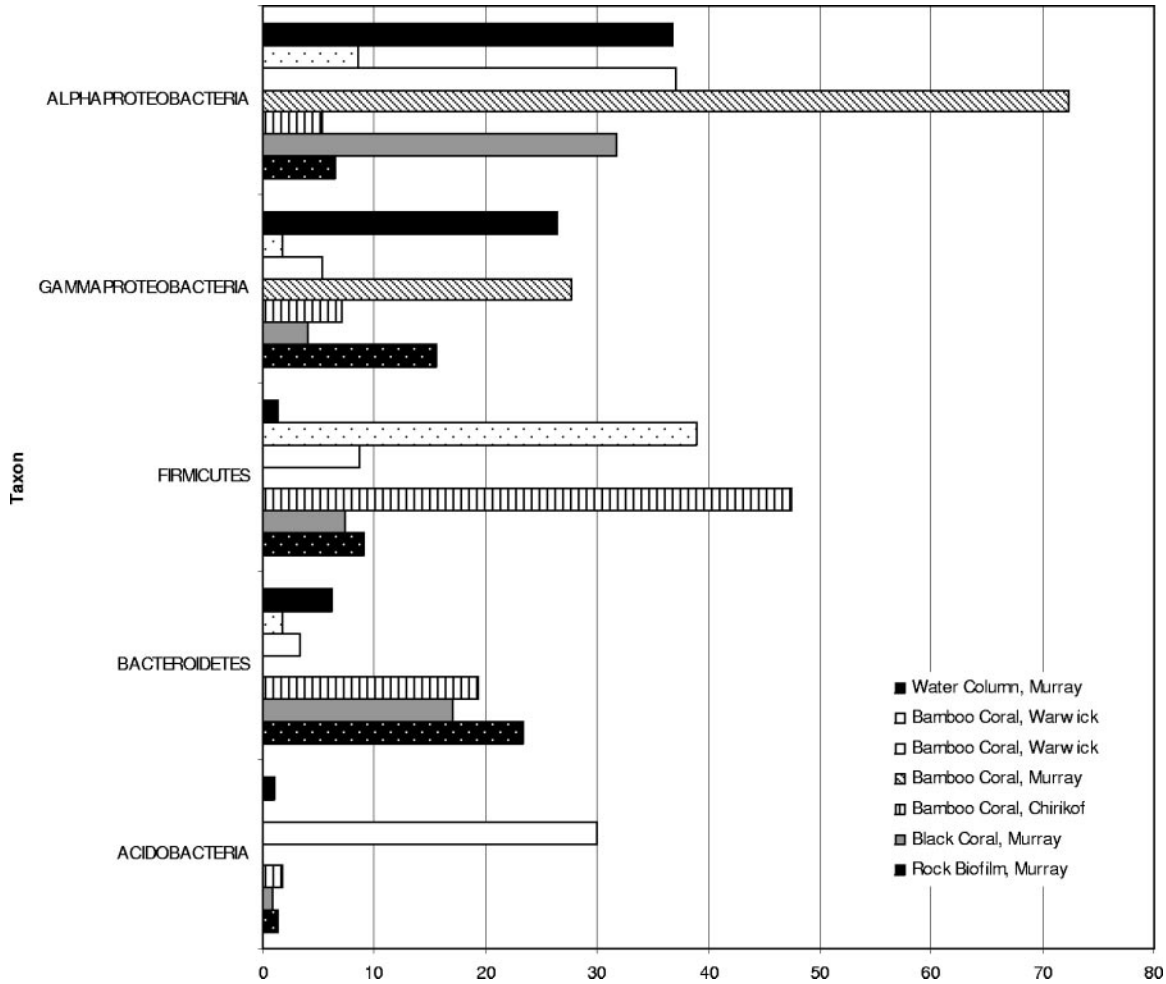

FIG. 1. Histogram showing percentages of composition (by taxon) for 16S rRNA gene libraries generated for this study, showing only taxa comprising at least 20% of sequences in at least one clone library.

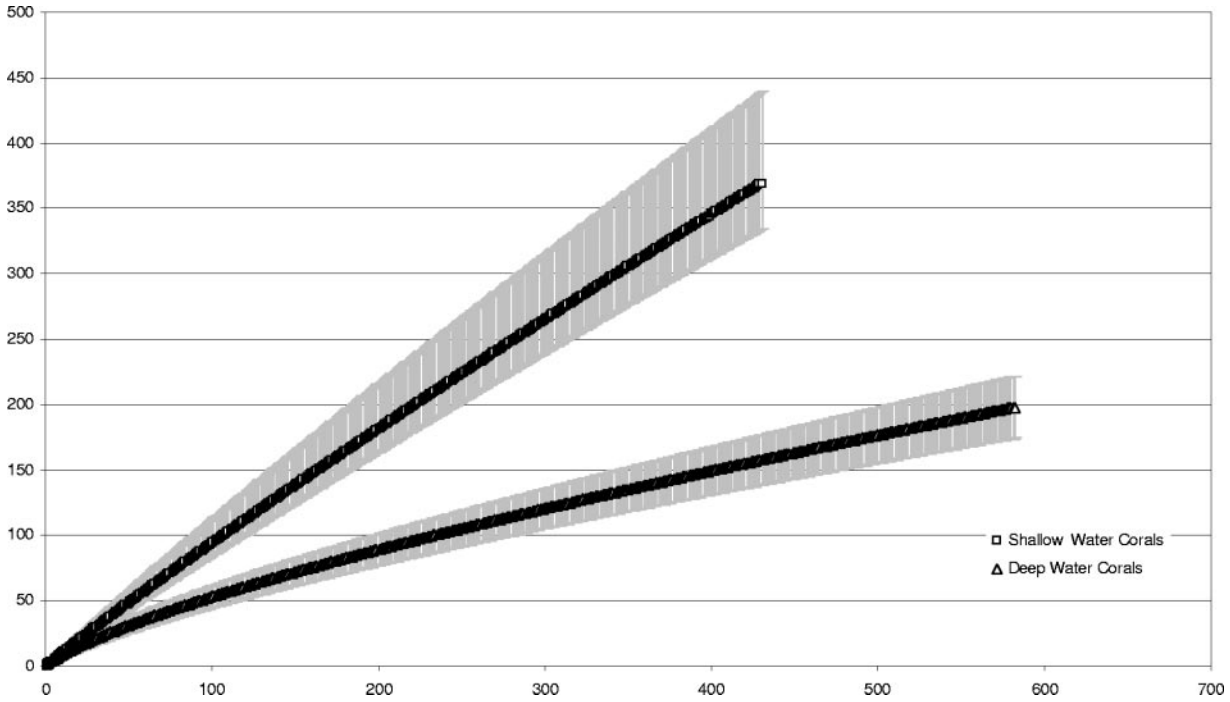

FIG. 2. Rarefaction curves for the accumulated coral-associated 16S rRNA gene sequences generated for this study (CGOA, -C, -D, -F, and -G) and the sequences of Rohwer et al. (12, 13). Bars indicate 95% confidence intervals. Statistical resampling was performed using EstimateS.

containing at least 10% gammaproteobacteria. Sequences falling within the pseudomonad tree (see Fig. S2 in the supplemental material) appear most closely related to the oligotrophic marine gammaproteobacteria (OMG) (2). The lack of a close phylogenetic relationship between representatives of the described major OMG clades and our coral sequences suggests that the latter represent new OMG clades.

Rarefaction analysis of our data and of sequence data from shallow-water scleractinian coral communities (12) suggested that our accumulated deepwater octocoral samples showed less diversity than their shallow-water counterparts (Fig. 2); with 350 sequences sampled, the shallow-water data set contained approximately twice as many observed operational taxonomic units (97% threshold for operational taxonomic unit definition) as the deepwater set.

This study provides a first glimpse of the deep-sea octocoral microflora. The results suggest that these populations are dominated by several major groups but that the relative proportions of these groups vary (bearing in mind that known methodological biases [5] limit the extent to which clone library compositions reflect community compositions). Phylotypes clustered according to sample origin, and we did not observe much overlap between coral-associated phylotypes and those recovered from the water column and rock surfaces (see Fig. S1 and S2 in the supplemental material), suggesting characteristic coral-associated assemblages with minimal influence of transient water-column microbes. Future sampling of multiple individuals and their immediate environment is clearly needed to perform a more comprehensive survey and to address questions regarding the nutritional relationships, evolution, and biogeography of these populations.

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