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Tropical Archaea: diversity associated with the surface microlayer of corals

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ABSTRACT: Recent 16S rDNA studies have focused on detecting uncultivated bacteria associated with Caribbean reef corals in an effort to address the ecological roles of coral-associated microbes. Reports of Archaea associated with fishes and marine invertebrates raised the question of whether Archaea might also be part of the coral-associated microbial community. DNA analysis of mucus from 3 reef-building species of Caribbean corals, *Montastraea annularis* complex, *Diploria strigosa* and *D. labyrinthiformis* in the US Virgin Islands yielded 34 groups of archaeal 16S ribotypes (defined at the level of 97% similarity). The majority (75%) was most closely matched by BLAST searches to sequences derived from marine water column samples, whereas the remaining ribotypes were most similar to sequences isolated from anoxic environments (15%) and hydrothermal vents (9%). Unlike previous 16S studies of coral-associated Bacteria, the results do not suggest specific associations between particular archaeal sequences and individual coral species. Marine Archaea (Groups I, II and III) in addition to *Thermoplasma*-like, methanogen, and marine benthic crenarchaeote phylotypes, were detected in the mucus of tropical corals. The finding of sequences from coral-associated Archaea that are closely related to strict and facultative anaerobes, as well as to uncultivated Archaea from other types of anoxic environments, suggests that anaerobic micro-niches may exist in coral mucus layers. Archaea, with their unique biogeochemical capabilities, broaden the scope of possible interactions between corals and their associated microbial communities.

KEY WORDS: Coral · Archaea · Microbes · Mucus · 16S rDNA · Diversity · Virgin Islands · Caribbean

INTRODUCTION

Coral reefs around the world are in decline, and infectious diseases are one of the most visible causes (Richardson & Aronson 2002). As a result, more attention has been focused on the microbial ecology of corals; specifically, the roles coral-associated microbes may be playing in preventing or establishing diseases, and what connections exist between the microbial community and the overall health of the coral.

Many coral-associated microbes live in the heavily colonized surface mucus layer (also known as the coral surface microlayer, CSM; surface mucopolysaccharide layer, SML, mucopolysaccharide layer, MPSL) (DiSalvo 1971, Sieburth 1975, Paul et al. 1986, Coffroth 1990). The amount and composition of mucus produced can vary significantly between coral species (Ducklow & Mitchell 1979a, Meikle et al. 1988), and serves a variety of purposes: sloughing cleanses the coral of sediments (Hubbard & Pocock 1972); its viscous nature can contribute to prey capture and feeding (Lewis & Price 1976), and it provides a substrate for microbial growth (Ducklow & Mitchell 1979b, Rublee et al. 1980, Herndl & Velimirov 1986). In some ways this microlayer resembles a biofilm or microbial mat, with the microbial population both numerically and metabolically exceeding that of the surrounding water (Ducklow & Mitchell 1979b, Herndl & Velimirov 1986, Paul et al. 1986, Ritchie et al. 1996).

Both classic microbiological and molecular methods have been used to investigate the bacterial communities associated with healthy corals (DiSalvo & Gundersen 1971, Segel & Ducklow 1982, Ritchie & Smith 1995, Santavy 1995, Lyons et al. 1998, Rohwer et al. 2001,

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2002, Frias-Lopez et al. 2002). The results have yielded tantalizing hints of symbiotic relationships between Bacteria and corals. There is clear evidence that different coral species have significantly different bacterial communities (Ritchie & Smith 1997, Frias-Lopez et al. 2002, Rohwer et al. 2002), and it seems that there can be specific bacterial species associated with a given coral species (Santavy 1995, Rohwer et al. 2001).

Archaea, prokaryotes originally cultured only from extreme environments, were previously regarded as 'ecologically insignificant' due to the perception of their being limited to a few unusual habitats. Archaea have since been detected by molecular techniques in many cold and temperate aerobic marine environments around the world (DeLong 1992, DeLong et al. 1994, Massana et al. 1997, 2000, López-García et al. 2001) and have been shown to constitute a significant portion of the marine picoplankton (Fuhrman & Ouverney 1998, Karner et al. 2001, Church et al. 2003). Marine Archaea have also been detected in the guts of a deep-sea deposit feeder (sea cucumber) and fish species from the North Sea, although it is not clear if they are symbiotic members of the gut flora or are present in the ingested sediment/seawater (McInerney et al. 1995, van der Maarel et al. 1998, 1999). Archaea have been found associated with marine sponges in waters of the coastal Pacific (Preston et al. 1996), Mediterranean (Margot et al. 2002), and Great Barrier Reef (Webster et al. 2001). Reports of Archaea associated with fishes and marine invertebrates, combined with the rising interest in coral microbial ecology, raised the question of whether Archaea might be detectable in scleractinian corals.

The intent of this and a related study (Wegley et al. 2004, this volume) was to screen samples from healthy Caribbean corals for the presence and diversity of marine Archaea. In this study, archaeal 16S rDNA libraries were assembled from coral mucus samples collected from 3 species of reef-building corals, *Montastraea annularis* complex, *Diploria strigosa* and *D. labyrinthiformis*, in the US Virgin Islands. This article reports on the association of Archaea from Marine Groups I, II and III, as well as on some novel phylotypes outside these groups, with corals in a shallow tropical marine environment.

MATERIALS AND METHODS

Sampling. Sterile 60 ml syringes were used to collect the CSM from healthy corals (no visible disease) located in 2 to 5 m of water. Preliminary samples to test the methodology were collected from a nearshore hard-bottom environment near Long Key in the Florida Keys. Corals sampled from Long Key comprised 2 colonies of *Solenastrea* sp., 2 colonies of *Diploria clivosa* and 1 colony of *Porites astreoides*. All of the samples subjected to DNA analysis beyond PCR amplification were collected from a fringing reef in Hawksnest Bay, St. John, US Virgin Islands (Fig. 1), within the boundaries of the Virgin Islands National Park (VINP). The Virgin Islands (VI) samples were collected from 2 colonies of *Montastraea annularis* complex, 2 colonies of *D. strigosa*, and 1 colony of *D. labyrinthiformis*. Colonies sampled were at least 5 m apart.

The syringes containing the CSM were sealed under water, brought to the surface, and immediately placed on ice.Within 1 h, the samples were centrifuged at 1500 rpm (465 × *g*) for 10 min to concentrate the CSM. The supernatant was poured off, and microbial DNA was extracted from the concentrated CSM (ca. 0.5 ml) using an Ultraclean Soil DNA Kit (MoBio Laboratories), following the manufacturer's protocol. DNA extracts were frozen (–20°C) for transport back to the US Geological Survey's microbiology laboratory in St. Petersburg, Florida.

Two 1 l samples of overlying water (temperature 30°C, salinity 35 ppt) were collected in sterile Whirlpak bags as controls. These samples were filtered within 4 h of collection, using sterile filtration funnels containing 47 mm diameter 0.2 µm pore-size Supor filters (#PN4803; Pall Corporation). These filters were frozen (–20°C) in 1 ml of STE buffer (Sambrook et al. 1989) for transport back to the laboratory.

Library construction and sequencing. The archaeal 16S rDNA was amplified from the DNA extracts using Archaea-specific primers 21f (5′-TTCCGGTTGATCCYGCCGGA) and 958r (5′-YCCGGCGTTGAMTCCAATT) (DeLong 1992). The PCR master-mix recipe per reaction was: 10 µl of GeneAmp 10X PCR buffer

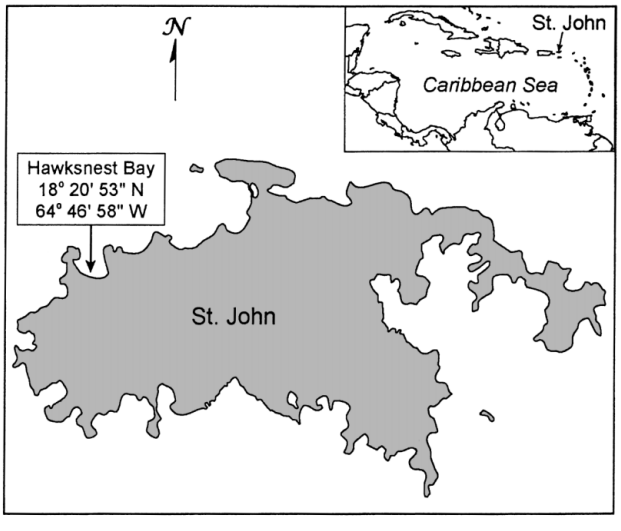

Fig. 1. Map of sampling site on the island of St. John, US Virgin Islands

(Applied Biosystems), 12 µl of 25 mM $MgCl_2$ (Applied Biosystems), 2 µl of 10 mM dNTP mix (Promega), 0.5 µl of 5 U $µl^{-1}$ Taq polymerase (Promega), 1 µl each of 10 nM upstream and downstream primers (synthesized by Qiagen Operon), and 26.5 µl sterile deionized water. Template DNA from the extractions (45 µl each) brought the reactions to their final volume of 100 µl each. The PCR amplification profile used for these reactions was 1 cycle for 2 min at 94°C; 30 cycles of 90 s at 95°C; 90 s at 55°C; 90 s at 72°C; 1 cycle of 10 min at 72°C; and hold at 4°C. After PCR, the amplification products from the VI samples were cloned into Plasmid Vector pCR2.1 using the TOPO-TA cloning kit as recommended by the manufacturer (Invitrogen). Transformants were selected on S-Gal™/LB agar/Kanamycin blend (Sigma). White colonies were picked (50 each for the 2 *Montastraea annularis* samples [VIM1, VIM2] and the 2 *Diploria strigosa* samples [VIDSA, VIDS1]; 47 for the *D. labyrinthiformis* sample [VIDL]; total 247 clones) and inoculated into 1 × yeast tryptone (YT) broth (Sambrook et al. 1989) containing 100 µg ml^{-1} ampicillin and 50 µg ml^{-1} kanamycin, and grown overnight at 37°C in a shaking incubator. Plasmid DNA was extracted from each of these clones using the Wizard® Plus SV Minipreps DNA Purification System (Promega) following the manufacturer's protocol. Plasmids were restriction-digested with *Eco* RI and submitted to gel electrophoresis to confirm the presence of inserts. Of the 247 clones, 206 contained the correct amplicon (42 from VIM1, 50 from VIM2, 43 from VIDSA, 33 from VIDS1, and 46 from VIDL). The confirmed samples were sequenced using the 21f primer (Northwoods DNA). The sequences (size range 700 to 800 bp) were analyzed by BLASTN (Altschul et al. 1990, 1997) to identify the nearest previously described archaeal sequence in GenBank.

Seawater control sample filters were thawed in sterile petri dishes, cut into strips with sterile scissors, and placed into individual tubes from the Ultraclean Soil DNA Kit (MoBio Laboratories). The same protocol used to process the mucus samples was followed, except that instead of using a 10 min vortex step to disrupt the samples, 2 cycles in the FastPrep FP120 (Qbiogene) at a speed setting of 5.5 for 30 s were used to remove and disrupt cells. Prior tests using filters had shown that vortexing alone was not sufficient (data not shown).

Sequence analysis. Of the 206 inserts sequenced, 156 were unique, although many differed by only a few base pairs. Considering any 2 16S rDNA sequences that were ≥97% identical with no gaps >2 bp to be the same ribotype (97% criterion: Stackebrandt & Goebel 1994, Hagström et al. 2000, Rohwer et al. 2002) resulted in 58 unique ribotypes. The 58 sequences were aligned using Clustal X (Thompson et al. 1994, 1997), and positions not sequenced in all isolates or with alignment uncertainties were removed. The phylogenetic analyses (neighbor-joining, parsimony and maximum likelihood) were performed using Phylogenetic Analysis Using Parsimony (PAUP) (Swofford 2000).

Nucleotide sequence accession numbers. The sequences from this study are available through GenBank under Accession Nos. AY380582 to AY380741.

RESULTS AND DISCUSSION

Marine archaeal 16S rDNA sequences have been found in the water column (DeLong 1992, DeLong et al. 1994, Fuhrman & Davis 1997, Massana et al. 1997, 2000, Fuhrman & Ouverney 1998, Murray et al. 1998, López-García et al. 2001, Moeseneder et al. 2001), coastal and deep-water sediments (Abreu et al. 2001, Inagaki et al. 2001), marine invertebrates (McInerney et al. 1995, Preston et al. 1996, Webster et al. 2001, Margot et al. 2002), and fishes (van der Maarel et al. 1998, 1999). To explore the possibility that marine Archaea are also associated with tropical corals, samples of the CSM were collected from 6 species of Caribbean corals and assayed for the presence of Archaea by PCR using Archaea-specific primers. The PCR reactions yielded positive results for archaeal 16S DNA from 2 colonies each of *Solenastrea* sp., *Montastraea annularis* complex, and *Diploria strigosa*. Positive amplification was detected from 1 of 2 colonies in the cases of *D. clivosa* and *D. labyrinthiformis*. No amplification product was produced from the *Porites astreoides* sample. A concurrent study (Wegley et al. 2004) investigated similar coral species from reefs in Panama and Bermuda, and was also able to amplify archaeal sequences by PCR from some but not all colonies sampled. However, it was able to amplify archaeal DNA successfully from a larger sample set of *Porites astreoides*.

A choice was made to focus on reef-building species of coral. Therefore, clone libraries were constructed from the *Montastraea annularis* complex, *Diploria strigosa*, and *D. labyrinthiformis* samples collected in the Virgin Islands. These samples yielded 206 clones that were sequenced and analyzed (Table 1). Based on similarity to a nearest-neighbor sequence in GenBank, the 58 unique ribotypes clustered into 34 groups, of which 44% were crenarchaeotes and 56% euryarchaeotes. The single most abundant group of ribotypes (OARB-like; see DeLong et al. 1994) made up approximately half (48%) of the sequenced clones, was related to euryarchaeotes, and was present in all 3 coral species. This is in marked contrast to Wegley et al.'s (2004) findings for similar corals (*D. strigosa*, *Montastraea* spp. and *Porites astreoides*) sampled in Panama and Bermuda. Of 353 sequences containing 93

Table 1. 16S rDNA archaeal sequencing results for coral samples from Hawksnest Bay, St. John, US Virgin Islands. Nearest-neighbor: designation of nearest-neighbor sequence in GenBank; ID%: percent identity with previously identified sequences; No. clones: numbers of similar clones; No. base pairs: numbers of base pairs sequenced; % clones: percentage of archaeal ribotypes associated with samples from *Montastraea annularis* complex (M1, M2), *Diploria strigosa* (DSA, DS1), and *D. labyrinthiformis* (DL1), based on number of clones sequenced for each coral sample (n = number of clones sequenced)

Nearest-neighbor sequence	GenBank Accession No.	ID%	No. clones	No. base pairs	Source	% clones				
						M1	M2	DSA	DS1	DL1
Crenarchaeota										
19a-4	AJ294875	97–98	3	801–856	M. B. Brehmer (unpubl.)	0	2	0	0	4.5
19a-5	AJ294876	97–99	5	801–859	M. B. Brehmer (unpubl.)	5	4	0	0	2
19a-18	AJ294878	95	1	854	M. B. Brehmer (unpubl.)	0	0	0	1	0
19a-27	AJ294881	96–97	2	814–843	M. B. Brehmer (unpubl.)	2.5	0	2.5	0	0
19b-52	AJ294873	97–98	4	771–832	M. B. Brehmer (unpubl.)	0	0	10.5	0	0
19c-51	AJ294896	95	1	844	M. B. Brehmer (unpubl.)	2.5	0	0	0	0
AEGEAN-70	AF290533	98	1	833	Moeseneder et al. (2001)	0	0	2.5	0	0
AEGEAN-67	AF290529	96–98	11	646–860	Moeseneder et al. (2001)	5	0	5	3	9
BBA6	AF004345	87–94	5	776–849	Vetriani et al. (1998)	7.5	0	0	0	4.5
Cenarchaem	AF083072	95	2	819–845	Schleper et al. (1998)	2.5	0	2.5	0	0
DOUR03	AF201357	96	1	821	Abreu et al. (2001)	0	0	2.5	0	0
pPCA4.21	AB049032	95	1	824	Inagaki et al. (2001)	0	0	2.5	0	0
SB95-20	AF223121	95	1	732	Massana et al. (1997)	2.5	0	0	0	0
TS235C306	AF052948	98	1	830	van der Maarel et al. (1998)	2.5	0	0	0	0
VC2.1Arc31	AF068822	93	1	863	Reysenbach et al. (2000)	0	2	0	0	0
Euryarchaeota										
19b-30	AJ294863	95	1	829	M. B. Brehmer (unpubl.)	0	0	0	0	2
19c-10	AJ294885	95	1	788	M. B. Brehmer (unpubl.)	0	0	0	0	2
33-P27A98	AF355901	92	2	827	Huber et al. (2002)	0	0	0	0	4
AEGEAN-60	AF290531	89–90	2	793–818	Moeseneder et al. (2001)	2.5	2	0	0	0
AEGEAN-71	AF290535	98	4	757–867	Moeseneder et al. (2001)	7.5	0	2.5	0	0
AFRICA3/13-2	AY225433	99	1	661	C. Winter et al. (unpubl.)	0	0	0	1	0
DCM65231	AF121990	99	1	844	M. J. E. C. van der Maarel et al. (unpubl.)	0	0	0	0	2
DH148-W1	AF257277	92–98	12	707–856	López-García et al. (2001)	7.5	4	10.5	1	4.5
Eel-TA1e6	AF134389	93	1	851	Hinrichs et al. (1999)	0	2	0	0	0
HTA-H9	AF418940	89–90	2	852–853	Stein et al. (2002)	0	4	0	0	0
KUA16	AB077226	87	1	839	Watanabe et al. (2002)	2.5	0	0	0	0
OARB	U11042	89–98	99	676–855	DeLong et al. (1994)	45	68	38	15	37
pCIRA110	AB108847	90–92	10	827–850	K. Takai et al. (unpubl.)	0	0	8	3	9
pCIRA112	AB108849	96–97	5	845–848	K. Takai et al. (unpubl.)	0	0	2.5	4	0
PVA-OTU-1	U46677	99	1	837	C. L. Moyer et al. (unpubl.)	0	2	0	0	0
SB95_35	AF223144	93	1	626	Massana et al. (1997)	2.5	0	0	0	0
SB95_72	U78206	98	6	821–853	Massana et al. (1997)	2.5	4	0	1	4.5
SBAR1A	M88074	97	1	853	DeLong (1992)	0	2	0	0	0
SBAR16	M88077	94–97	15	737–844	DeLong (1992)	0	4	10.5	2	15
n =						**40**	**50**	**39**	**31**	**46**

archaeal ribotypes, Wegley et al. (2004) found the majority was related to crenarchaeotes, including the 2 most abundant ribotypes. This may be a biogeographic distinction, or may be due to differences in sample processing (extracting DNA from the coral mucus only versus from a combination of mucus, tissue and skeleton).

Two 1 l seawater samples were taken at the same time as the CSM samples as background controls. No archaeal clones were obtained from either of these samples. Because it is known that Bacteria are concentrated in the coral mucus compared to the surrounding water (Ducklow & Mitchell 1979b, Herndl & Velimirov 1986, Paul et al. 1986), it is likely that Archaea are also. Archaea may be present in such low numbers in the tropical water-column that their ribosomal signature cannot be detected in a 1 l volume. Previous studies comparing bacterial 16S rDNA sequences between seawater and corals by DGGE have found the profiles to be significantly different, and have concluded that the communities were quite separate (Rohwer et al. 2001, Cooney et al. 2002, Frias-Lopez et al. 2002). This suggests that even if archaeal 16S genes had been detected in the overlying water column, they would have been distinct from those associated with the corals.

BLAST searches of the coral-associated archaeal sequences against GenBank revealed that the majority (75%) most closely matched sequences derived from marine water column samples (Table 1) (DeLong 1992, DeLong et al. 1994, Massana et al. 1997, van der Maarel et al. 1998, López-García et al. 2001, Moeseneder et al. 2001). The remaining ribotypes were most similar to sequences isolated from anoxic sediments or water (15%) (Vetriani et al. 1998, Hinrichs et al. 1999, Reysenbach et al. 2000, Abreu et al. 2001, Inagaki et al. 2001, Stein et al. 2002, Watanabe et al. 2002) and hydrothermal vent environments (9%) (Reysenbach et al. 2000, Huber et al. 2002).

Unlike previous molecular studies of coral-associated Bacteria (Rohwer et al. 2001, 2002), the results of this study do not suggest specific associations between particular archaeal ribotypes and individual coral species. The most prevalent group of ribotypes (OARB-like; see Table 1 in DeLong et al. 1994) was present on all coral species tested. There were a few instances where a ribotype was only present on 1 species or 1 genus of coral (Table 1), but they were always rare. A greater number of samples would be required to confirm whether these few ribotypes reflect a true species-specific association. Sponge-associated Archaea seem to be very highly species-specific with regard to the genus *Axinella*, with only one or two 16S rDNA sequences per sponge species (Preston et al. 1996, Margot et al. 2002). These *Axinella*-associated Archaea are closely related to the Marine Group I crenarchaeotes. A study of Archaea in the sponge *Rhopaloeides odorabile* on the Great Barrier Reef, however, revealed the presence of both euryarchaeotes and crenarchaeotes (Webster et al. 2001). The latter situation more closely resembles the coral-associated archaeal community detected in the Virgin Island corals.

This study is 1 of 2 independent efforts (Wegley et al. 2004) to detect marine Archaea Groups I and II associated with Caribbean corals (Fig. 2). Both Marine Group I and II archaeal sequences were detected in all 3 species of reef-building coral analyzed in this study. There was a greater diversity of euryarchaeotes than crenarchaeotes present in the CSM. The Group III Euryarchaeota have been detected only rarely in the water column (Fuhrman & Davis 1997, Massana et al. 2000, Moeseneder et al. 2001). This work, however, is the first to report Group III Euryarchaeota in warm shallow waters and, more specifically, associated with corals. In this study, 6 Group III euryarchaeal sequences were recovered from the CSM 3; are shown in Fig. 2.

Several 16S rDNA euryarchaeal sequences from the coral mucus fall somewhere between Group III euryarchaeotes and *Thermoplasma acidophilum* (VIM2-23, VIM1-28, VIDL-38; Fig. 2). VIM1-28 is most closely related to a sequence recovered from anoxic oil-contaminated ground water (Watanabe et al. 2002) and VIM2-23 is similar to a sequence from the anoxic bottom layer of a metal-rich freshwater reservoir (Stein et al. 2002). VIDL-38 is most similar to sequences extracted from marine sediments in the Aegean Sea (M. B. Brehmer; direct submission to GenBank).

Clone VIDL-2 occurs on a very deep branch of the phylogenetic tree, nearest to the methanogen *Methanococcus voltae*. This sequence's nearest neighbors in the public domain are phylogenetically distant, but include a sequence from a hydrothermal vent on the Juan de Fuca Ridge (Huber et al. 2002). The vent phylotype was considered to be unique to the vent environment and is possibly an example of an unknown euryarchaeote Group VI (Huber et al. 2002). Methanogens metabolize the fermentation products of other anaerobes and are also capable of sulfur reduction (Stanier et al. 1986).

Several ribotypes (VIDL-39, VIM1-36, VIM1-43) form a clade with *Desulfurococcus mobilis*. The coral-derived sequences are most closely matched to sequences from anoxic marine sediments, already shown to form a separate clade from the planktonic Group I marine crenarchaeotes (Vetriani et al. 1998). It has been suggested that these benthic phylotypes may share overlapping ecological niches with sulfate-reducing bacteria in anaerobic environments (Vetriani et al. 1998).

One coral-associated crenarchaeal clone, VIM2-31, is deeply rooted and does not group with either of the known marine or benthic low-temperature clades. This sequence is distantly related (93% identity) to a sequence from a mid-Atlantic Ridge hydrothermal vent, which does group with the marine benthic crenarchaeotes (Reysenbach et al. 2000). VIM2-31 is proposed as a novel phylotype of a new group of marine low-temperature crenarchaeotes.

Previous findings of coral-associated Bacteria that are typically found in anaerobic environments (e.g. *Bacillus*/*Clostridium* and nitrogen-fixers) have led to speculations that they function when corals become anoxic at night (Carlton & Richardson 1995, Kuhl et al. 1995, Rohwer et al. 2002), or that the prokaryotes are maintained in anaerobic niches in coral colonies (Williams et al. 1987, Shashar et al. 1994, Carlton & Richardson 1995, Rohwer et al. 2002). Finding sequences from coral-associated archaeal organisms related to strict anaerobes (methanogens, *Desulfurococcus*), facultative anaerobes (*Thermoplasma*), as well as uncultivated Archaea from other types of anoxic environments, suggest that anaerobic zones may also exist in the coral mucus layer.

The microbial ecology of corals is just beginning to be characterized. It has been speculated that coral-

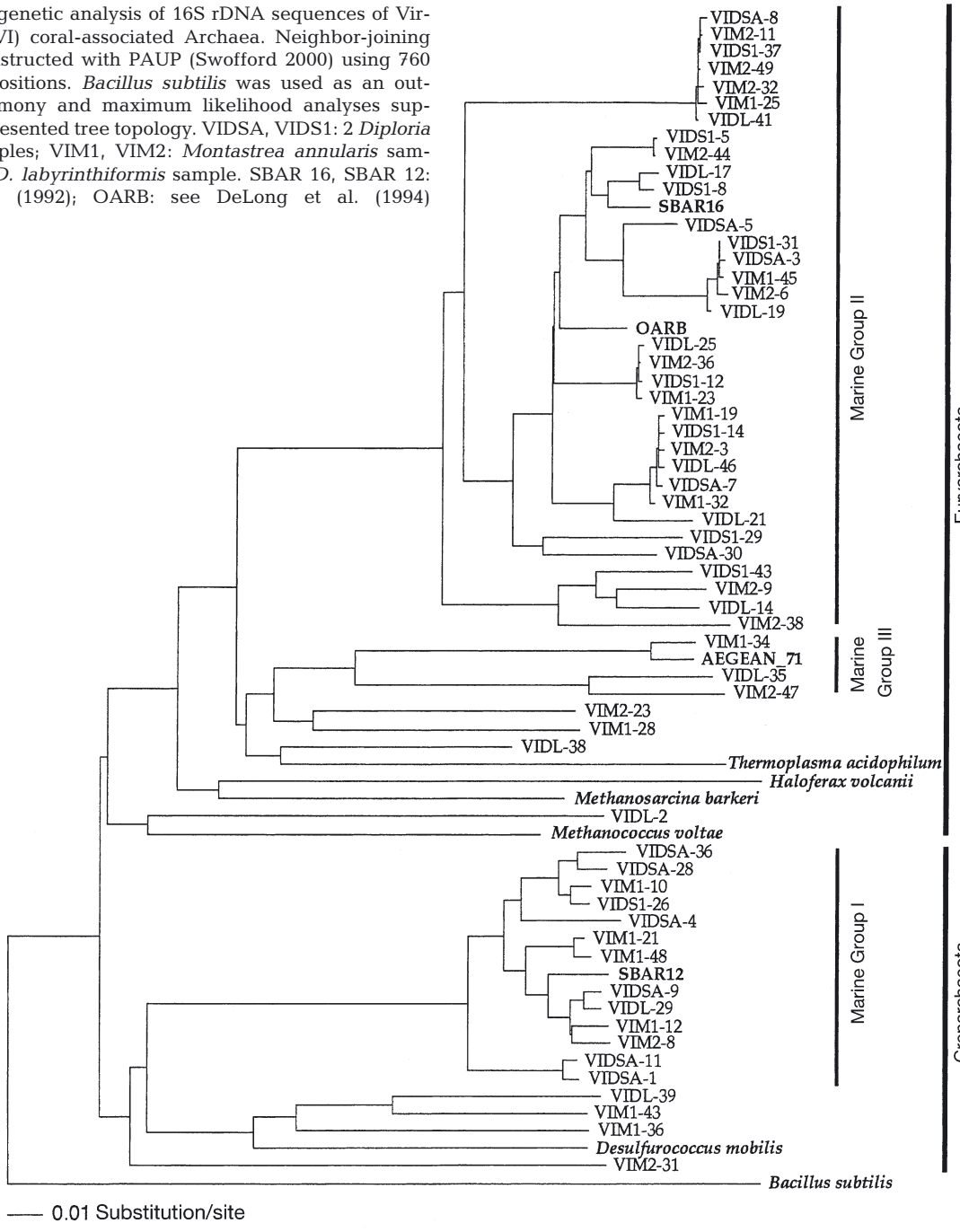

Fig. 2. Phylogenetic analysis of 16S rDNA sequences of Virgin Island (VI) coral-associated Archaea. Neighbor-joining tree was constructed with PAUP (Swofford 2000) using 760 nucleotide positions. *Bacillus subtilis* was used as an outgroup. Parsimony and maximum likelihood analyses supported the presented tree topology. VIDSA, VIDS1: 2 *Diploria strigosa* samples; VIM1, VIM2: *Montastrea annularis* samples; VIDL: *D. labyrinthiformis* sample. SBAR 16, SBAR 12: see DeLong (1992); OARB: see DeLong et al. (1994)

associated Bacteria benefit the host by functions such as nitrogen fixation (Williams et al. 1987, Shashar et al. 1994, Rohwer et al. 2002) and antibiotic production or niche-filling to ward off pathogens (Rohwer et al. 2002, Harder et al. 2003). Microbes may be breaking down coral/zooxanthellae waste products, cycling basic nutrients back to the algal symbiont, in a modified version of the marine microbial loop (Azam et al. 1983). Additionally, bacteria 'farms' may be maintained by the coral as a backup source of nutrients (Rohwer et al. 2002). In return, bacteria benefit from the ready source of nutrients (Rublee et al. 1980, Herndl & Velimirov 1986), as well as from some level of protection from ultraviolet radiation afforded by the coral mucus (Lyons et al. 1998). Studies have found that the bacterial communities associated with stressed or diseased coral tissues differ in both number and composition from those in healthy tissue of the same species (Ducklow & Mitchell 1979b, Pantos et al. 2003). This suggests that the microbes are at least attuned to host metabo-

lism and, at most, may have an active role in maintaining the overall health of the organism. The introduction of Archaea, with their varied (and for the uncultivated specimens, mostly unknown) metabolic capabilities, adds to the likelihood of unique biogeochemical processes occurring. These processes and their hosts must be identified in order to integrate microbial functions with the biology of the coral animal and algal symbiont.

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