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FEATURE ARTICLE

Regulation of microbial populations by coral surface mucus and mucus-associated bacteria

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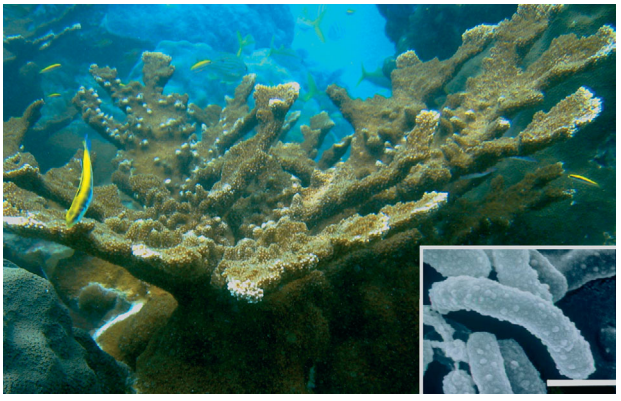
ABSTRACT: Caribbean populations of the elkhorn coral *Acropora palmata* have declined due to environmental stress, bleaching, and disease. Potential sources of coral mortality include invasive microbes that become trapped in the surface mucus and thrive under conditions of increased coral stress. In this study, mucus from healthy *A. palmata* inhibited growth of potentially invasive microbes by up to 10-fold. Among cultured bacteria from the mucus of *A. palmata*, 20% displayed antibiotic activity against one or more tester strains, including the pathogen implicated in white pox disease. A novel mucus-mediated selection for coral symbionts revealed a discrete subset of bacteria and selected for isolates that produce antibiotics. This result suggests that coral mucus plays a role in the structuring of beneficial coral-associated microbial communities and implies a microbial contribution to the antibacterial activity described for coral mucus. Interestingly, antibiotic activity was lost when mucus was collected during a summer bleaching event. Isolates from apparently healthy *A. palmata* tissue during this event lacked antibiotic-producing bacteria and were dominated by members of the genus *Vibrio*, including species implicated in temperature-dependent bleaching of corals worldwide. This indicates an environmental shift from beneficial bacteria, and variability in the protective qualities of coral mucus, which may lead to an overgrowth of opportunistic microbes when temperatures increase. Finally, coral mucus inhibited antibiotic activity and pigment production in a potentially invasive bacterium, illustrating that coral mucus may inactivate mechanisms used for bacterial niche establishment.

KEY WORDS: Surface mucopolysaccharide layer · Coral symbionts · Antibiotics · Coral bleaching · Disease · Pigment production · Chemical defense



This study found that mucus from healthy *Acropora palmata* (*photo*) inhibits the growth of potentially invasive microbes by up to 10-fold. Conversely, mucus collected during a summer bleaching event lacked antibiotic properties, suggesting that high temperatures reduce the protective function of coral mucus. Inset: *Vibrio* sp., which at high temperatures replace the community of beneficial bacteria (scale bar = 500 nm).

Photos: Kiho Kim (coral), Shawn Polson (inset)

INTRODUCTION

The decline of corals world-wide has led to a need for a better understanding of disease susceptibility in cnidarians (Harvell et al. 1999). This study focused on protective properties of mucus produced by the elkhorn coral *Acropora palmata*—one of the first corals proposed for listing as an endangered species in the United States—due to its high susceptibility to environmental stress (e.g. bleaching), disease and physical damage. Coral mucus provides protection from UV, desiccation and increased sediment loading (Brown & Bythell 2005), but little is known about the protective properties of mucus in disease resistance. Coral mucus is proposed to enhance resistance by a number of mechanisms, including providing a physical barrier between

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the coral and the environment, mucociliary transport of microbes for removal via ingestion by the coral host, sloughing to avoid colonization by invasive microbes, and acting as a medium for secreted allelochemicals with antimicrobial properties (reviewed in Brown & Bythell 2005). Extracts from soft corals and gorgonian corals have antimicrobial properties (Burkholder & Burkholder 1958, Kim 1994, Slattery et al. 1995, 1997, Kelman et al. 1998, 2006). Koh (1997) described antimicrobial compounds present in numerous Pacific scleractinian coral species and recent work by Geffen & Rosenberg (2005) shows that the Red Sea scleractinian coral *Pocillopora damicornis* exhibits antimicrobial activity. However, the origin of these allelochemicals is unknown.

Corals harbor a diverse array of bacterial associates (reviewed in Brown & Bythell 2005), some of which are thought to be species-specific (Ritchie & Smith 1997, Rohwer et al. 2002). To date, very little is known of the metabolic capabilities of these bacteria, their function on the coral surface, and their potential benefit to the coral, zooxanthellae, or both. Bacterial symbionts have been shown to be responsible for the production of secondary metabolites previously attributed to the host organism (Yasumoto et al. 1986, Elyakov et al. 1991). Some marine macroorganisms combat microbial fouling by producing compounds that inhibit bacterial growth or attachment, while others rely on microbial production of defense compounds (Gil-Turnes et al. 1989, Holmstrom et al. 1992, Armstrong et al. 2001). Commensal relationships between bacteria in multispecies biofilms can play a role in determining the spatial distribution of microbial populations within the biofilm (Neilson et al. 2000, Rao et al. 2005). Castillo et al. (2001) determined that 30% of bacteria isolated from coral species have antibiotic capabilities. The roles played by coral mucus and mucus-associated bacteria in determining the compositions of coral-associated microbial communities are currently unknown.

This study addressed antibiotic activity associated with *Acropora palmata* mucus and mucus-associated bacteria. Here, antibiotic refers to a substance that selectively kills or inhibits the growth of a microorganism. In addition, an experimental approach was used to investigate the potential of coral mucus as a selection medium for coral symbionts. For the purpose of this study, a symbiont is defined as a bacterium that benefits from properties of the coral mucus while providing a benefit to the coral in return (a mutualistic relationship).

MATERIALS AND METHODS

Bacterial strains. To isolate culturable bacteria, *Acropora palmata* mucus samples were diluted in sterile seawater and plated onto glycerol artificial seawater agar (GASWA; Smith & Hayasaka 1982), followed by incubation at 24°C. Microorganisms exhibiting a unique colony or cellular morphology (as compared to other colonies on a single plate) were subcultured to purification under the same growth conditions. For ease of performing medium through-put antibiotic assays, 96-well microtiter plate libraries of culturable bacterial isolates were generated. Each library consisted of 96 individually purified coral bacterial isolates stored frozen at –80°C in liquid GASWA medium containing 30% glycerol. Culturable bacterial libraries were archived for further antibiotic screening and species identification. Strain VBR 7 was isolated from *A. palmata* mucus in a screen for putative invasive microbes (described below). Other sources of potentially invasive microbes used in this study included canal water from Big Pine canal (Sands, 24° 40.31′ N, 81° 20.40′ W), dust from Mali, Africa (12° 37.22′ N, 7° 59.40′ E; provided by V. Garrison, USGS), and water from Looe Key Reef (24° 33.78′ N, 81° 24.05′ W). Tester strains used for antibiotic assays are described below.

Sampling. Samples were taken from 12 *Acropora palmata* colonies at various locations at Looe Key Reef, in the Florida Keys (24° 32.76′ N, 81° 24.21′ W and 24° 32.75′ N, 81° 24.35′ W), between January and December of 2005 and were used to generate culturable bacterial libraries for antibiotic production assays. Samples for coral mucus selection experiments were taken from 3 *A. palmata* colonies in April 2005 (mean water temperature of 24°C, sustained at 22 to 25°C for 2 mo prior to sampling) and 3 *A. palmata* colonies in September of 2005 (mean water temperature of 30°C, sustained at 28 to 30°C for 2 mo prior to sampling). September samples were taken during a bleaching event in which all *A. palmata* colonies observed were affected by hurricane damage, bleaching or disease. During September sampling, mucus from apparently healthy areas of *A. palmata* colonies was collected. These were areas that appeared healthy, but that were adjacent to entirely bleached areas, or areas with sloughed tissue, in each colony sampled.

To collect samples under field conditions, 30 ml of the surface mucopolysaccharide layer from apparently healthy *Acropora palmata* were harvested from the skyward-facing, sun-exposed portion of the colony, 5 to 10 cm from the actively growing edge. Mucus from each colony was consistently collected over an area of 5 × 5 cm after gentle agitation with a sterile syringe. Agitation encourages sloughing of the viscous mucus matrix for ease of syringe aspiration, while reducing aspiration of water. Samples were maintained at 24°C and processed within 2 h of collection. Water samples were taken by briefly opening a sterile 15 ml conical tube 1 m from the coral colony prior to mucus sampling. Water samples were used

in both mucus challenge experiments and bacterial composition comparisons.

Inhibition of potentially invasive microbes. Sources of potentially invasive microbes were challenged with mucus collected from *A. palmata*. Potential sources of invasive microbes were chosen based on previous implications to disease causation (Patterson et al. 2002, Garrison et al. 2003). These sources included Florida Keys canal water, African dust, and water column microbes. Mucus-treated media were used to test growth inhibition by plating out 400 µl of undiluted coral mucus onto GASWA medium and allowing it to dry for 10 min. Mucus treated plates were sterilized via UV irradiation by placing uncovered Petri plates face downward onto a M-26 UVP transilluminator for 10 min at 302 nm wavelength. UV irradiation was chosen for sterilization, as mucus is too viscous for filter sterilization and heat/pressure sterilization would greatly alter components, including antibiotic properties, of coral mucus. UV irradiated mucus-treated plates that were left un-inoculated were used to control for complete UV killing in each experiment. To address potential UV alteration of media, inoculates from African dust and canal water were used in control comparisons measuring growth on UV irradiated versus non-irradiated GASWA medium.

Seawater was tested on mucus-treated GASWA medium to address growth inhibition of marine bacteria present in the water column. In order to address coral resistance to potential human-derived water quality contaminants, such as *Serratia marscecens* or *Escherichia coli*, mucus-treated Luria Bertani (LB) agar was used when testing Florida Keys canal water-associated microbes. Dust from Mali, Africa (collected by V. Garrison) was tested on GASWA medium to address inhibition of microbes from dust events with the potential to remain viable in sea water. Both sea water and canal water were concentrated by centrifugation, resuspended in 1/5 volume of supernatant, and inoculated onto each media treatment; 50 µg of dust were added to 1 ml of sterile seawater, serially diluted and plated onto GASWA mucus-treated and GASWA control plates.

In addition to potential sources of invasive microbes, both Gram-positive and Gram-negative tester strains were used in mucus inhibition assays to address the range of bacterial inhibition by *Acropora palmata* mucus. Exponentially growing cultures of each tester strain were serially diluted and assayed as described above. Tester strains included *Bacillus subtilis* (ATCC 6633 Km resistant), *Staphylococcus aureus* (MRSA, ATCC 43300), *Salmonella typhimurium* (ATCC 6994), and the *Serratia marsescens* white pox isolate, PDL100 (ATCC BAA-632).

Individual experiments were repeated 4 times. Experimental and control plates were incubated at 24°C for 48 h. Dilutions containing between 200 and 400 colonies on control media (GASWA + UV treatment) were used in comparison with the corresponding tester plates (GASWA + Mucus + UV treatment). Colony count, mean and SD were recorded for each experiment. To derive measures of fold-inhibition, the experimental mean was divided into the control mean for each experiment.

Antibacterial production assays. *Acropora palmata* mucus-associated bacteria were used in a primary screen to test for the production of anti-bacterial compounds against a range of tester strains, including: *Serratia marcescens* PDL100, a pathogen implicated in white pox disease of *A. palmata* (ATCC BAA-632; Patterson et al. 2002), methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC 43300), methicillin-sensitive *S. aureus* (MSSA; ATCC 29213), vancomycin-resistant *Enterococcus* (VRE; Microgenomics), *Bacillus subtilis* (ATCC 6633 Km resistant), *Salmonella typhimurium* (ATCC 6994), *Enterococcus faecalis* (ATCC 29212), *Shigella* (Microgenomics), *Escherichia coli* O157 (Microgenomics), and *Agrobacterium tumefaciens* (Microgenomics). Culturable bacterial libraries, stored in 96-well plate format, were inoculated onto rectangular Petri plates containing GASWA solid medium and grown for 2 d at 24°C. Growth of coral bacteria was followed by UV irradiation for 15 to 20 min to inhibit cross contamination from coral-derived bacteria during antibiotic testing. Overnight cultures of tester strains were grown in LB, Tryptic Soy Broth (TSB) or GASWA liquid medium, as appropriate. Strain-specific soft agar medium (0.8%) was melted and cooled to 42°C; 8 ml of soft agar was inoculated with individual log-phase tester strains and poured over UV irradiated library plates. Plates were incubated for 2 d at 30°C. Growth inhibition resulted in a clear zone of inhibition (non-growth of the overlayed tester strain) around a coral-derived bacterial colony that produced an active antibacterial compound. Zones of inhibition were measured using calipers (0.02 mm) and scored as distance (in mm) from the outside of the clearing zone to the outer edge of the coral-derived bacterial colony tested. Antibiotic spectra of library isolates were used to further dereplicate marine isolates.

Selection for coral bacterial symbionts. To select for antibiotic-producing coral bacteria, a culture-based approach was designed to take advantage of the antibiotic properties associated with *Acropora palmata* mucus. This method was hypothesized to eliminate bacteria that are inhibited by mucus-associated antibiotic activity (trapped microbes that may become invasive), while selecting for individuals resistant to antibiotic properties of mucus (potentially beneficial coral bacteria, including antibiotic producers).

This assay was carried out as described above for mucus inhibitory assays. However, instead of challenging mucus-treated plates with sources of potentially invasive microbes, coral mucus dilutions from the same coral colony used to prepare plates were inoculated onto GASWA mucus-treated or GASWA control plates. Colonies were counted and colony forming units (CFUs) per ml estimated for each dilution to represent fold-inhibition. Individual colonies were picked from mucus treated plates to represent potential coral bacterial symbionts (designated as 'residents'). Isolates from the control plates were selected as putative transient bacteria, or bacteria that are potentially invasive under the right conditions ('visitors'). Water column isolates were purified after growth on GASWA medium for comparison to residents and visitors pools.

Mucus inhibition of antibiotic properties in a potentially invasive bacterium. Mucus used in challenge experiments with a potentially invasive microbe (VBR7) was taken from *Acropora palmata* (April and September 2005, Florida Keys), *Pseudopterogorgia americana* (April 2005, Florida Keys) and *Montastraea faveolata* (April 2005, Florida Keys and Flower Garden Banks). From each coral source, 400 µl of undiluted coral mucus were plated onto GASWA solid media, allowed to dry for 10 min, and UV irradiated for 10 min to prevent outgrowth of mucus-associated microbes. Two resulting colony morphologies of VBR7 were dark purple (after growth on GASWA control medium) and white (after growth on each mucus-treated medium). The 2 morphologies of the VBR 7 strain were patched onto replica GASWA plates and assayed for antibiotic activity as described above.

Bacterial identification. For species identification of culturable coral isolates, DNA extraction was performed on each strain via a chemical lysis protocol detailed in Weidner et al. (1996). PCR amplification was carried out on genomic DNA with oligonucleotide forward primer R1n, corresponding to position 22 to 41 of the *Escherichia coli* 16S rRNA gene, and reverse primer U2 corresponding to complementary position 1085 to 1066 (Weidner et al. 1996). PCR products were electrophoresed on a 1% agarose gel, and verified using the AlphaImager 3300. A ~1100 bp fragment was purified from the PCR reactions using the Qiagen PCR purification kit. PCR products were directly sequenced via BigDye™ terminator cycling and automated sequencing (Macrogen) using R1n and U2 for forward and reverse strand synthesis (Weidner et al. 1996). Consensus sequences from forward and reverse strands were generated and GenBank BLAST searches were performed in order to demonstrate percentage identity to known bacteria (Altschul et al. 1997). DNA sequences were deposited into GenBank. Accession numbers are provided in Tables 2 & 3.

RESULTS

Antibiotic properties of *Acropora palmata* mucus

UV irradiation for 10 min at a wavelength of 302 nm inhibited the growth of all mucus-associated microbes for over 2 wk (data not shown). UV irradiation did not significantly affect CFUs per ml of associated microbes compared to non-irradiated control plates (data not shown).

Acropora palmata mucus collected in April 2005 inhibited growth of microbes from Florida Keys canal water 10-fold, and water column and African dust microbes roughly 4-fold (Table 1). In addition, *A. palmata* mucus inhibited the growth of both Gram-positive and Gram-negative tester strains, including *Bacillus subtilis* (8-fold), *Staphylococcus aureus* (5-fold), *Salmonella typhimurium* (4-fold), and the *Serratia marcescens* white pox isolate PDL100 (2-fold; Table 1).

In contrast, mucus collected from apparently healthy areas of *Acropora palmata* in September 2005, during a bleaching event, had no significant inhibitory effects against the *Serratia marcescens* isolate PDL100, or microbes from Florida Keys canal water, African dust, or the water column (Table 1).

Antibiotic production by coral associated bacteria

Initial antibiotic testing of libraries containing 776 culturable bacteria strains collected from *Acropora palmata* throughout 2004 revealed that 155 isolates (20%) inhibited the growth of 1 or more tester strains and 62 (8%) of these isolates showed antibacterial activity against the *Serratia marcescens* isolate, PDL100 (data not shown).

Selection for coral bacterial symbionts

April 2005 sampling

Selection of putative coral symbionts from mucus dilutions plated onto mucus-treated media consistently resulted in 50- to 80-fold growth inhibition as compared to GASWA control medium (Fig. 1). From mucus-treated plates, 96 potential symbionts were selected, and designated as putative coral 'residents'; 95 bacteria were isolated from control plates and designated as putative 'visitors'. For comparison, 50 water column bacteria were isolated from GASWA control medium. Of the 96 resident bacteria tested for antibiotic production in this experiment, 39 (41%) displayed antimicrobial activity against 1 or more tester strains. Of the 95 visitor bacteria tested for antibiotic production, 15 (16%) produced antibiotic activity

Table 1. *Acropora palmata* mucus inhibition assays. Inhibition of tester strains and potential sources of invasive microbes tested by plating mucus (M) collected at two different times of the year (with differing mean daily water temperatures) onto glycerol artificial seawater (GASWA) medium (G), followed by UV irradiation to inhibit out-growth of mucus-associated microbes. Various dilutions or concentrations of inoculum were plated onto each treatment medium (see 'Materials & methods' for further details); dilutions producing between 200 and 400 colonies on control plates (G+UV) were compared to corresponding tester plates (G+M+UV). Numbers based on mean (±SD) of 4 plates for each experiment. Fold inhibition estimated by dividing experimental mean into control mean. (–) = no data available for a particular source

Inoculum	April 2005			September 2005		
	No. of colonies		Fold inhibition	No. of colonies		Fold inhibition
	G+UV (ctrl)	G+M+UV (expt)		G+UV (ctrl)	G+M+UV (expt)	
Bacillus subtilis	395 (±22)	51 (±11)	7.8	–	–	–
Staphylococcus aureus	245 (±40)	46 (±10)	5.4	–	–	–
Salmonella typhimurium	217 (±34)	55 (±12)	3.9	–	–	–
Serratia marcescens	193 (±33)	91 (±13)	2.1	233 (±26)	277 (±25)	–0.8
Water column	305 (±29)	76 (±7)	4.0	188 (±27)	231 (±14)	–0.8
Canal water	269 (±24)	27 (±9)	10.0	328 (±28)	274 (±20)	1.2
African dust	278 (±51)	65 (±9)	4.3	206 (±24)	191 (±22)	1.1

against 1 or more tester strain. Bacterial numbers were condensed in group pools by dereplication based on antibiotic spectrum and by loss upon repeated subculturings. This reduced the residents pool to 30 viable strains, the visitors pool to 31 viable strains, and the water column pool to 25 viable strains. Genetic analysis based on partial sequencing of the 16S rDNA gene further dereplicated pools to 17, 17, and 12 unique isolates, from the residents, the visitors, and the water-column pools, respectively. Isolates that appeared genetically identical based on 16S sequencing and colony morphology, but displayed different antibiotic spectra, were counted as unique. Genetic identities, antibiotic spectra, and GenBank accession numbers of this subset of isolates are shown in Table 2. Fig. 2 illustrates isolates that are unique within the residents, visitors, and water column pools.

September 2005 sampling

Selection of putative coral symbionts from apparently healthy areas of *Acropora palmata* during a late summer bleaching event resulted in no growth inhibition on mucus-treated media as compared to GASWA control media (Table 1). Nevertheless, for the purpose of comparison to spring 2005 selections, 21 isolates were chosen from mucus treated plates and were designated as putative coral residents. From the control plates, 27 bacteria were isolated and designated as putative visitors. For comparison, 59 water column isolates were chosen from GASWA control plates. Results show that *A. palmata* mucus collected during the September 2005 bleaching event lost the ability to select a discrete set of isolates (Table 3; Fig. 3). Genetic analysis based on partial sequencing of the 16S rRNA gene

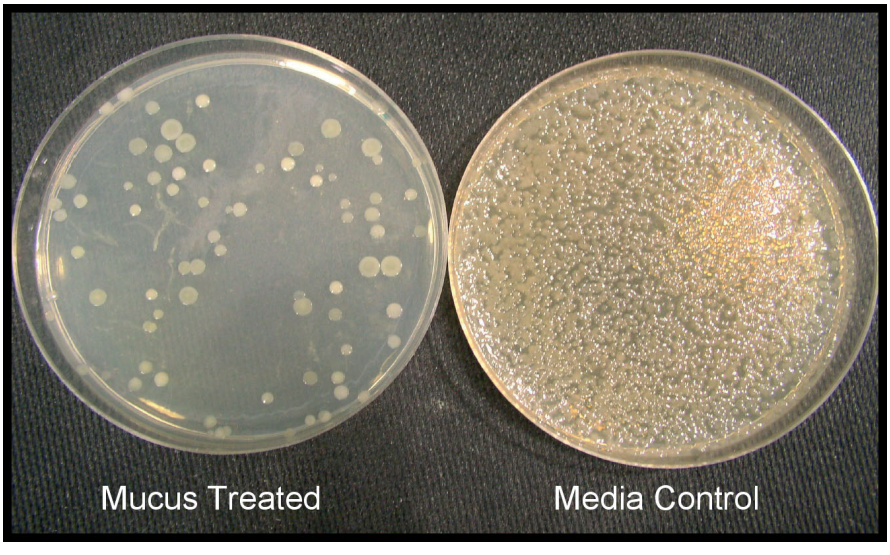

Fig. 1. Selection scheme. Elkhorn coral *Acropora palmata* mucus dilutions (1:100) inoculated onto glycerol artificial seawater (GASWA) solid medium treated with coral mucus (left) or GASWA control medium (right). Bacterial growth was inhibited 50- to 80-fold on GASWA medium treated with mucus collected in spring 2005

Table 2. Partial list of bacterial strains isolated from April 2005 selection for coral symbionts. For 2 mo prior to and during mucus sampling, mean daytime water temperature = 22 to 25°C. Not all isolates initially tested for antibiotic activity are included, due to loss of viability during repeated sub-culturing and/or frozen storage. Tester strains: methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC 43300); methicillin-sensitive *Staphylococcus aureus* (MSSA; ATCC 29213); vancomycin-resistant *Enterococcus* (VRE); *Bacillus subtilis* (ATCC 6633 Km resistant); *Salmonella typhimurium* (ATCC 6994); *Enterococcus faecalis* (ATCC 29212); *Shigella* (MicroGenomics); *Escherichia coli* O157; *Agrobacterium tumefaciens*; and coral pathogen *Serratia marcescens* strain PDL100. Residents: coral associates grown on mucus-treated experimental medium (GASWA+mucus+UV) as potential symbionts. Visitors: coral associates grown on control medium (GASWA+UV) as transient and/or potentially invasive microbes. Water column bacteria: water column isolates grown on control medium (GASWA). bp = no. of base pairs sequenced. % ID = percentage identity to closest-match bacterial representative in GenBank database. Isolates = no. of similar strains isolated. AB spectrum = antibiotic spectrum, based on inhibition against listed tester strains. ZI = zone of inhibition, scored as distance (mm) from edge of colony to end of clearing zone. (–) = antibiotic activity not detected. Accession # = GenBank accession number of coral isolate

Strain	bp	% ID	Closest match	Isolates	AB spectrum (ZI)	Accession #
Residents						
R1ma	802	100	*Bacillus megaterium*	1	*B. subtilis* (*5*)	DQ530511
R1mb	830	99	*Agrobacterium tumefaciens*	2	–	DQ530512
R2te	744	99	*Photobacterium phosphoreum*	2	–	DQ530513
R1m1	801	99	*Photobacterium damselae*	1	–	DQ530514
R1m2	682	99	*Peligiobacter variabilis*	1	–	DQ530515
R1m3	807	99	*Photobacterium* sp. YS27-3	2	–	DQ530516
R1t2	700	98	*Alteromonas* sp.	1	–	DQ530517
R1t3	789	99	*Halomonas meridiana*	1	–	DQ530518
R1t4	690	100	*Halomonas meridiana*	2	*B. subtilis* (*1.5*)	DQ530519
R1t6	682	100	*Photobacterium mandapumensis*	2	*B. subtilis* (*5*)	DQ530520
R1t9	770	99	*Exiguonobacterium sp*	1	*S. marscesens* (*1.5*)	DQ530521
R1m5	804	100	*Photobacterium mandapumensis*	4	–	DQ530522
R2m1	825	99	Uncultured *Alteromonas*	1	*S. marscesens* (*1.5*)	DQ530523
R2m2	805	*99*	*Photobacterium leiognathis*	1	*S. marscesens* (*1.5*)	DQ530524
R2t1	831	99	*Photobacterium damselae*	3	*S. marscesens* (*1.5*)	DQ530525
R2t2	816	99	*Photobacterium* sp. *YS27-3*	2	*S. marscesens* (*1.5*)	DQ530526
R2t3	833	100	*Photobacterium* sp *YS27-3*	3	*B. subtilis* (*1.5*), *S. marscesens* (*1*)	DQ530527
Visitors						
V1mt1	869	99	*Staphylococcus* sp.	2	–	DQ530528
V2mt2	811	99	Arctic sea ice bacterium	2	–	DQ530529
V2mt3	866	99	*Agrobacterium* sp.	2	–	DQ530530
VBR1	738	100	*Vibrio fortis*	2	–	DQ530531
VBR2	720	100	*Vibrio shilonii*	2	–	DQ530532
VBR5	806	99	*Enterovibrio coralii*	3	–	DQ530533
VBR6	787	98	*Vibrio harveyii*	5	–	DQ530534
VBR7	676	100	*Pseudoalteromonadaceae* bacterium	1	*B. subtilis* (*8*), MRSA (*6*), MSSA (*6*), *VRE* (*5*), *E. faecalis* (*5*), *S. typhimurium* (*7*), *Shigella* (*7*), *A. tumefaciens* (*6*), *E. coli* (*4.5*), *S. marscesens* (*4*)	DQ530535
VBR8	835	100	*Pseudoalteromonadaceae* bacterium	1	–	DQ530536
VBR10	807	100	*Vibrio olivaceus*	3	–	DQ530537
VBR12	654	99	*Vibrio nigripulchritudo*	1	MRSA (1), MSSA (1)	DQ530538
VBR16	730	99	*Pseudoalteromonas* sp.	1	*B. subtilis*	DQ530539
VBR19	797	100	*Pseudovibrio*/Alpha proteobacterium	1	MRSA (4)	DQ530540
VBR22	798	100	Alpha proteobacterium Z143-1	2	*B. subtilis* (*2.5*), MRSA (*6*), MSSA (*7*), *S. typhimurium* (*2*), *Shigella* (*5*), *A. tumefaciens* (4)	DQ530541
VBR27	648	96	*Vibrio hollisae*	1	–	DQ530542
VAP1-8	726	99	*Vibrio harveyii*	1	*B. subtilis* (*1*), *S. typhimurium* (*4*)	DQ530543
VAP1-9	718	99	*Vibrio shilonii*	1	*B. subtilis* (*1*), *S. typhimurium* (4)	DQ530544
Water column bacteria						
W1	868	99	*Staphylococcus saprophyticus*	4	–	DQ530545
W2	745	99	*Staphylococcus aureus*	2	–	DQ530546
W3	837	99	*Agrobacterium* sp.	3	–	DQ530547
W4	652	99	*Vibrio nigripulchritudo*	3	–	DQ530548
W5	680	100	*Vibrio harveyii*	2	–	DQ530549
W6	787	99	Alpha proteobacterium	3	–	DQ530550
W7	875	99	*Pseudoalteromonas* sp.	1	*B. subtilis* (*3*)	DQ530551
W8	573	99	*Pseudoalteromonas* sp.	2	–	DQ530552
W9	689	100	Alpha proteobacterium	1	–	DQ530553
W10	401	99	*Vibrio alginolyticus*	1	–	DQ530554
W11	717	99	*Kocuria* sp.	1	–	DQ530555
W12	613	98	*Photobacterium eurosenbergii*	2	–	DQ530556

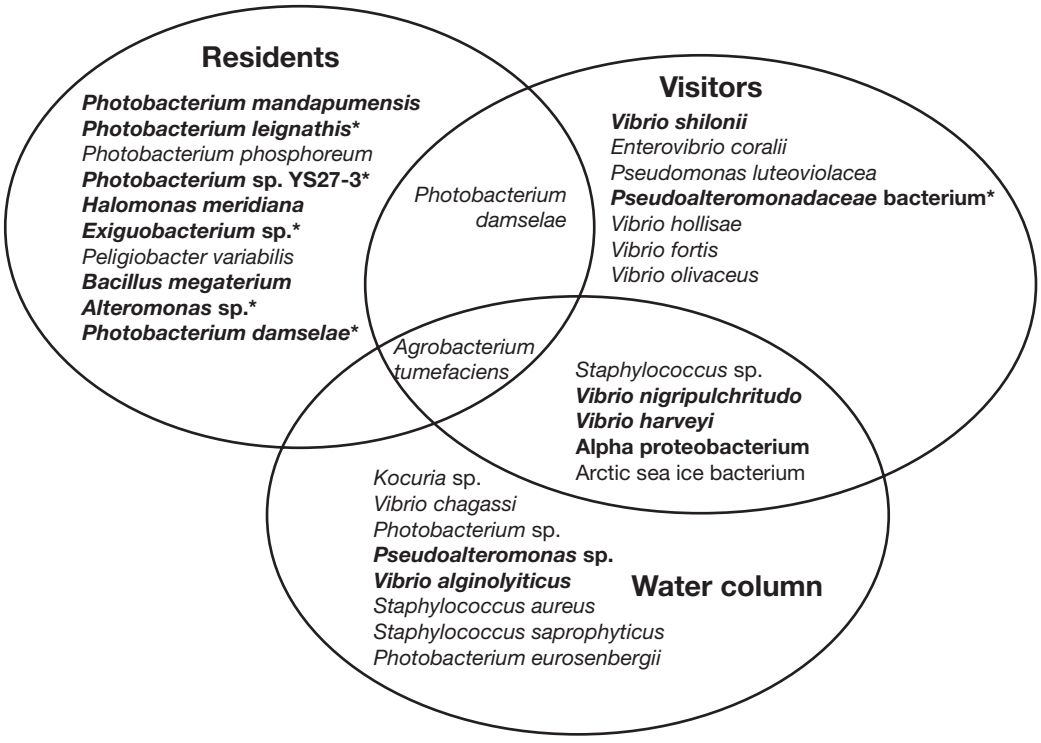

Fig. 2. Mucus selection scheme to enrich for coral symbionts (April 2005). Coral mucus sampled when mean daytime water temperature = 24°C. Residents (top left): representative bacteria selected on mucus-treated medium (GASWA + mucus + UV) as putative coral symbionts. Visitors (top right): selected on control GASWA medium. Water column (bottom): selected from the water column on control medium. Overlap = bacteria common to different treatments and sources. **Bold**: bacteria producing an antimicrobial compound (residents, 41% total; visitors, 16% total; not all representatives shown). *Bacteria producing antibiotic activity against *Serratia marcescens* strain PDL110

placed 100% of resident, 92% (22 of 24) of visitor and 86% (49 of 58) of water-column isolates within the genus *Vibrio*. No resident isolates displayed antibiotic activity. Only one isolate, most similar to *V. nigripulchritudo* and common to both the visitor pool and seawater, had antibiotic activity (Table 3).

Coral mucus regulates metabolic activities in a coral-associated bacterium

One visitor isolate (VBR7), from *Acropora palmata* mucus produced a dark purple, non-diffusible, pigment on control plates, but lost the capability to produce the pigment when grown on *A. palmata* mucus-treated media (Fig. 4A). VBR7 also produced a broad-spectrum antibiotic (Table 2). Partial sequencing of the 16S rRNA gene identified this bacterium as 99% identical to *Pseudoalteromonaceae* bacterium (Table 2). Antibiotic tests were performed on this isolate during pigment production and after pigment loss. Fig. 4B shows isolate VBR7 producing pigment on control media (left side, each panel) and after loss of pigment upon growth on coral mucus (right side, each panel). Each replica panel was overlaid with a different tester strain. The antibacterial compound associated with VBR7 was active against all Gram-positive and Gram-negative tester strains, with zones of inhibition ranging from 3 to 8 mm. This indicated the presence of a readily diffusible, broad-spectrum antibiotic. Loss of pigment and antibiotic activity in VBR7 was also demonstrated when VBR7 was grown in the presence of mucus isolated from the gorgonian coral *Pseudopterogorgia americana* (collected from the Florida Keys), and the star boulder coral *Montastraea faveolata* (collected from both the Florida Keys and Flower Garden Banks).

DISCUSSION

While coral health appears to be declining world wide, the Caribbean elkhorn coral *Acropora palmata* has suffered the greatest losses, to the extent that it has recently been listed as a threatened species under the US Endangered Species Act. *A. palmata* appears particularly sensitive to stress and disease, although little is known of its physiological response to stressors

Table 3. September 2005 (bleaching event) selection of coral symbionts. For 2 mo prior to and during mucus sampling, mean daytime water temperature = 28 to 30°C. Tester strains: methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC 43300); methicillin-sensitive *Staphylococcus aureus* (MSSA; ATCC 29213); vancomycin-resistant *Enterococcus* (VRE); *Bacillus subtilis* (ATCC 6633 Km resistant); *Salmonella typhimurium* (ATCC 6994); *Enterococcus faecalis* (ATCC 29212); *Shigella* (MicroGenomics); *Escherichia coli* O157; *Agrobacterium tumefaciens*; and coral pathogen *Serratia marcescens* strain PDL100. bp = no. of base pairs sequenced. % ID = percentage identity to closest-match bacterial representative in GenBank database. Isolates = no. of similar strains isolated. AB spectrum = antibiotic spectrum, based on inhibition against listed tester strains. ZI = zone of inhibition, scored as distance (mm) from edge of colony to end of clearing zone. (–) = antibiotic activity not detected. Accession # = GenBank accession number of coral isolate

Strain	bp	% ID	Closest match	Isolates	AB spectrum (ZI)	Accession #
Coral associates grown on mucus-treated experimental media (GASWA+mucus+UV)						
R1	693	100	*Vibrio harveyi*	*6*	–	DQ521046
R2	693	99	*Vibrio coralllilyticus*	*4*	–	DQ521047
R3	849	100	*Vibrio alginolyticus*	*4*	–	DQ521048
R4	692	100	*Vibrio campbelli*	*2*	–	DQ521049
R5	690	98	*Vibrio shilonii*	*1*	–	DQ521050
R6	682	99	*Vibrio neptunius*	*1*	–	DQ521051
R7	692	99	*Vibrio* sp. PH1	*1*	–	DQ521052
R8	692	100	*Vibrio chagasii*	*1*	–	DQ521053
R9	683	99	*Vibrio probioticus*	*1*	–	DQ521054
Coral mucus associates grown on control media (GASWA+UV)						
V1	690	100	*Vibrio corallilyticus*	*4*	–	DQ521055
V2	720	98	*Vibrio* sp. PH1	*4*	–	DQ521056
V3	833	99	*Vibrio alginolyticus*	*2*	–	DQ521057
V4	691	98–99	*Vibrio shilonii*	*2*	–	DQ521058
V5	694	99	*Vibrio harveyi*	*3*	–	DQ521059
V6	691	99	*Vibrio* sp. ME2-03	*3*	–	DQ521060
V7	690	100	*Vibrio* sp. HB-8	*2*	–	DQ521061
V8	683	100	*Vibrio olivaceus*	*1*	–	DQ521062
V9	690	100	*Vibrio* sp. A356	*1*	–	DQ521063
V10	689	100	*Vibrio parahaemolyticus*	*1*	–	DQ521064
V11	554	98	*Shewanella* sp.	*1*	–	DQ521065
V12	684	99	*Vibrio nigripulchritudo*	*1*	MSSA (1), MRSA (1)	DQ521066
V13	676	100	*Photobacterium mandapamensis*	2	–	DQ521067
Water column isolates grown on control media (GASWA)						
W1	693	100	*Vibrio harveyi*	*9*	–	DQ521068
W2	691	98	*Vibrio shilonii*	*5*	–	DQ521069
W3	689	100	*Vibrio ponticus*	*5*	–	DQ521070
W4	692	99	*Vibrio* sp. *PH1*	*5*	–	DQ521071
W5	690	99	*Vibrio nigripulchritudo*	*5*	MSSA (1), MRSA (1)	DQ521072
W6	823	99	*Vibrio olivaceus*	*4*	–	DQ521073
W7	690	98–99	*Vibrio neptunius*	*3*	–	DQ521074
W8	700	97	*Shewanella* sp. *L-10*	*3*	–	DQ521075
W9	686	100	*Vibrio campbelli*	*3*	–	DQ521076
W10	609	99	*Vibrio corallilyticus*	*2*	–	DQ521077
W11	689	100	*Photobacterium mandapamensis*	*2*	–	DQ521078
W12	690	100	*Vibrio alginolyticus*	*3*	–	DQ521079
W13	675	96	*Photobacterium ganghwensis*	*2*	–	DQ521080
W14	823	98	*Vibrio* sp. BL1-41	*1*	–	DQ521081
W15	694	100	*Vibrio* sp. *YASM15*	*1*	–	DQ521082
W16	609	99	*Vibrio gallicus*	*1*	–	DQ521083
W17	607	98	*Vibrio* sp. *Absa37*	*1*	–	DQ521084
W18	645	98	*Vibrio* sp. *ME2-03*	*1*	–	DQ521085
W19	691	100	*Vibrio* sp. *HB-8*	*1*	–	DQ521086
W20	696	98	*Pseudoalteromonas sagamiensis*	*1*	–	DQ521087
W21	607	100	*Rhodobacteraceae* sp.	*1*	–	DQ521088

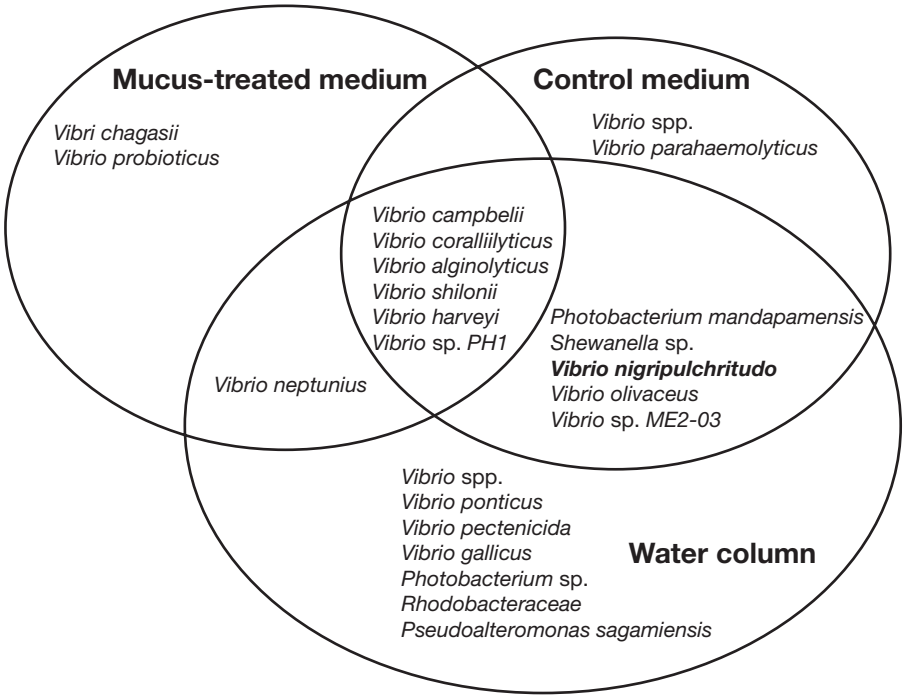

Fig. 3. Mucus selection scheme (September, 2005). Coral mucus sampled when mean daytime water temperature = 30°C. Representative bacteria selected on mucus-treated medium (GASWA + mucus + UV; top left); control medium (GASWA + UV; top right); or water column bacteria isolated on control medium (GASWA; bottom). Note that applied selection scheme falls apart (cf. Fig. 2), with loss of diversity in all treatments and sources tested; high incidence of *Vibrio* spp., including numerous vibrios implicated in coral bleaching and opportunistic diseases of marine organisms. Overlap = bacteria common to different treatments and sources. **Bold** = bacteria producing an antibacterial compound

(Antonius 1977, 1981, Gladfelter et al. 1977, Bruckner 2002, Precht 2004, Sutherland & Ritchie 2004). Some suggested sources of coral decline include increased sea surface temperatures, coastal degradation, pollution, diseases, ecosystem imbalance caused by anthropogenic influences, and the synergistic effect of multiple stressors (Harvell et al. 2002, Rosenberg & Ben-Haim 2002, Sutherland et al. 2004). One possible explanation for an increased incidence of coral diseases is stress-induced susceptibility to opportunistic microbes trapped in the coral mucus. Numerous sources of pathogenic microbes have been suggested

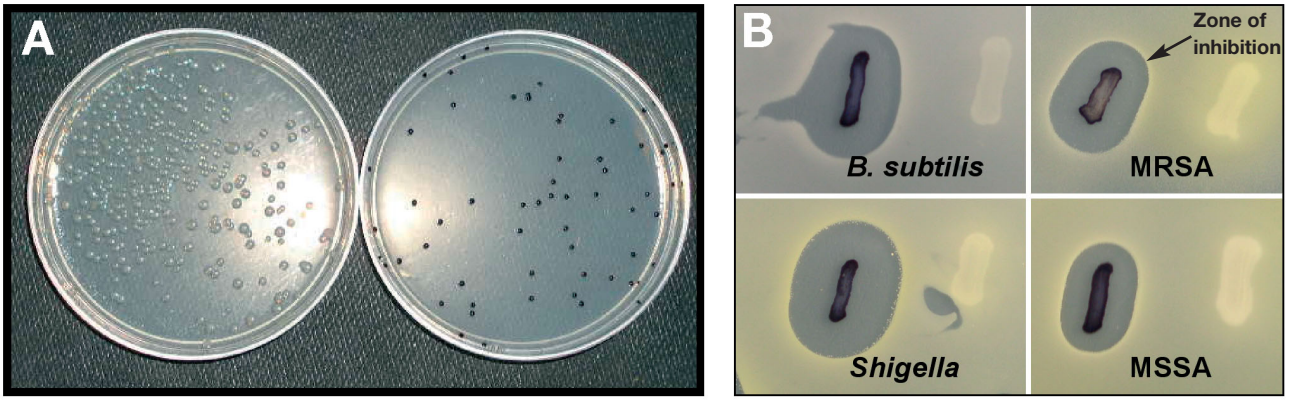

Fig. 4. Coral mucus inactivates metabolic properties of potentially invasive bacterium. (A) Putative 'visitor' bacterium VBR7 isolated from elkhorn coral *Acropora palmata* mucus produced a deep purple pigment when grown on GASWA control medium (right) but not when grown on medium treated with mucus collected from *A. palmata* (left), scleractinian coral *Montastrea faveolata*, or gorgonian coral *Pseudopterogorgia americana* (not shown). (B) Each panel shows antibiotic activity of replicas of isolate VBR7 before (control medium; left side, each panel) and after pigment loss on mucus treated medium (right side, each panel). Tester strains used were *Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA), and *Shigella*. Arrow: MRSA growth inhibition zone produced by VBR7

as possible threats to the health of corals in the Florida Keys. These include canal water, which is a source of human enteric bacteria such as *Serratia marscesens* (Patterson et al. 2002); African dust, hundreds of millions of tons of which are transported to the Caribbean each year along with associated bacteria, fungi and viruses (Garrison et al. 2003); and water column microbes that may become opportunistic under conditions of increased temperature and nutrient load.

Acropora palmata mucus as a biochemical defense

This study shows that mucus collected from *Acropora palmata* has antibiotic activity against (1) Gram-positive and Gram-negative bacteria, (2) a number of potentially invasive microbes (including microbes from Florida Keys canal water, African dust, and surrounding sea water), and (3) a pathogen implicated in white pox disease of *A. palmata*. This result suggests that healthy *A. palmata* employ a biochemical mechanism for disease resistance that may act as a primary defense against pathogens. In contrast, mucus collected from *A. palmata* during a period of increased water temperature did not show significant antibiotic activity against the same suite of sources and tester strains, suggesting that the protective mechanism employed by *A. palmata* is lost when temperatures increase. This observation suggests a mechanism to the hypothesis that increased temperatures lower coral resistance, resulting in an increased susceptibility to disease.

Beneficial coral bacteria

Both culture-dependent and independent methods have been used to understand microbial communities on the surface of corals (reviewed in Brown & Bythell 2005). Both methods show that corals favor specific populations of bacterial associates that are predicted to be mutualistic with the coral host (Ritchie & Smith 1997, 2004, Rohwer et al. 2002). While culture-based methods are biased toward the small percentage of microbial associates that are able to be cultivated in the laboratory, culture-independent studies based on 16S rDNA are limited in their prediction of compound production and metabolic potential of coral-associated bacteria. This study did not attempt to address total microbial community structure of *Acropora palmata*, but focused on a subset of marine bacteria cultured under specific growth conditions to investigate a potential bacterial contribution to the antibiotic activity seen in coral mucus.

Initial testing of culturable bacteria collected from *A. palmata* mucus throughout the course of 2004 illustrated that roughly 20% displayed antibacterial activities. This result suggests a potential role for coral bacterial associates in the production of mucus-associated antibiotic activity. *In situ* antibiotic production by associated bacteria is a means of securing a niche by controlling microbial populations competing for the same resources (Neilson et al. 2000, Rao et al. 2005). The level of antibiotic contribution by these bacteria *in situ* remains to be determined.

Selection for coral symbionts

Coral mucus traps particles and microbes that pass by in the water column (Wild et al. 2004). Therefore, attempts at understanding the importance of coral-associated microbial communities may be misleading due to the fact that coral mucus is indiscriminate, retaining microbes from a fluctuating water mass that may not be present in the water column at the time of collection. These dynamic fluctuations include coastal upwelling, countless local and regional influences, and local flora and fauna. Thus, many microbes trapped in coral mucus are less likely to be true 'residents,' or mutualists, but rather 'visitors' consisting of transient commensal microorganisms that do no good or harm, or organisms that can potentially become opportunistic under the right conditions.

The phylogenetic approaches used in most comprehensive analyses of unculturable coral microbes provide information on the identity of microbes present, but very little information relative to microbial interactions, or information necessary for the elucidation of true coral residents (microbes beneficial to the coral host, zooxanthellae, or other resident microbes). Here, a culture-based approach was developed to address this challenge by using sterile coral mucus as a selection medium for coral symbionts: bacteria that benefit from nutrients available on the coral surface while providing a benefit to the coral in return. This approach is based on the hypothesis that true coral symbionts will be resistant to the antibiotic properties of *Acropora palmata* mucus, while many trapped bacteria may be sensitive to the bacteriocidal, or bacteriostatic, properties in coral mucus.

This selection scheme was first applied using *Acropora palmata* mucus collected in spring 2005 under temperatures that were sustained at 22 to 24°C for 2 mo prior to sampling. Bacteria isolated on mucus-treated plates as putative symbionts were designated as coral 'residents,' while bacteria isolated from control media were designated as potential 'visitors,' or transient bacteria trapped from the water column. Fig. 2 illustrates that the vast majority of bacteria cultured, using either mucus-treated selection media or control

media, belonged to the γ-proteobacteria. Members of this group are abundant on corals and have been identified using a range of methods, including culture-based (Ritchie & Smith 2004), molecular (Rohwer et al. 2002), and fluorescence *in situ* hybridization and spectral imaging (Ainsworth et al. 2006). Therefore, I think that this subset of culturable associates represents a valid group for the study of symbiotic interactions.

Results show that the mucus-based selection method enriched for *Photobacterium* spp. (60%), *Halomonas* spp. (10%), and a range of bacterial species that have antibiotic activities (Table 2, Fig. 2). The significance of *Photobacterium* and *Halomonas* spp. enrichment is not clear, although a subset of each is shown to produce antibiotics (Table 2). It is possible that these bacteria additionally play a role in the health of the coral holobiont via production of vitamins or cofactors necessary for the growth of beneficial bacteria, or by providing other regulatory compounds. In addition, some strains designated as coral residents were active against the *Serratia marcescens* PDL100 coral disease pathogen. That this method enriched for bacteria that produce antibiotics provides additional support for a bacterial contribution to the antibiotic activity of coral mucus while providing a novel method for the enrichment of marine bioactive compounds.

Bacteria isolated as visitors are representative of bacteria documented in earlier studies, including a subset of *Vibrio* species consistently found in association with healthy corals (Ritchie & Smith 1995a,b, 2004). As partial sequencing of the 16S gene is not sufficient for a thorough identification of members of the genus *Vibrio*, these isolates will require further genetic delineation. Table 2 shows that there is a higher percentage of vibrios (48%) when comparing control plates to the coral mucus selection scheme (no vibrios). A subset of vibrios isolated from control plates (visitor microbes *Vibrio shilonii* and *Enterovibrio* sp.) are most similar to species associated with coral bleaching in the Mediterranean Sea and in the Great Barrier Reef (Kushmaro et al. 2001, Thompson et al. 2005), suggesting that these bacterial species are ubiquitous in tropical oceans. Several visitor and water column isolates produced antibiotics. Isolate VBR7 produced a broad spectrum antibiotic with large zones of inhibition and isolate VAP1-9, which displayed 99% identity to *Vibrio shilonii*, exhibited antibiotic activity against 2 Gram-positive tester strains. These isolates may represent bacteria capable of becoming opportunistic under conditions of coral stress.

Collectively, these results suggest that coral mucus provides a hostile environment for some bacteria and a nurturing environment for others, illustrating that the mucus plays an important role in structuring microbial communities on the coral surface. The use of coral mucus as a selection medium is experimental. It is possible that UV sterilization alters the composition of the mucus in a manner not addressed by the UV controls. However, the results using UV irradiated summer mucus, described below, argue against significant alteration of the mucus by UV treatment.

Loss of antibiotic activity and shift to *Vibrio* spp. during a summer bleaching event

The antibiotic properties of coral mucus, and the potential for mucus to select a discrete set of commensal bacteria, were lost at increased temperatures during a bleaching event (Table 3; Fig. 3). Mucus was taken from corals sustained at a mean daytime sea surface temperature of 28 to 30°C for 2 mo prior to collection. Vibrios were the predominant species cultured from the mucus of apparently healthy *Acropora palmata* tissue during this event. Vibrios were also predominant in the water column during this period, representing 85% of the cultured isolates (Table 3). Less than 2% of bacteria isolated from the surface of *A. palmata* during this period produced antibiotic activity. These findings illustrate a temporal shift in the protective qualities of coral mucus, and a composition shift from beneficial bacteria to vibrio dominance under conditions of increased temperature. Vibrios present during this event included those involved in temperature dependent bleaching of corals, such as *Vibrio shilonii* (= *V. shiloi*; Kushmaro et al. 2001) and *V. coralliilyticus* (= *V. coralyticus*; Ben-Haim et al. 2003) as well as numerous vibrios known to be opportunistic to other marine organisms (Table 3). These findings are consistent with a shift of the equilibrium between *Pseudomonas* spp. and *Vibrio* spp. in healthy corals, to vibrio dominance when corals are bleached (Ritchie & Smith 1995a, 2004). However, as mucus was collected from apparently healthy coral tissue, and not bleached tissue, this provides evidence that a community shift to vibrio dominance may occur prior to zooxanthellae loss.

During bleaching, coral mucus production changes in quality and can decrease in quantity, depending on the cellular damage caused by the environmental factor(s) that initiated bleaching (Lasker et al. 1984, Glynn et al. 1986). Thus, microbes that depend on particular substrates in healthy mucus may be reduced in number when these substrates are no longer available. One model that addresses coral bleaching and disease susceptibility is that antimicrobial properties of coral mucus are compromised by temperature-dependent alteration of resident microbes, or other factors influencing antibiotic activity, followed by an overgrowth of vibrios, which are abundant in the water column during warmer months. A similar model is that bene-

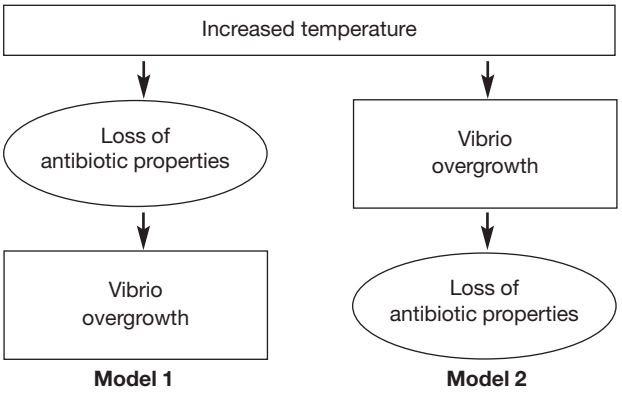

Fig. 5. Models of bacterial overgrowth on coral surfaces. In Model 1, rising water temperature compromises antibiotic production by the coral holobiont, either by affecting antibiotic production by the coral or by affecting composition and/or activity of resident microbiota. This results in opportunistic overgrowth of transient microbes in coral mucus, increasing the probability of bleaching or disease. In Model 2, overgrowth by transient microbes out-competes the resident population of microbiota, with subsequent loss of antibiotic activity associated with coral mucus. This model requires additional evidence that resident coral-associated microbes contribute significantly to antibiotic activity described for coral mucus

ficial microbes are simply out-competed by vibrios, many of which thrive under conditions of increased temperature (Lipp et al. 2002, Rosenberg & Ben-Haim 2002, Thompson et al. 2005). Both models (Fig. 5) are supported by observations in this study and others. Kuntz et al. (2005) reported that growth stimulation of bacterial communities present on corals may directly result in coral mortality. In addition, it has been shown that 4 vibrio strains, inoculated as a group, were able to cause signs of yellow band disease when temperatures were increased (Cervino et al. 2004).

Another possible mechanism that supports both models is cooperation among similar bacteria (Foster 2005). This theory is distinct from a one-pathogen model for coral bleaching by *Vibrio* species (Kushmaro et al. 2001, Ben-Haim et al. 2003), in that it is based on a bacterial version of kin-selection theory, where similar bacteria are able to co-operate to secure a niche because they share like genes (Foster 2005). This may also apply to vibrios associated with corals. In this scenario, vibrios may compete for space on the coral surface, reducing relatedness among beneficial surface bacteria by sharing similar gene products involved in securing a niche (such as feeding enzymes and virulence factors, among others). The result is the swamping of resident beneficial microbes, the initiation of coral bleaching and, perhaps ultimately, an increase in disease susceptibility.

Regardless of the precise ordering of events, during times of increased sea surface temperature, corals are more susceptible to disease (Rosenberg & Ben-Haim 2002), which is demonstrated in this study to be correlated with a loss of a protective function provided in the coral mucus and an increase in the number of vibrios, both in the water column and on the surface of apparently healthy coral tissue. These observations may offer further clarification for the increase in coral disease incidence that occurs following bleaching events (Causey 2001, Harvell et al. 2002, Weil et al. 2006).

Undocumented function of coral mucus

While studying the interaction of *Acropora palmata* mucus with potentially invasive microbes, it was noted that one visitor isolate (VBR7), a *Pseudoalteromonaceae* bacterium isolated from *A. palmata* mucus, produced a dark purple, non-diffusible pigment on control plates, but lost the ability to produce the pigment when grown on *A. palmata* mucus-treated media (Fig. 4a). This isolate was initially shown to produce a powerful, broad-spectrum antibiotic (Table 2). As pigment production is often correlated with antibiotic activity (Ritchie, pers. obs.), antibiotic tests were performed on this isolate both during pigment production and after pigment loss. Results showed that VBR7 produced a broad-spectrum, readily diffusible antibiotic when grown on control media, during pigment expression. Conversely, antibiotic activity was absent when the pigment was lost in this strain. Loss of pigment production and antibiotic activity in VBR7 was also demonstrated when VBR7 was grown in the presence of *A. palmata* mucus collected during summer months, mucus isolated from the gorgonian coral *Pseudopterogorgia americana* (collected from the Florida Keys), and mucus from the star boulder coral *Montastrea faveolata* (collected from both the Florida Keys and Flower Garden Banks). This result illustrates that a component universal to coral mucus, independent of species, location, and season, is capable of inhibiting pigment and antibiotic production associated with VBR7. The production of cell signaling molecules by many microbes regulates bacterial processes in a population density-dependent manner (Miller & Bassler 2001, Teplitski et al. 2004). This type of communication, called 'quorum sensing,' is common in bacterial biofilms and regulates processes such as adhesion, antibiotic production, and virulence (Miller & Bassler 2001). Quorum sensing molecules, if present in coral mucus, could result in the pigment and antibiotic suppression seen in VBR7. Although further studies will reveal whether these regulatory processes within the mucus are attributed to the coral, zooxanthellae, or coral associated bacteria, the regulation of bacterial

gene expression by mucus is likely to play a significant role in both the determination of microbial community structure and in the establishment of pathogenic bacteria.

CONCLUSIONS

In this study, *Acropora palmata* mucus was shown to have antibiotic properties that are likely to play a role in ordering beneficial microbial communities on the coral surface. In addition, mucus from healthy *A. palmata* harbors bacteria capable of producing antibiotics, implicating a microbial contribution to the protective properties of coral mucus. Coral mucus inactivated pigment production and antibiotic activity in a mucus-associated bacterium, illustrating an undocumented role of coral mucus in the control of associated microbes. Mucus-associated antibiotic activity was reduced when mucus was collected during a period of increased sea surface temperature. This finding suggests a seasonal variability in the protective qualities of coral mucus that may result in increased susceptibility to bleaching and disease. Beneficial coral bacteria were replaced by *Vibrio* spp. during this event, which resulted in a loss of antibiotic producing bacteria on coral surfaces. Future studies will reveal temporal and environmental changes on the coral surface, and the precise role of coral mucus and beneficial microbes in coral health and disease susceptibility.

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