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Sulfate-Reducing Bacteria in Tubes Constructed by the Marine Infaunal Polychaete *Diopatra cuprea*

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Marine infaunal burrows and tubes greatly enhance solute transport between sediments and the overlying water column and are sites of elevated microbial activity. Biotic and abiotic controls of the compositions and activities of burrow and tube microbial communities are poorly understood. The microbial communities in tubes of the marine infaunal polychaete *Diopatria cuprea* collected from two different sediment habitats were examined. The bacterial communities in the tubes from a sandy sediment differed from those in the tubes from a muddy sediment. The difference in community structure also extended to the sulfate-reducing bacterial (SRB) assemblage, although it was not as pronounced for this functional group of species. PCR-amplified 16S rRNA gene sequences recovered from *Diopatra* tube SRB by clonal library construction and screening were all related to the family *Desulfobacteriaceae*. This finding was supported by phospholipid fatty acid analysis and by hybridization of 16S rRNA probes specific for members of the genera *Desulfosarcina*, *Desulfobacter*, *Desulfobacterium*, *Desulfobotulus*, *Desulfococcus*, and *Desulfovibrio* and some members of the genera *Desulfomonas*, *Desulfuromonas*, and *Desulfomicrobium* with 16S rRNA gene sequences resolved by denaturing gradient gel electrophoresis. Two of six SRB clones from the clone library were not detected in tubes from the sandy sediment. The habitat in which the *D. cuprea* tubes were constructed had a strong influence on the tube bacterial community as a whole, as well as on the SRB assemblage.

Abiotic and biotic characteristics of estuarine sediments strongly affect the distributions and activities of the resident microbial communities. Undisturbed marine sediments are often highly stratified, with oxygen-consuming processes occurring near the sediment surface and a variety of anaerobic processes occurring in deeper sediment layers (3, 72). Chemocline microenvironments that contribute to and are derived from the stratification of microbial activities are well-known sites of enhanced microbial activity; this is particularly true of the sediment-water interface, in which numerous chemical gradients are sharply defined (65). The depth of the oxic-anoxic boundary is dependent on the sediments in which it exists and the biota inhabiting the sediments. The depth of oxygen penetration in nearshore sediments composed of fine particles, silts, and clays is typically not as great as that in sediments composed of coarser materials (9, 14), and this strongly influences microbial distributions. Sediment grain size and total organic content have also been correlated with bacterial abundance. In general, as grain size decreases, the organic matter content of sediments and bacterial numbers increase (17, 21).

Stable, sharply defined sediment stratification, as described above, is often transient in nearshore marine systems due to intense physical and biological disturbance (31, 86). Although sediment mixing may have negative effects on some bacteria, exposing obligate anaerobes to oxygen and physically disrupting microbial consortia (66), it may also stimulate the growth and activity of other bacteria by introducing solutes, including oxygen, into anoxic sediments (28, 36, 45). Enhanced solute exchange is also an important property of macroinfaunal tubes and burrows (6, 38, 49, 78). The dwellings of marine infauna vary greatly in structure, ranging from simple tubes composed of loosely packed sediment lined with mucopolysaccharides (e.g., *Amphitrite ornata* [4]) to structurally cohesive tubes constructed from polysaccharides and proteins (e.g., *Diopatra cuprea* [64]). Large burrow complexes can be constructed by burrowing crustaceans and may have packed sediment walls (e.g., *Upogebia affinis* [5]). These burrow and tube structures increase the surface area across which solutes can diffuse into or out of the sediments and provide a more stable physical environment compared to surficial sediments.

When infaunal host organisms irrigate their burrows or tubes with seawater, oxygen and other solutes are introduced into formerly anoxic sediments (29, 38, 48), and potentially inhibitory compounds are removed (47). Radial chemoclines, analogous to the planar chemoclines found in undisturbed surficial sediments, are thus extended vertically into the sediments (2, 3, 4, 12, 60). These stable burrow structures with their radial chemoclines of potential electron donors and acceptors support microbial activity and biomass at levels that are elevated relative to those in the surrounding bulk sediments (3, 6, 48, 49, 78).

Sulfate-reducing bacteria (SRB) are key participants in the biogeochemical cycling of sulfur and carbon in marine sediments and are responsible for as much as 50% of the total carbon oxidation in shallow-water coastal marine sediments (40). It is believed that the tubes and burrows of marine infauna may be significant foci of sulfate reduction. For example, the sulfate reduction rates in the irrigated burrows of the marine polychaete *A. ornata* were estimated to be higher than

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those in the surrounding anoxic sediment (4). SRB have been detected in the burrows of several species of marine infauna by using phospholipid fatty acid (PLFA) analysis (24, 78). This method allows detection of some SRB groups on the basis of specific signature biomarker PLFAs (26, 44, 82), but not all SRB groups have specific biomarker PLFAs and PLFA analysis does not provide detailed, phylogenetically based information on the types of SRB that may be present in burrows.

D. cuprea (referred to as *Diopatra* below) actively irrigates its tubes, and the irrigation rates are as high as 52 ml g (fresh weight) of worm^{-1} h^{-1} (58). Oxygen from incurrent seawater diffuses through the tube wall and into the surrounding sediment, as indicated by orange to light brown oxidized bands of sediment around the tubes. *Diopatra* tubes found within the intertidal zone are exposed at low tide, during which irrigation activity ceases, and oxygen in the burrow water is rapidly depleted and remains depleted until the incoming high tide. PLFA analysis of several other common marine infaunal tubes and burrows has shown that SRB are present in these structures (78), but some of the animals are relatively sluggish irrigators and oxygenation of the burrow water may not be very efficient. Previous work by Phillips and Lovell (68) showed that *Diopatra* tubes support large and highly active bacterial communities. Whether SRB can occur in tubes of an active irrigator, such as *Diopatra*, and, if so, what types of SRB occur there have not been determined.

We examined the diversity of the bacterial community, specifically the SRB, in the tubes of *Diopatra* and in surrounding sediments by 16S rRNA gene sequence recovery and analysis, employing clonal library construction, denaturing gradient gel electrophoresis (DGGE), and DNA-DNA hybridization methods. PLFA analysis was also conducted to provide a more inclusive view of the composition and physiological status of the bacterial community inhabiting *Diopatra* tubes. The goals of this study were to determine the types of SRB present in *Diopatra* tubes and to examine whether the texture of the sediment around the *Diopatra* tubes influences the types of bacteria (specifically, SRB) present in the tubes.

MATERIALS AND METHODS

Study sites. Samples were collected from three sites in the North Inlet estuary (33°N, 79°W), Georgetown, S.C. Study site A was located on the sheltered side of a large intertidal shoal. Site B was located on an intertidal mud flat sheltered by the shoal used for site A. *Diopatra* tubes and sediment samples were collected from both of these sites. Site C was located on a small intertidal sand bar, and *Diopatra* tubes were collected from it as well.

Characteristics of the *Diopatra* tubes. The tubes of *Diopatra* are built by solitary onuphid polychaetes and are composed of sulfated polysaccharide mucus that is secreted from glands and hardens upon contact with seawater (64). Each tube has two structurally different portions. The unreinforced portion of the tube is built below the sediment surface by secretion of mucus as the worm burrows vertically through the sediment. The tube cap, which is in the form of an inverted hook, is reinforced with debris that is cemented to the exterior of the tube wall. It is typically found above the sediment surface, but it may extend below the surface, particularly when the sediment is frequently disturbed. The lengths of the cap and the portion of the reinforced tube section that extends into the sediment depend upon erosion and deposition of sediment (64).

Sample collection. Only the unreinforced portions of the tubes were used for analysis as debris cemented onto the outside of the reinforced portions of *Diopatra* tubes made them unsuitable. The tubes were exhumed until 20 to 25 cm of unreinforced tube was exposed. Branched tubes, tubes having subsurface reinforced sections longer than 10 cm, and tubes with subsurface reinforced sections interspersed with unreinforced sections were excluded from the analysis. The tube cap and reinforced tube associated with it were removed, and 12 to 16 cm of unreinforced tube was collected for analysis. Tube samples were placed in sterile disposable 50-ml screw-cap polypropylene tubes containing seawater. Sediment samples were collected by using cut-off 10-ml syringes (interior diameter, 1.4 cm; length, 8.5 cm) from the top 8 cm of sediment and from 8 to 16 cm below the surface for analysis of sediment physical characteristics, for bacterial cell counting, and for genomic DNA extraction. Sediment samples collected for cell counting were fixed in filtered 2.5% formaldehyde in sterile 50-ml tubes. Sediment and tube samples were transported to Columbia, S.C., on ice for processing. *Diopatra* tubes for PLFA analysis were collected from site C as described above, transferred to sterile WhirlPak bags (Nasco, Fort Atkinson, Wis.), and immediately frozen on dry ice in the field. These samples were stored at −70°C pending shipment to the Waterway Experiment Station for processing.

Sediment analysis and bacterial cell counting. The sediment grain size distribution and organic matter content for sample site C have been presented previously (56). Sediment samples from sites A and B were collected and dried to constant weight at 55°C. The sediment was then dry sieved through a Wentworth series of sieves by using a mechanical shaker to facilitate size fractionation. Porosity was calculated from the water loss after drying of the sediment. The organic matter content of the sediment was determined by determining the ash-free dry weight after combustion at 500°C in a muffle furnace for 4 h. Bacterial cell numbers were determined by using the epifluorescence direct count method of Hobbie et al. (37), as modified for use with sediments (77, 87), and by using Sytox Green (Molecular Probes, Eugene, Oreg.) instead of acridine orange (68).

Amplification, cloning, and amplified ribosomal DNA (rDNA) restriction analysis of 16S rRNA genes. Genomic DNA was purified from sediment and *Diopatra* tube samples by using the methods of Lovell and Piceno (56), as modified by Piceno et al. (69). Nearly full-length 16S rRNA gene sequences were then PCR amplified by using the domain *Bacteria*-specific oligonucleotide primers 27F (forward primer) and 1492R (reverse primer) designed by Lane (53) (Table 1). The PCR system consisted of the following: 1 ng of template DNA μl^{-1}, 0.025 U of Expand High Fidelity DNA polymerase (Boehringer Mannheim, Indianapolis, Ind.) μl^{-1}, 1× Expand High Fidelity PCR buffer, 1.5 mM $MgCl_2$, each deoxynucleoside triphosphate at a concentration of 0.2 mM, and 0.25 pmol of each primer μl^{-1} in a 25-μl (final volume) mixture. Amplification and screening of amplicons were performed as described by Dang and Lovell (19). Following amplification, PCR amplimers were purified by using a GENECLEAN kit (Bio 101, Vista, Calif.) and were cloned by using the pGEM-T vector system (Promega, Madison, Wis.).

Amplified rDNA restriction analysis was performed with clones by using the procedure described by Dang and Lovell (19), with the following modifications: (i) *Taq* DNA polymerase (QIAGEN) was used instead of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Foster City, Calif.), and (ii) amplimers were digested with HaeIII and MspI.

16S rDNA sequencing. Plasmid DNA was purified from each unique clone (QIAGEN), and plasmid quality and quantity were determined by agarose gel electrophoresis and fluorometry, respectively. The DNA insert was sequenced in both directions by using forward bacterial primer GM5F (*Escherichia coli* sequence positions 341 to 357) (62) and universal reverse primer 907R (*E. coli* sequence positions 907 to 928) (8) (Table 1). The sequences were determined by using a SequiTherm EXCEL II Long-Read LC DNA sequencing kit (Epicentre Technologies, Madison, Wis.) and a Li-Cor DNA4000LS sequencer (Li-Cor, Lincoln, Nebr.) or an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, Calif.) with BigDye version 2.0 chemistry.

Analysis of 16S rDNA sequences. Previously reported 16S rDNA gene sequences most similar to those determined in this study were identified by using Sequence_Match (version 2.7) via Ribosomal Database Project II (57) and the advanced BLAST search program (7) of the National Center for Biotechnology Information GenBank database (11). Sequences were aligned by using Clustal X (version 1.81) (81). Distance matrices were constructed by using Jukes-Cantor or Kimura models, and neighbor-joining trees (bootstrapped 1,000 times) and maximum-parsimony phylogenetic trees (utilizing the close-neighbor-interchange search method with a search level of 3, initial random addition trees with 10 replicates, and bootstrapping 1,000 times) were constructed from aligned sequences by using MEGA (version 2.1) (51). Maximum-likelihood trees were constructed by using Tree-puzzle (version 5.0) (79) with 1,000 puzzling steps and Jukes-Cantor and Kimura substitution models. The major microbial phyla and proposed phyla used for phylogenetic analyses are listed in Table 2.

DGGE analysis of samples. Clones of SRB 16S rDNA, 16S rDNA amplicons from sediment, and *Diopatra* tube DNA extracts were analyzed by using DGGE. Sample DNA was PCR amplified by using the GM5F primer (62) modified with a GC clamp at the 5′ end (63) and universal primer 907R (8) (Table 1). The reaction mixture was similar to that used for amplified rDNA restriction analysis,

TABLE 1. Oligonucleotides used in this study

Oligonucleotide	Sequence	Position[a]	Specificity	Reference
27F[b]	5′-AGAGTTTGATCMTGGCTC-3′[e]	27–44	Domain *Bacteria*	53
1492R[b]	5′-GGTTACCTTGTTACGACTT-3′	1492–1510	Domain *Bacteria*	53
GM5F[b,d]	5′-CCTACGGGAGGCAGCAG-3′	341–357	Domain *Bacteria*	62
907R[b]	5′-CCGTCAATTCCTTTGAGTTT-3′	907–928	Domain *Bacteria*	8
Probe 687[c]	5′-TACGGATTTCACTCCT-3′	687–702	*Desulfovibrio* plus some members of the genera *Geobacter*, *Desulfomonas*, *Desulfuromonas*, *Desulfomicrobium*, *Bilophila*, and *Pelobacter*	23
Probe 804[c]	5′-CAACGTTTACTGCGTGGA-3′	804–821	*Desulfobacter*, *Desulfobacterium*, *Desulfobotulus*, *Desulfococcus*, and *Desulfosarcina*	23
Probe DSS658[c]	5′-TCCACTTCCCTCTCCCAT-3′	658–678	*Desulfosarcina*	59

[a] Based on the 16S rDNA of *E. coli.*
[b] 16S rDNA primer.
[c] Biotinylated 16S rDNA probe.
[d] When used for DGGE, this primer has the following GC clamp at its 5′ end: 5′-CGCCCGCCGCGCGCGCGGGCGGGGCGGGGGCACGGGGGG-3′.
[e] M = A or C.

except that 0.1 ng of sample DNA μl^{-1} was used in each reaction. The touchdown PCR protocol used was as follows: initial denaturation at 94°C for 3 min; 20 cycles of 95°C for 60 s, 65°C for 60 s decreasing by 0.5°C/cycle, and 72°C for 60 s; 10 cycles of 95°C for 60 s, 55°C for 60 s, and 72°C for 60 s; and a final extension step at 72°C for 5 min. Six 25-μl reaction mixtures were used for each sample. The reaction mixtures were pooled, and 15 μl was used to screen for amplification efficiency and amplimer size by electrophoresis on a 1.5% agarose–Tris-borate-EDTA gel. Amplimers were concentrated by isopropyl alcohol precipitation, recovered by centrifugation at 10,000 × *g* for 30 min, washed with 70% ethanol, air dried, and dissolved in 10 μl of Tris-EDTA.

DGGE was performed by using the D-Code universal mutation detection system (Bio-Rad, Hercules, Calif.) with 1-mm-thick, 8% polyacrylamide gels and a denaturing solution gradient of 40 to 60% urea-formamide (0% denaturing solution contained 20% [vol/vol] 40% acrylamide/bisacrylamide in 1× Tris-acetate-EDTA (TAE) buffer, and 100% denaturing solution contained 20% [vol/vol] 40% acrylamide/bisacrylamide, 40% formamide [deionized], and 42% [wt/vol] urea in 1× TAE buffer). The samples were electrophoresed in 1× TAE buffer at 60°C and a constant voltage of 100 V for 19 h. DGGE gels were stained with ethidium bromide, and digital images were collected by using an Alpha Imager gel documentation system (AlphaInnotech Corp., San Leandro, Calif.).

SRB probes. DNA oligonucleotide probes were selected to provide coverage of SRB types known to occur in marine sediments, with particular emphasis on coverage of SRB sequences recovered from the clonal libraries. Probes were made and biotinylated by Integrated DNA Technologies, Inc. (Coralville, Iowa). Probe 804 hybridizes with the genera *Desulfobacter*, *Desulfobacterium*, *Desulfobotulus*, *Desulfococcus*, and *Desulfosarcina* (23). Probe DSS658 hybridizes with only *Desulfosarcina* (59). Probe 687 hybridizes with members of the genus *Desulfovibrio* (Table 1) (23).

Southern blotting and hybridization analysis of DGGE gels. DGGE gels were electroblotted onto positively charged nylon membranes (Tropilon-plus; Tropix, Bedford, Maine) by using a GENIE electrophoretic transfer apparatus (Idea Scientific, Corvallis, Oreg.). DNA was transferred in 0.5× Tris-borate-EDTA at 6 V for 1 h and then denatured by washing the membrane with denaturing solution (0.4 M NaOH, 0.6 M NaCl) for 15 min. The membrane was transferred to neutralizing solution (0.5 M Tris-HCl [pH 8.0], 0.6 M NaCl) and washed twice for 10 min each time. It was then transferred to 5× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate) for 5 min. Following the washes, the membrane was thoroughly air dried and then baked under a vacuum at 80°C for 2 h. Hybridization and detection of SRB sequences were performed by using the Southern Lights hybridization and detection protocol (Tropix) for biotinylated probes, modified as follows: the membrane was prehybridized and hybridized at 49, 50.3, and 37°C for probes 804, DSS658, and 687, respectively. The biotinylated probe concentration was 2.5 pmol/ml, and the hybridization buffer (100 $\mu l/cm^2$ of membrane) was amended with boiled salmon sperm DNA (23 ng/cm^2 of membrane).

Purification and quantification of phospholipid fatty acids. Solvents were obtained from Burdick & Jackson (Muskegon, Mich.) and were residue analysis grade (GC^2). Standards and derivitizing reagents were purchased from Supelco Inc. (Bellefonte, Pa.), Nu Chek Prep (Elysian, Minn.), and Pierce Chemical Co. (Rockford, Ill.). The *Diopatra* tube samples were extracted, and PLFAs were recovered and derivitized for identification and quantification as described by White and Ringelberg (85). The resulting phospholipid fatty acid methyl esters were dissolved in hexane containing methyl nonadecanoate (50 pmol μl^{-1}) as an internal standard and were analyzed by using a gas chromatograph equipped with a DB-5MS capillary column (60 m by 0.25 mm [inside diameter]; film thickness, 0.1 μm; J&W Scientific, Folsom, Calif.) coupled to a mass selective detector (Hewlett-Packard GC6890-5973) by using electron impact ionization at 70 eV. The areas under the peaks were converted to concentrations, added, and then normalized to the weight of the sample extracted for biomass determinations. Fatty acid double bond positions and geometry were confirmed by gas chromatography-mass spectrometry analysis of the dimethyl disulfide adducts of the monounsaturated polar fatty acid methyl esters.

Fatty acid nomenclature. Fatty acids are designated below as follows: A:BωC, where A is the total number of carbon atoms, B is the number of double bonds, and C is the position of the double bond from the aliphatic end of the molecule. The geometry of this bond is indicated by c (for *cis*) or t (for *trans*). The prefixes i and a refer to iso- and anteisomethyl branching, respectively. Midchain methyl branches are designated by me preceded by the position of the methyl group from the acid end of the molecule. Cyclopropyl fatty acids are designated cy.

Statistical analysis. Gel images and blots from hybridizations were analyzed by using Gel-Pro Analyzer 4.0 (MediaCybernetics, Carlsbad, Calif.). The resulting banding patterns were converted to binary data (presence or absence) and were compared by using the unweighted pair group method with arithmetic mean (UPGMA) with the simple matching coefficient in order to calculate similarities and to perform principal-component analysis (Multi-Variate Statistical Package 3.13g; Kovach Computing Services, Wales, United Kingdom).

RESULTS

Sediment characteristics and bacterial cell counts. The upper and lower samples from each site were pooled for sediment analysis due to the small sample sizes. The two Skimmer Shoals sites were close to each other, yet the sediments differed markedly (Table 3). Site A was located on the sheltered side of a large shoal, and the sediment was composed primarily of very fine sand, as described with the Wentworth scale. The site B sediment, from the protected mudflat flanking the shoal, was more mixed. This sediment consisted primarily of silt, but there was also a substantial content of very fine sand and clay. The sediment at site B also had a higher organic matter content and porosity. The sediment from site C at the Oyster Landing sand bar was composed primarily of sand larger than that found at Skimmer Shoals (56). The silt and clay content and the organic carbon content were lower than those at either of the Skimmer Shoals sites. Significantly higher numbers of bacterial cells

TABLE 2. Organisms used for phylogenetic analysis and their accession numbers

Taxon	Species	Accession no.
Archaea	*Archaeoglobus fulgidus*	X05567
	Thermococcus celer	M21529
Bacteroidetes	*Bacteroides fragilis*	M11656
	Cytophaga diffluens	M58765
	Flavobacterium aquatile	M62797
	Flexibacter flexilis	M62794
α-*Proteobacteria*	*Roseobacter litoralis*	X78312
	Hyphomonas polymorpha	AJ227813
β-*Proteobacteria*	*Nitrosomonas europaea*	AF037106
	Rhodocyclus purpureus	M34132
δ-*Proteobacteria*	*Desulfacinum hydrothermale*	AF170417
	Desulfacinum infermum	L27426
	Desulfoarculus baarsii	M34403
	Desulfobacca acetoxidans	AF002671
	Desulfobacter postgatei	M26633
	Desulfobacterium autotrophicum	M34409
	Desulfobotulus sapovorans	M34402
	Desulfobulbus propionicus	M34410
	Desulfocapsa thiozymogenes	X95181
	Desulfocella halophila	AF022936
	Desulfococcus multivorans	M34405
	Desulfofaba gelida	AF099063
	Desulfofrigus oceanense	AF099064
	Desulfohalobium rerbaense	U48244
	Desulfomicrobium baculatum	AF030438
	Desulfovibrio piger	AF192152
	Desulfomonile limimaris	AF282177
	Desulfonatronum lacustre	Y14594
	Desulfonema limicola	U45990
	Desulforhopalus vacuolatus	L42613
	Desulfosarcina variabilis	M34407
	Desulfostipes sapovorans	AF148141
	Desulfotalea arctica	AF099061
	Desulfotomaculum nigrificans	AB026550
	Desulfovibrio desulfuricans	AF098671
γ-*Proteobacteria*	*Aeromonas hydrophila*	X60404
	Pseudomonas putida	L28676
	Thiorhodovibrio winogradskyi	AJ006214
	Vibrio parahaemolyticus	M59161
ε-*Proteobacteria*	*Arcobacter nitrofigilis*	L14627
	Compylobacter jejuni	L04315
Aquifex	*Aquifex pyrophilus*	M83548
Chlorobium	*Chlorobium limicola*	Y08102
Chloroflexus	*Chloroflexus aurantiacus*	M34116
Chrysiogenes	*Chrysiogenes arsenatis*	X81319
Clostridium	*Clostridium butyricum*	AJ289704
Fusobacterium	*Fusobacterium nucleatum*	AJ133496
Holophaga	*Holophaga foetida*	X77215
Nitrospira	*Nitrospira marina*	X82559
Planctomyces	*Planctomyces maris*	X62910
Spirochaeta	*Spirochaeta halophila*	M88722
Verrucomicrobium	*Verrucomicrobium spinosum*	X90515
Candidate phylum OD1	Uncultured bacterium	AY193145
Candidate phylum OP11	Uncultured OP11	AF424454

were found in the upper 8 cm of sediment at site B than at site A ($P = 0.016$, as determined by the Student t test). However, the cell counts in the deeper subsurface sediments did not differ significantly (Table 3).

Clonal library composition. A total of 123 clones were recovered from two clonal libraries; 78 clones were recovered from Skimmer Shoals site A, and 45 clones were recovered from site B. Twenty unique clones were recovered from the site A library, and each of these clones was designated by the letter A and a number; 19 unique clones were recovered from the site B library, and each of these clones was designated by the letter B and a number. These libraries had coverage values of 78.4 and 57.8%, respectively.

16S rDNA sequence analysis. Skimmer Shoals site A clones yielded 15 valid sequences, and site B clones yielded 17 sequences. The other clones either could not be sequenced or contained chimeric sequences. All valid sequences were aligned, and their phylogenetic affiliations were determined. The majority of the sequences belonged to four major bacterial phyla or subdivisions. Three clones belonged to the *Bacteroidetes*, five clones belonged to the *Verrucomicrobia*, 10 clones belonged to the gamma-*Proteobacteria*, and six clones belonged to SRB in the delta-*Proteobacteria*. Three clones grouped with two proposed bacterial phyla; one clone grouped with OP11, and two clones grouped with OD1.

Clones from both sites were affiliated with the δ-*Proteobacteria*, γ-*Proteobacteria*, and *Verrucomicrobiales*. All clones affiliated with the *Bacteroidetes* were recovered from site B tubes, as were the clones falling into the proposed phyla OD1 and OP11. Two clones, A17 and B3, which were most similar to *Chloroflexus* (84.9 and 70.2% similar, respectively) failed to group with *Chloroflexus aurantiacus* in phylogenetic trees. Other clones that failed to group with known bacterial phyla or each other were B2 and B15 (most similar to the *Acidobacteriaceae*; 86.6 and 71.3% similar, respectively), B10 (*Firmicutes*; 74.3% similar), and A16 (*Planctomycetales*; 75.8% similar) (Fig. 1). PLFAs recovered from *Diopatra* tubes from both sites also indicated that there were diverse bacterial communities dominated by gram-negative bacteria, and there was a low level of eukaryote-specific PLFAs (Table 4).

SRB clones belonged to the *Desulfobacteriaceae*, as determined by BLAST searches and phylogenetic tree construction (Fig. 2). Two clones were similar to *Desulfobacter* (clones A6 and B5). Clone B5 was 93% similar to both *Desulfobacter vibrioforme* and *Desulfotignum balticum*, a newly described sulfate-reducing bacterium (50). Clone A6 was most similar to *Desulfospira joergensenii*, *Desulfobacula toluolica*, and *D. vibrioforme* (96, 95, and 94% similarity, respectively). Both of these clones had a one-base mismatch with the 804 probe at the same location and with the same substitution. This mismatch is consistent with *Desulfospira*, *Desulfobacula*, *and Desulfotignum* 16S rDNA sequences. Clones A4, A12, and B4 were most similar to *Desulfosarcina* (98, 98, and 91% similarity to *Desulfosarcina variabilis*, respectively). Clone B14 was most similar to *Desul-*

TABLE 3. Sediment characteristics at Skimmer Shoals sites A and B

Parameter	Site A		Site B	
	Mean	SD	Mean	SD
Grain size (% composition)[a]				
Medium sand (0.5–0.25 mm)[b]	0.80	0.05	0.00	0.00
Fine sand (0.25–0.125 mm)	0.46	0.15	0.65	0.92
Very fine sand (0.125–0.0625 mm)	73.69	1.80	27.31	1.49
Silt (0.0625–0.0039 mm)	21.61	1.48	48.80	1.75
Clay (<0.0039 mm)	4.15	0.77	23.25	3.18
Organic matter content (%)	2.15	0.21	7.23	0.96
Porosity	0.56	0.03	0.82	0.05
Bacterial counts (10^8 cells/ml) in:				
Surface sediment (top 8 cm)	30	6.6	41	5.1
Subsurface sediment (8 cm below surface)	24	1.2	24	1.2

[a] Wentworth classification.
[b] The numbers in parentheses are size fractions.

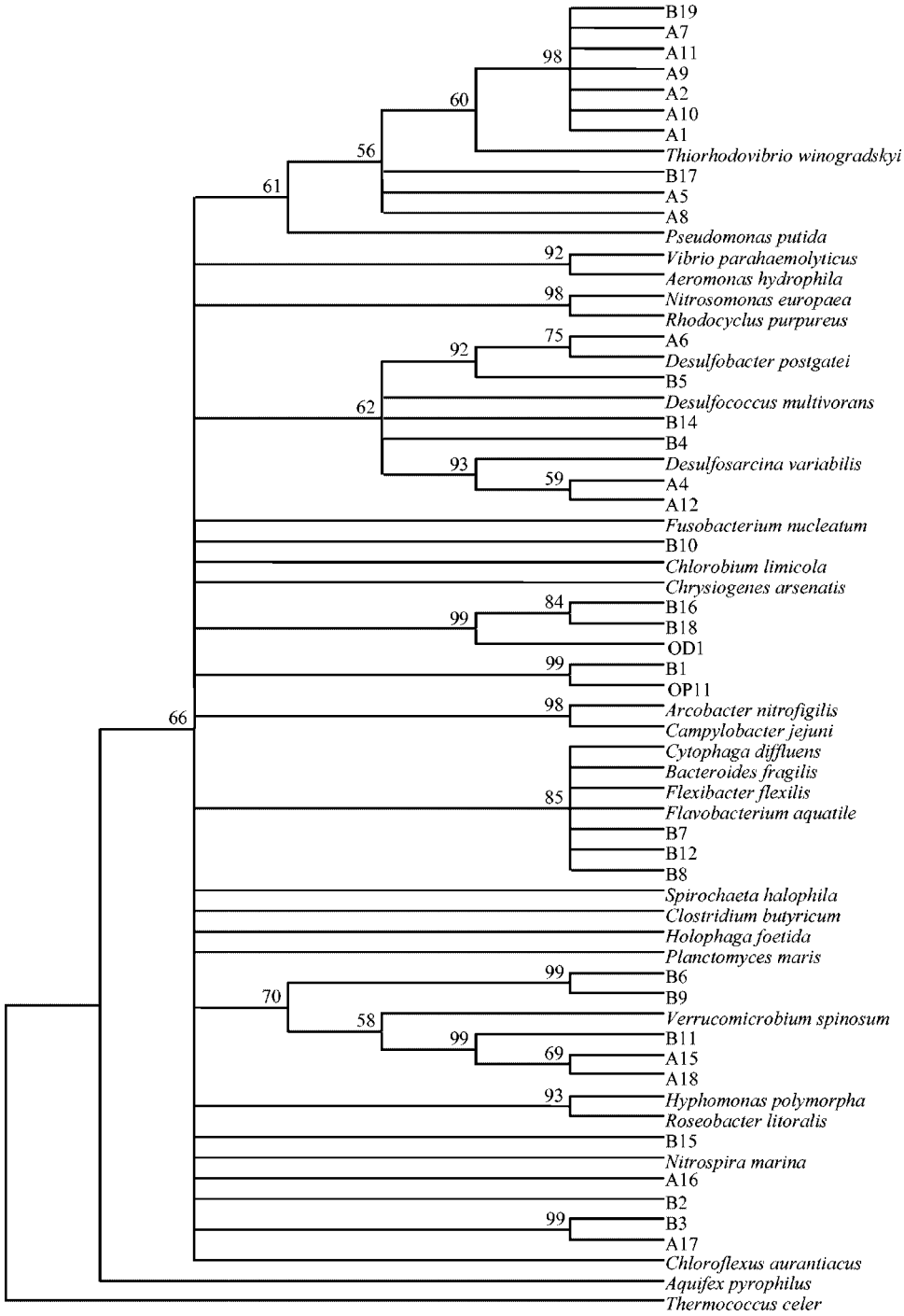

FIG. 1. Unrooted phylogenetic tree showing the affiliations of cloned sequences with bacterial phyla and subdivisions. Approximately 610 bp, comprising the region of 16S rDNA sequenced from cloned DNA, was used to construct the maximum-parsimony tree by utilizing the close-neighbor-interchange search method with a search level of 3, initial random addition trees with 10 replicates, and bootstrapping 1,000 times. Nodes supported above 50% are indicated. The *Thermococcus celer* 16S rDNA sequence was used as the outgroup.

fobacterium indolicum (81% similarity). The similarity of most clone sequences to the sequences of known SRB was reinforced by their positions in phylogenetic trees. Clones A6 and B5 clustered with *Desulfobacter*, and clones A4 and A12 clustered with *Desulfosarcina*. However, clones B4 and B14 did not cluster with the SRB sequences to which they were most similar. These sequences clustered together and were 92% similar to each other. The discrepancy between the position in the phylogenetic tree and the simple level of similarity may be in part a function of the short lengths of the sequences.

The SRB clone sequences were analyzed by DGGE to determine the relative positions of the sequences in the gels (Fig. 3). All of the clones except clones A4 and A12 produced clear bands at different positions in the gel. Clones A4 and A12

TABLE 4. PLFA values for five replicate *D. cuprea* tubes exhumed from site C[a]

Compound or ratio[b]	Mol% or ratio						
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Avg	SD
Saturated PLFA							
14:0	2.13	3.59	3.31	0.48	1.16	2.13	1.34
15:0	1.38	2.08	2.09	0.68	0.33	1.31	0.80
16:0	15.98	18.25	17.39	11.55	6.91	14.02	4.74
17:0	1.14	1.34	1.38	1.09	1.12	1.21	0.14
18:00	2.36	2.47	2.55	4.14	10.81	4.47	3.62
20:00	0.48	0.51	0.57	0	0.4	0.39	0.23
21:00	0.14	0.38	0.31	0	0	0.17	0.17
22:00	0.14	0.18	0.16	0	0.14	0.12	0.07
24.0	0.1	0.14	0.22	0	0	0.09	0.09
Total	23.85	28.94	27.98	17.94	20.87	23.92	4.66
Terminally branched saturated PLFA							
i14:0	1.17	1.33	1.39	0	0.07	0.79	0.70
i15:0	7.41	6.31	6.42	2.69	0.29	4.62	3.01
a15:0	8.72	6.23	6.9	4.88	0.29	5.40	3.18
i16:0	2.26	2.1	2.12	1.24	0.13	1.57	0.90
i17:0	1.64	1.47	1.44	1.62	0.25	1.28	0.58
a17.0/17:1ω8c	2.7	2.38	2.48	2.13	0.32	2.00	0.96
Total	23.9	19.82	20.75	12.57	1.36	15.68	9.02
Midbranched saturated PLFA							
12me 15:0	0	0.83	0.81	1.01	0	0.53	0.49
10me 16:0/unk 17:1	3.93	3.22	2.37	3.12	0.52	2.63	1.30
unk18:0	0	0	0	0	0.65	0.13	0.29
10me18	0.36	0.26	0.26	0	0	0.18	0.17
Total	4.29	4.32	3.44	4.13	1.17	3.47	1.33
Monounsaturated and cyclopropyl PLFA							
16:1ω9c	0.92	1.06	1.09	0	0.19	0.65	0.52
16:1ω7c	14.23	12.22	11.19	9.38	1.68	9.74	4.83
16:1ω7t	1.04	0.73	0.69	0	0	0.49	0.47
16:1ω5c	3.41	1.88	1.99	5.09	0.21	2.52	1.83
16:1ω5t	0	0	0	0	0.23	0.05	0.10
17:1ω6	0.6	0.67	0.69	0	0	0.39	0.36
cy17:0	1	0.9	0.82	0	0	0.54	0.50
18:1ω7c	11.67	12.44	12.83	18.63	4.73	12.06	4.94
18:1ω7t	0.7	0.65	0.77	1.5	0.31	0.79	0.44
18:1ω5c	1.14	1.1	1.49	0.96	0.5	1.04	0.36
18:1ω9c	1.71	2.92	2.86	2.59	1.38	2.29	0.70
19:1b	0	0	0	0	0.41	0.08	0.18
19:1c	0	0	0	0	0.24	0.05	0.11
20:1ω11c	0.21	0.08	0.19	0	0.67	0.23	0.26
20:1ω9c	0.34	0.15	0.34	3.73	6.5	2.21	2.83
20:1ω7c	0.13	0.28	0.18	0	0.4	0.20	0.15
unk20:1	0	0	0	0	0.26	0.05	0.12
22:1ω11c	0	0	0	0	0.43	0.09	0.19
20:1ω9c	0	0	0	2.3	0.33	0.53	1.00
22:1ω7c	0	0	0	0	0.13	0.03	0.06
cy19:0	4.14	2.86	2.36	3.24	0.07	2.53	1.52
Total	41.24	37.94	37.49	47.42	18.67	36.55	10.75
Branched monounsaturated PLFA							
i17:1ω7c	0.76	0.08	0.89	0	0	0.35	0.44
unk18:1	0	0	0	0	2.91	0.58	1.30
br18:1/19:1a	0	0	0	0	0.19	0.04	0.08
br19:1	0.75	1.5	1.1	0	0	0.67	0.67
Total	1.51	1.58	1.99	0	3.1	1.64	1.11
Poly-PLFA							
18:4ω3/10me17:0	0.29	0.49	0.43	0	0.47	0.34	0.20
18:2/unk1	0.6	0.63	1.14	1.79	0.81	0.99	0.49

Continued on facing page

TABLE 4—*Continued*

Compound or ratio[b]	Mol% or ratio						
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Avg	SD
18:2ω6	0	0	0.53	1.6	0.56	0.54	0.65
18:3ω3/i18:0	0.88	0.91	0.75	0	1.55	0.82	0.55
20:4ω6/20:5ω3	0	0	0	0	29.6	5.92	13.24
20:4ω6	0.69	2.35	1.96	6.33	0	2.27	2.46
20:5ω3	0.36	1.21	0.99	3.22	0	1.16	1.25
20:3ω3	0.11	0.26	0.23	0	0.24	0.17	0.11
poly(20:0)	0	0.25	0.2	0	0	0.09	0.12
20:2	0	0	0	0	0.24	0.05	0.11
20:2ω3	0	0	0	0	1.74	0.35	0.78
22:6ω3	0.24	0.46	0.33	0	1.24	0.45	0.47
22:4ω6	0.28	0	0.32	3.73	2.03	1.27	1.59
22:5ω3	0	0	0	0	4.94	0.99	2.21
22:3ω3	0	0	0	0	1.19	0.24	0.53
22:2a	0	0	0	0	5.2	1.04	2.33
22:2b	0	0	0	0	0.55	0.11	0.25
24:5ω3	0	0	0	0	0.2	0.04	0.09
Total	3.45	6.56	6.88	16.67	50.56	16.82	19.50
Other compounds							
Cyclotetradecane	1.7	0.83	1.48	1.28	1.41	1.34	0.32
Oleyl alcohol	0	0	0	0	2.09	0.42	0.93
unk-2	0	0	0	0	0.36	0.07	0.16
unk-3	0	0	0	0	0.4	0.08	0.18
Total	1.7	0.83	1.48	1.28	4.26	1.91	1.35
Total	99.94	99.98	100.01	100	99.98	99.982	0.03
Cy/MUFA[c]							
cy17:0/16:1ω7c	0.07	0.07	0.07	0.00	0.00	0.04	0.04
cy19:0/18:1ω7c	0.35	0.23	0.18	0.17	0.01	0.19	0.12
trans/*cis*[d]							
16:1ω7t/16:1ω7c	0.07	0.06	0.06	0.00	0.00	0.04	0.04
18:1ω7t/18:1ω7c	0.06	0.05	0.06	0.08	0.07	0.06	0.01

[a] The total amounts for tubes 1, 2, 3, 4, and 5 were 43,874, 130,569, 46,475, 5,199, and 112,733 pmol g (dry weight)$^{-1}$, respectively (average ± standard deviation, 67,770 ± 52,213.53 pmol g [dry weight]$^{-1}$).
[b] br, branched; unk, unknown.
[c] Ratio of cyclopropyl PLFA to monounsaturated fatty acid precursor.
[d] Ratio of trans isomer to *cis* isomer.

produced bands that comigrated. This was not unexpected considering the similarity of these sequences to each other. The B14 sequence did not produce a band in any of the samples.

Bacterial communities in sediment and *Diopatra* tubes. The DGGE profiles of sediment samples from the two Skimmer Shoals sites were very similar to each other and were considerably more complex than those of samples from *Diopatra* tubes at either site (Fig. 4). The profiles were very reproducible, as indicated by the similarity of patterns from replicate samples. The profiles of sediment samples were virtually identical both for the sites and for the depths sampled (data not shown). The *Diopatra* tube communities were less complex than the sediment sample communities. They displayed greater variability between sites than the sediment communities and were confirmed to be different at sites A and B by UPGMA and principal-component analysis. The DGGE profiles for tubes from the two sites clustered separately in both analyses (Fig. 4).

SRB assemblage analysis. Bands whose DGGE gel positions corresponded to the positions of the bands for all cloned SRB sequences except B14 were found at both Skimmer Shoals sites in all sediment samples and most *Diopatra* tube samples. B14 was not found in tube or sediment samples from either site. However, not all of these bands hybridized with probe 804, and in some cases probes hybridized with bands that were not readily visible in the gel. Probes 804 and DSS658 hybridized with bands corresponding to related clones. In site B samples, probe 804 hybridized with bands corresponding to clones B4, A4, and A12 in all site B *Diopatra* tubes. Bands corresponding to B5 were found in two tube samples. Two unknown bands that did not correspond to any clone were also found in site B samples. Unknown band 1 was found in three tubes, and unknown band 2 hybridized in all tube samples. In samples from site A, probe 804 hybridized to bands corresponding to clones A6 and B4 and unknown band 2 in all tube samples. Bands at the positions of bands for clones A4 and A12 and unknown band 1 were found in one tube sample. The clone B5 band was not found in site A samples. Probe DSS658 hybridized with bands whose positions corresponded to the positions of bands for clones B4, A4, and A12 and unknown bands 1 and 2 in all tube samples (Table 5). Probe DSS658 also hybridized with multiple bands that probe 804 did not detect in all samples. These bands were likely not SRB sequences as probe 804 should have

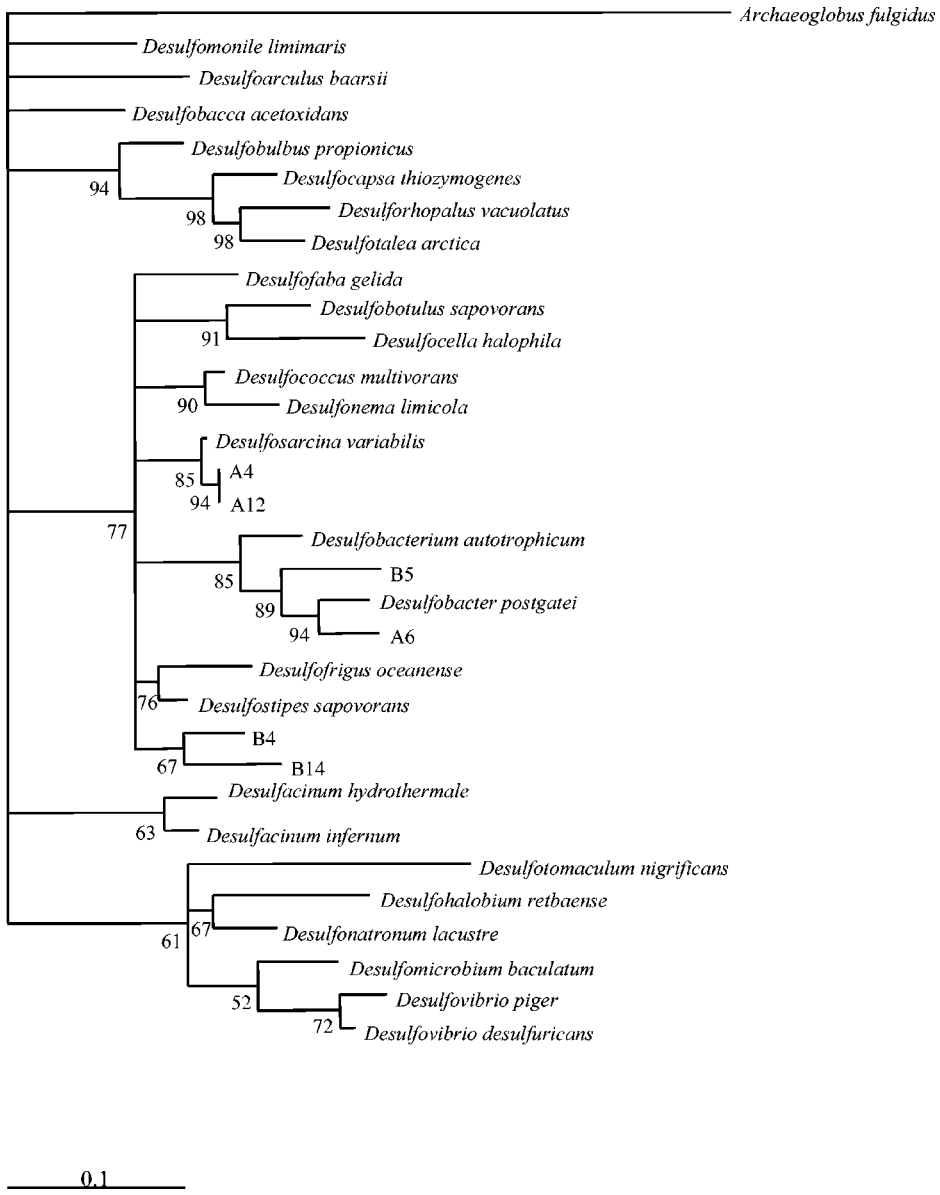

FIG. 2. Unrooted phylogenetic tree showing the affiliations of sequences that were similar, as indicated by the results of BLAST searches, to sequences of known sulfate-reducing bacteria. Approximately 610 bp, comprising the region of 16S rDNA sequenced from cloned DNA, was used to construct the quartet puzzling maximum-likelihood tree. The Jukes-Cantor substitution model and 1,000 puzzling steps were employed in tree construction. Nodes supported above 50% are indicated. The *Archaeoglobus fulgidus* 16S rDNA sequence was used as the outgroup.

hybridized with them if they were true SRB sequences. The resulting bands were probably the result of incompletely homologous hybridization. UPGMA (Fig. 5) of the probe 804 hybridization results grouped the majority of the SRB assemblages from site A together. Although the assemblages from site B did not cluster together as strongly as those from site A, most of these assemblages were more similar to each other than to those from site A.

The presence of *Desulfobacter* or SRB closely related to *Desulfobacter* was also supported by the presence of the PLFA 10me16:0 in all *Diopatra* tubes analyzed (Table 4). Although specific PLFAs that are considered signatures for *Desulfovibrio* and *Desulfobulbus* (i17:1ω7c and 17:1ω6, respectively [67]) were found, there was substantially less of these phospholipids than of 10me16:0. No 16S rDNA sequences from *Desulfovibrio* or *Desulfobulbus* or related organisms were recovered or identified either by clonal library screening or by hybridization of probe 687 to DGGE gel blots.

Physiological status. PLFA *trans*/*cis* ratios greater than 0.1 for the common membrane PLFAs 16:1ω7 and 18:1ω7 have been used as an indicator of physiological stress in bacteria (33). The *Diopatra* tube PLFAs had *trans*/*cis* ratios less than 0.1 but greater than the ratios determined for 16:1ω7 and 18:1ω7 from other infaunal worm tube and burrow microbiota (78). The cyclopropyl fatty acids and the ratios of cyclopropyl fatty acids to precursor monounsaturated fatty acids (cy17:0/16:

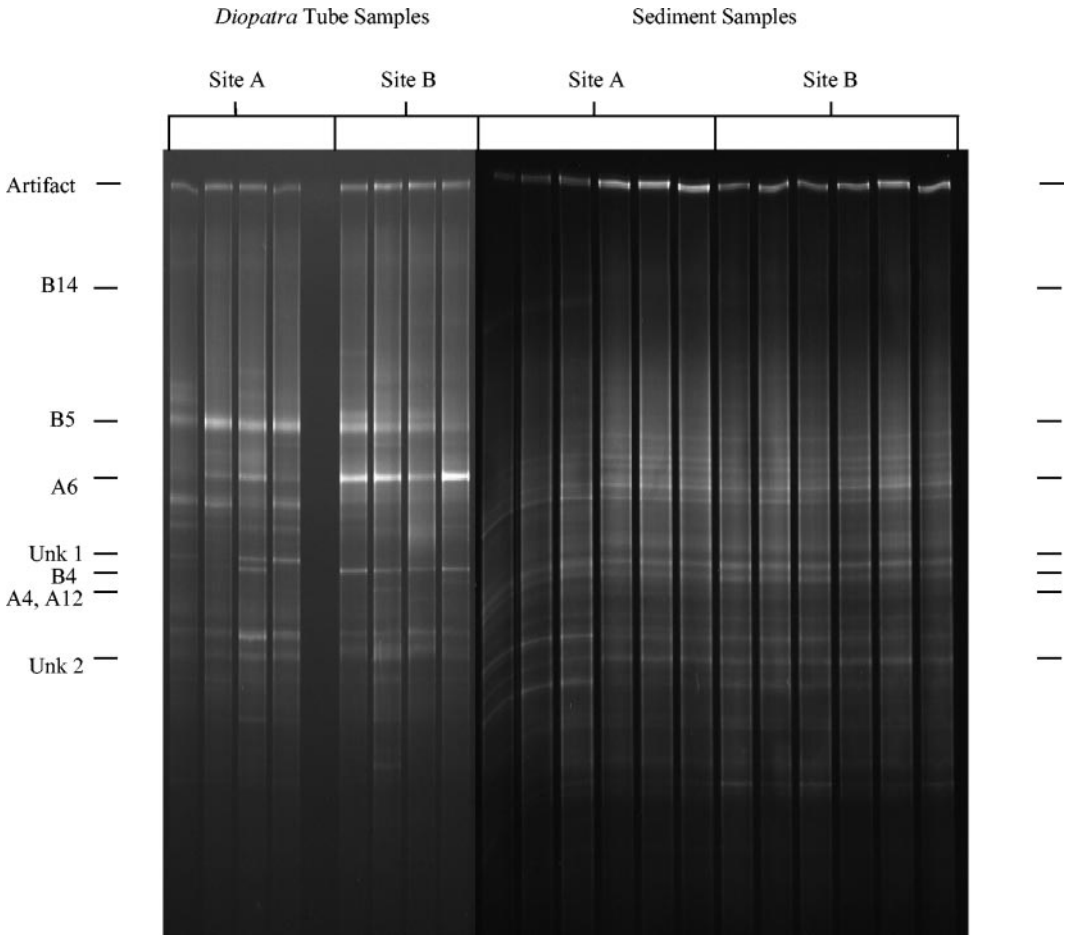

FIG. 3. DGGE analysis of sediment and *D. cuprea* tube samples from Skimmer Shoals sites A and B. The approximate locations of bands that hybridized and the clones to which they corresponded are indicated. Unk indicates bands representing unknown but presumptive SRB.

1ω7c and cy19:0/18:1ω7c) have also been used as indicators of physiological changes due to stress or anoxia (32). The levels of cy17:0 and cy19:0 and the ratios of these acids to their precursors (cy17:0/16:1ω7c and cy19:0/18:1ω7c, respectively) were comparable to the levels and ratios found in structures of other infaunal species (78). The levels of these acids may reflect suboxic conditions in the burrow water of *Diopatra* tubes when they are collected at low tide, which is a period during which the worms do not ventilate their tubes (18).

DISCUSSION

Although SRB have been classically described as obligate anaerobes (70), they are ubiquitous inhabitants of marine sediments and clearly occur in habitats that are at least intermittently oxic. The marine habitats in which SRB have been detected include the oxic upper few millimeters of intertidal sediments (42, 75), microbial mats (15, 30, 61, 76), the rhizospheres of marine plants (52, 35, 73), and the tubes and burrows of various infaunal species (78; this study). It is clear that SRB can maintain activity in such environments. Sulfate reduction has been measured at the oxic-anoxic interface and within oxic zones in marine sediments (42) and cyanobacterial mats (30, 41, 80), demonstrating that there are active SRB in these locations.

Members of the *Desulfobacteriaceae* were found in the tubes of *Diopatra*, which are actively irrigated with oxic seawater during periods of tidal submersion. The survival of a variety of cultured SRB under oxic to suboxic conditions has been explained on the basis of enzymes that may aid in reducing the impact of toxic oxygen species, such as superoxide and hydrogen peroxide. Several SRB possess catalase and superoxide dismutase activities (25, 34), as well as a variety of other enzymes and cofactors that may be involved in neutralizing toxic oxygen species (1, 54, 83, 84). The mechanisms by which sulfate reduction may occur in oxic environments are not as clear, although several possible mechanisms have been proposed.

SRB in nature often occur within biofilms or aggregates, which may promote the formation of reduced microniches. Aggregate formation has been demonstrated to be a response to oxygen stress by some SRB (74). Bianchi et al. (10) and Jørgensen (39) have shown that even relatively small bacterially colonized organic aggregates can maintain internal anoxic conditions and support sulfate reduction. Phillips and Lovell (68) found that the inner lining of the *Diopatra* tube was covered by a microbial biofilm having high cell densities and

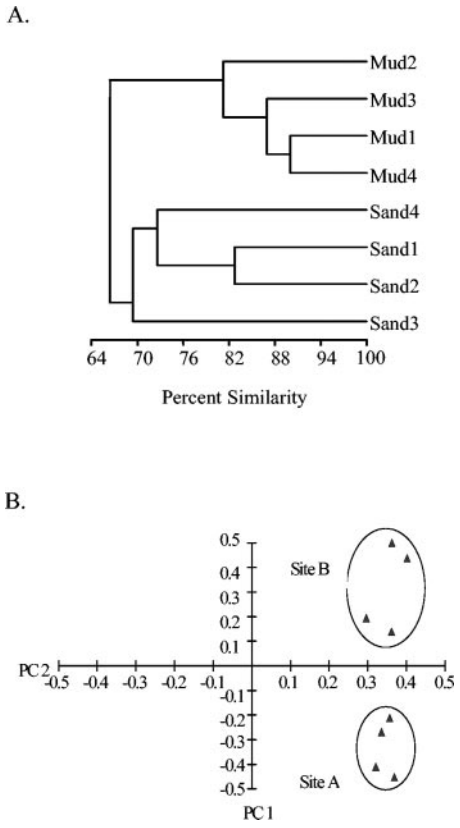

FIG. 4. UPGMA dendrogram (A) and principal-component analysis (B) based on presence-absence data for banding patterns from denaturing gradient gels containing all *D. cuprea* tube samples from Skimmer Shoals sites A and B.

unusually high proportions of cells having intact, polarized membranes and thus the potential for metabolic activity. In many cases, these cells were arranged in microcolonies, some larger than 100,000 μm^3 (68). Microcolonies such as these would be large enough to support the formation of anaerobic microniches (39). Aggregations of SRB with other bacteria have been demonstrated in bioreactor biofilms (59), and it certainly seems reasonable to hypothesize that they may be found in *Diopatra* tube biofilms. Growth of SRB in close association with other bacteria, including oxygen-consuming bacteria, may occur in burrow-lining biofilms and possibly even in intertidal sediments. If it does, this consortial mode of growth could provide the SRB with substantial protection from oxygen.

Consortial growth may not be necessary for SRB to produce reduced microniches in biofilms. Some anaerobic bacteria are capable of reducing their growth medium or milieu if the entry of oxygen is somewhat restricted (43). In addition, Cypionka et al. (16) suggested that chemical reduction of O_2 by H_2S may be a significant process at the oxic-anoxic interface in sediments. By this method, some SRB may be able to reduce their immediate environs. On the other hand, oxygen may be quite toxic to sulfate reducers and may limit their growth and activity in intermittently oxic environments. Although it has been shown that SRB are able to survive a wide range of oxygen tensions and sulfate reduction has been measured in aerobic habitats,

TABLE 5. Results of hybridization of denaturing gradient gels of *D. cuprea* tube samples from sites A and B

Sample	Probe	SRB-specific band[a]					
		B5	A6	Unknown 1	B4	A4, A12	Unknown 2
Site A							
Tube 1	804		1		1		1
	DSS658				1		1
Tube 2	804		1	1	1	1	1
	DSS658			1	1	1	1
Tube 3	804		1		1		1
	DSS658				1		1
Tube 4	804		1		1		1
	DSS658				1		1
Site B							
Tube 1	804				1	1	1
	DSS658				1	1	1
Tube 2	804	1	1	1	1	1	1
	DSS658			1	1	1	1
Tube 3	804	1	1	1	1	1	1
	DSS658			1	1	1	1
Tube 4	804		1	1	1	1	1
	DSS658			1	1	1	1

[a] Presence of a band is indicated by 1.

neither sulfate reduction nor growth of SRB pure cultures has been demonstrated in the presence of oxygen to date (20, 46). It is possible that SRB are inactive and do not grow during periods of burrow ventilation and remain quiescent until conditions more suitable for sulfate reduction are present again.

The PLFA biomarker for *Desulfobacter* was found in substantially larger quantities than the PLFA biomarkers for *Desulfovibrio* or *Desulfobulbus*. *Desulfosarcina*-like 16S rRNA gene sequences were cloned and were detected by DNA-DNA hybridization analysis of *Diopatra* tube DNA from both sites. All 16S rRNA gene sequences belonging to SRB fell in the *Desulfobacteriaceae*, which is in agreement with the high level of the *Desulfobacter* biomarker. These results are consistent with those of Hines et al. (35), who found higher levels of 16S rRNA related to the *Desulfobacteriaceae* than of 16S rRNA related to *Desulfovibrio* in the rhizosphere of *Spartina alterniflora*. Lower levels of 16S rRNA of *Desulfobulbus* than of *Desulfobacteriaceae* 16S rRNA were found in most of the samples. The *Desulfobulbus* 16S rRNA concentrations were periodically higher than the concentrations of *Desulfobacteriaceae*

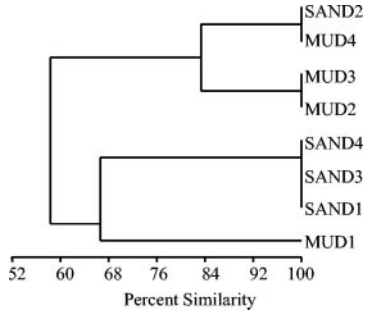

FIG. 5. UPGMA dendrogram constructed by using presence-absence data for banding patterns resulting from hybridization of probe 804 to denaturing gradient gels containing all *D. cuprea* tube samples from Skimmer Shoals sites A and B.

16S rRNA in the *S. alterniflora* rhizosphere and in the upper 2 cm of the salt marsh sediments (22, 35). Hines et al. (35) suggested that *Desulfovibrio* is not as well adapted to life in habitats likely to have steep chemical gradients, such as the burrows and tubes of marine infauna and the rhizosphere of *S. alterniflora*, and that these organisms may prefer habitats that are more anaerobic (or perhaps more consistently anaerobic) than those occupied by the *Desulfobacteriaceae*. However, members of the genus *Desulfovibrio* have been found on the intermittently oxic rhizoplane and within the root cortex of the sea grass *Halodule wrightii* (52). The metabolically diverse members of the *Desulfobacteriaceae* may also have a competitive advantage over members of the genus *Desulfovibrio* because of their ability to utilize a diverse array of electron donors (35). Raskin et al. (71) showed that members of the *Desulfobacteriaceae* were more competitive than other SRB under certain conditions and proposed that utilization of alternate metabolic pathways was responsible for this advantage. It is also possible that our failure to recover *Desulfovibrio* or *Desulfobulbus* 16S rRNA gene sequences may have been due to PCR biases, but the finding of low levels of these genera in the *S. alterniflora* rhizosphere by Hines et al. (35) did not result from a PCR analysis, nor did our finding of low levels of PLFA biomarkers of these organisms in *Diopatra* tubes. The exact conditions that are more advantageous for the growth of *Desulfovibrio* or *Desulfobulbus* than for the growth the *Desulfobacteriaceae* are not clear, but these conditions apparently did not occur in *Diopatra* tubes, while conditions which supported growth of the *Desulfobacteriaceae* did occur.

Study site A had coarser sediments, a lower organic matter content, and lower bacterial cell counts than site B. Oxygenated surface water more readily penetrates coarse sediments by advection and diffusion. In addition, the lower organic matter content of coarser sediment may reflect a poorer environment for bacterial activity than finer sediments, resulting in reduced growth. Surprisingly, there did not appear to be major differences in at least the most abundant members of the bacterial communities, as indicated by 16S rRNA gene sequence DGGE, between sites A and B or at different depths in the sediment. The bacterial assemblages within the *Diopatra* tubes were different at sites A and B. A greater diversity of clones was obtained from site B tubes, and many of these clones did not group with any known bacterial phylum. Only two clones from site A did not group with known phyla. Interestingly, two clones grouped with the proposed phylum OD1, which includes unidentified bacteria from very diverse habitats, such as deep-sea sediments (55) and forest soils (27). One clone grouped with OP11, a proposed phylum that includes bacteria isolated from Antarctic continental shelf sediment (13).

The SRB assemblages in site A and site B *Diopatra* tubes were also different. We propose that the SRB distributions in *Diopatra* tubes from different sediment habitats are strongly influenced by sediment characteristics, chiefly porosity and rates of solute (especially oxygen) transport and consumption. The irrigation rates of *Diopatra* tubes would not be expected to be different at the two the study sites. Also, the study sites were close to each other, and hence, the incurrent water should not have been very different at the two sites. However, the coarser sediment at site A is more conducive to oxygen penetration. The lower organic matter content of the coarser sediment may result in not only reduced growth but also lower rates of oxygen consumption by aerobic bacterial respiration. This may result in greater exposure of *Diopatra* tubes to oxygen. Further studies are required to determine the exact causes of the observed population differences and to examine the microscale distributions of specific SRB within *Diopatra* tubes.

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