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SYMPOSIUM

Coral-Associated Bacterial Assemblages: Current Knowledge and the Potential for Climate-Driven Impacts

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Synopsis The importance of associations between microorganisms and their invertebrate hosts is becoming increasingly apparent. An emerging field, driven by the necessity to understand the microbial relationships that both maximize coral health and cause coral disease, is the study of coral–bacteria interactions. In this article, we review our current understanding of the diversity, specificity, development, and functions of coral-associated bacteria. We also summarize what is known regarding the role of coral microbiota in the health and disease of coral. We conduct a meta-analysis to determine whether the presence of unique taxa correlates with the state of coral health (i.e. healthy, diseased or bleached), as well as whether coral reef habitats harbor clusters of distinct taxa. We find that healthy and bleached corals harbor similar dominant taxa, although bleached corals had higher proportions of *Vibrio* and *Acidobacteria*. Diseased corals generally had more *Rhodobacter*, *Clostridia*, and *Cyanobacteria* sequences, and fewer *Oceanospirillum* sequences. We caution, however, that while 16S rRNA is useful for microbial species identification, it is a poor predictor of habitat or lifestyle, and care should be taken in interpretation of 16S rRNA surveys to identify potential pathogens amongst complex coral–microbial assemblages. Finally, we highlight evidence that coral–bacterial assemblages could be sensitive to the effects of climatic change. We suggest that the relationship between coral and their bacterial associates represents a valuable model that can be applied to the broader discipline of invertebrate–microbial interactions.

Introduction

The close associations between animals and their microbiota have shaped the evolutionary paths of both host and symbiont alike. While interactions between microorganisms and vertebrates have been well studied, relatively little attention has been given to the examination of microbial–invertebrate associations. A frontier of invertebrate biology is the interaction between microorganisms and their hosts. Indeed, many of the biologically active compounds ascribed to marine invertebrates, like sponges (Flatt et al. 2005; Ridley et al. 2005) and bryozoans (Hildebrand et al. 2004) have been found to be produced by their bacterial associates. Increasingly, these associations show strong functional significance. For instance, the bacterial symbionts of sponges and bryozoans produce chemicals that protect their hosts from heterospecific settlement of larvae (Ridley et al. 2005) and from predation (Lopanik et al. 2004), respectively. Research on coral-associated bacteria is revealing important symbiotic functions, similar to that on other sessile invertebrates, and this system is emerging as one of the best-studied examples of invertebrate–microbial interactions. While corals have been found to harbor a wide variety of microbes, including heterotrophic eukaryotes, bacteria, archaea and viruses, the majority of studies thus far have centered on bacteria associated with coral.

The coral organism is a complex host that forms associations with both external and internal microbiota. The coral animal, its intracellular algal

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symbionts, and the diverse microorganisms found in association with coral tissues and exudates have been termed the ''holobiont'' (Rohwer et al. 2002; Reshef et al. 2006). While it has long been known that the algal symbiont is an obligate partner supplying up to 95% of the host's metabolic requirements for carbon and contributing to formation of the skeleton (Muscatine 1973), the roles of coral-associated bacteria have not been well elucidated. The structure of the coral host provides a multifaceted habitat, with distinct and diverse bacteria residing in the host skeleton, tissues, and surface mucus layer. As in terrestrial ecosystems, where bacterial assemblages play an essential role in ecosystem functioning (Balser et al. 2006), coral-associated bacteria are likely to drive biochemical and ecological processes within the reef environment. In this article, we review the literature concerning coral-associated bacteria, summarizing the diversity, specificity, development, and functional roles of coral microbiota. We also consider the relationship between coral-associated microorganisms and disease, conducting a meta-analysis to determine whether diseased or bleached coral harbor unique taxa, as well as whether clusters of taxa are distinct to the coral reef habitat. Finally, we examine evidence that these populations have the potential to be disrupted by climatic change. While this review focuses on coral-associated bacteria, we suggest it contains themes useful for a broader consideration of the importance of invertebrate–microbial interactions.

Diversity and specificity of coral-associated bacteria

Sequence-based assessments of microbial assemblages, which involve random sampling of bacterial rRNA genes amplified from nucleic acid (Olsen et al. 1986), provide high taxonomic resolution for environmental samples across large datasets based on nucleotide heterogeneity. While cultivation-based approaches provide important information on the metabolism of some microorganisms, the vast majority (>99%) of marine microorganisms do not grow on enriched media (Azam 1998). In coral, 16S rRNA surveys of bacteria have elucidated an astonishing diversity of bacterial ribotypes, many of which are not closely related to cultivated or uncultivated microorganisms identified in previous studies. For instance, Rohwer et al. (2002) characterized the bacterial assemblage of three Caribbean species and estimated the presence of 6000 ribotypes in libraries from 14 coral samples. Additional studies examining bacterial assemblages from multiple coral species and geographic regions have found similar results (Rohwer et al. 2001; Bourne and Munn 2005; Klaus et al. 2005; Koren and Rosenberg 2006; Sekar et al. 2006; Kapley et al. 2007; Wegley et al. 2007; Koren and Rosenberg 2008; Lampert et al. 2008; Hong et al. 2009; Littman et al. 2009b; Reis et al. 2009). Like most microbial assemblages in marine ecosystems, coral-associated microbial assemblages contain microdiverse clusters (i.e. organisms varying by a handful of nucleotides across entire 16S rRNA genes) of closely related taxa, where rarely is exactly the same sequence retrieved twice in surveys. Microbial assemblages in corals, like plankton communities, are dominated by a few different taxonomic units with a long tail of the species-distribution curve (Rohwer et al. 2002), suggesting that much of the diversity within the coral microbiome exists within the ''rare'' biosphere (Sogin et al. 2006).

A central question in microbial ecology is whether microorganisms fill defined niches within complex communities, or whether communities are comprised of functionally redundant, neutrally-selected taxa leading to random assemblages (Fuhrman et al. 2006). In marine plankton, microbial assemblages are heterogeneous between geochemical and productivity-defined habitats (Moeseneder et al. 2001; Hewson and Fuhrman 2004), yet in richer habitats, like sediments, spatially distinct communities in the same habitat type are more similar to each other than to those in adjacent habitats (Hewson et al. 2007). It is, therefore, not surprising to see a similar pattern in studies of coral-associated bacteria, which presumably inhabit a productive environment, with similar bacterial ribotypes associated with the same coral species, but distinct from those in surrounding seawater and sediments (Frias-Lopez et al. 2002; Rohwer et al. 2002; Bourne and Munn 2005; Pantos and Bythell 2006; Littman et al. 2009b; Reis et al. 2009). This is supported by the observation that some bacterial ribotypes form host-species–specific with coral (Rohwer et al. 2001; Frias-Lopez et al. 2002; Rohwer et al. 2002; Bourne 2005; Klaus et al. 2005; Sekar et al. 2006; Lampert et al. 2008; Reis et al. 2009). It is hypothesized that this specificity is indicative of the importance of certain interactions to holobiont functioning, and that these interactions are structured in ways that maximize the health of the holobiont (Rohwer et al. 2002; Reshef et al. 2006).

While the existence of such coral–bacterial (and therefore microbial habitat) specificity is widely accepted, the spatial and temporal stability of these interactions is debated. In seawater, for example, bacterial assemblages can be heterogeneous within

the same habitat at spatial scales ranging from micrometers to kilometers (Long and Azam 2001; Hewson et al. 2006a, 2006b). In coral, some studies have shown that species-specific bacteria are geographically consistent (Rohwer et al. 2001, 2002). For instance, Rohwer et al. (2002) showed that bacteria associated with three coral species in Panama contained similar ribotypes to those of the same coral species in Bermuda. The opposite trend has also been observed, in which bacterial assemblages contained different ribotypes between geographic locations, but similar corals were inhabited by similar ribotypes (Klaus et al. 2005; Guppy and Bythell 2006; Littman et al. 2009b). Trends observed by sequence library surveys of uncultivated communities are generally consistent with those using fingerprinting approaches, which have lower taxonomic resolution, but provide greater qualitative assessment of large numbers of samples or assemblages. These discrepancies could be explained, in part, by differences in methods (clone sequencing versus terminal restriction fragment length polymorphism [T-RFLP] and denaturing gradient gel electrophoresis [DGGE]), coral taxonomic resolution (comparing coral species within the same genus versus different genera) and the operator-defined taxonomic resolution of sequence analyses (''cutoffs'' of sequence identity defining operational taxonomic units to permit comparisons between communities) (Rohwer et al. 2001, 2002; Klaus et al. 2005; Guppy and Bythell 2006; Littman et al. 2009b). Microbial taxonomic resolution influences similarity between assemblages based on sequencing; it is currently unclear which nucleotide identity cutoffs are appropriate for defining ecologically meaningful taxonomic levels. The varied trends over geographic scales and with host species may also reflect differential species responses (host and/or microbiota) to site-specific factors (Hong et al. 2009; Littman et al. 2009b). Taken together, differences between studies highlight the multifaceted and dynamic nature of coral-associated microbiota, and caution should be taken not to over-simplify or over-generalize the nature of these associations.

The onset of coral–bacterial associations

Determining when and how coral–microbial assemblages are established is fundamental to a better understanding of the coral holobiont. Apprill et al. (2009) examined the onset of microbial associations in the coral, *Pocillopora meandrina*, by comparing bacterial T-RFLP profiles between pre-spawned oocyte bundles, spawned eggs, and week old planulae. They found that there were distinct ribotypes present within each stage, but that bacterial cells were not internally incorporated until the planulae were fully developed (Apprill et al. 2009). This suggests that, unlike the zooxanthellae, which are vertically transmitted in this system, bacteria that form associations with *P. meandrina* are acquired via horizontal uptake. As bacteria are internally incorporated during late development of the planulae, it is possible that bacteria play a role in processes specific to this life stage, such as benthic settlement (Apprill et al. 2009).

There is also evidence that coral-associated bacteria differ between adults and juveniles of coral. Nonmetric multidimensional scaling (nMDS) representations of bacterial profiles assessed through random sequencing of clone libraries, DGGE, and T-RFLP, were all consistent in demonstrating that adult *Acropora tenuis* and *Acropora millepora* displayed tight grouping, whereas there was no apparent relationship between profiles of juveniles (Littman et al. 2009a). The bacterial complement of juvenile corals was also more diverse, and while there was some conservation in bacterial ribotypes between adult and juvenile corals, the vast majority of adult-associated bacterial ribotypes were not found in juveniles. This suggests a successional process whereby associates of adult corals gradually replace the diverse bacterial consortia of juveniles (Littman et al. 2009a). Future studies are required to examine this successional process throughout the ontogeny of the coral to determine when and how species-specificity is established and whether these factors differ among coral species.

The role of coral-associated bacteria

While the presence of coral-associated bacteria has long been established (Di Salvo and Gundersen 1971), little is known about how these microorganisms contribute to the functioning of the coral holobiont. There is increasing evidence that coral microbiota are crucial to at least two aspects of the host's physiology: biogeochemical cycling and pathogen resistance. The tight nutrient cycling that enables corals to thrive in oligotrophic waters was originally attributed to the mutualism between the coral host and its photosynthetic dinoflagellates. Recently, however, both culture-dependent and independent techniques have demonstrated that coral microbiota likely play a role in coral reef biogeochemistry (Williams et al. 1987; Szmant et al. 1990; Shashar et al. 1994; Ferrier-Pages et al. 2000; Lesser et al. 2007; Wegley et al. 2007; Chimetto et al. 2008;

Olson et al. 2009; Raina et al. 2009; Kimes et al. 2010). For example, nitrogen fixation within the coral holobiont has been documented using acetylene reduction assays (Williams et al. 1987; Shashar et al. 1994; Lesser et al. 2007; Chimetto et al. 2008) and bacteria possessing genes for nitrogen fixation have been identified within multiple coral species from varying geographic regions (Lesser et al. 2004; Wegley et al. 2007; Olson et al. 2009; Kimes et al. 2010). In addition, recent studies have found evidence that members of coral-associated microbiota may also be involved in additional nitrogen cycling processes, including nitrification, ammonium assimilation, ammonification, and denitrification (Wegley et al. 2007; Kimes et al. 2010). There is also evidence that coral-associated microbial assemblages function in carbon and sulfur cycling (Ferrier-Pages et al. 1998; Ferrier-Pages et al. 2000; Wegley et al. 2007; Raina et al. 2009; Kimes et al. 2010). Genes that regulate carbon fixation, carbon degradation, and methanogenesis have been detected in coral-associated bacteria (Wegley et al. 2007; Kimes et al. 2010), as have those that regulate assimilation of organic and inorganic sulfur sources (Wegley et al. 2007; Raina et al. 2009; Kimes et al. 2010). The ability of microbes to subsize the nutrient budgets of their coral host is likely a driver in the establishment of coral-associated microbial assemblages. Furthermore, niche partitioning of bacterial assemblages is likely to be controlled by availability of nutrients at the microscale of the coral host structure (van Duyl and Gast 2001; Scheffers et al. 2005; Raina et al. 2009; Ainsworth et al. 2010). However, it should be noted that the presence of a functional gene or gene fragment does not necessearily imply functionality, and that additional *in situ* or expression-based studies are required to elucidate the role that microbes play in driving nutrient cycling on coral reefs. The role of microbes in biogeochemical cycling and their distribution at the scale of the holobiont micro-niche are important areas of future research.

It has also been hypothesized that coral-associated bacteria play a role in resistance to disease (Ritchie and Smith 2004; Rohwer and Kelley 2004; Reshef et al. 2006) via competition for nutrients and/or space, and/or production of antibiotics (Rohwer and Kelley 2004). Several studies have demonstrated the antibacterial activity of isolates of coral mucus against indicator bacteria (e.g. *Escherichia coli*, *Staphylococcus aureus*), potentially invasive microbes (from Florida Keys canal water, African dust, and surrounding seawater) and putative pathogens of coral (*Vibrio shiloi*, *V. coralliilyticus*, and *Serratia marsecens*) (Ritchie 2006; Nissimov et al. 2009; Rypien et al. 2009; Shnit-Orland and Kushmaro 2009). It has also been shown that the antibacterial properties of coral mucus select for a discrete set of commensal bacteria (Ritchie 2006) and that antagonistic interactions are prevalent among co-occurring coral-associated microbes (Rypien et al. 2009). However, it should also be noted that coral mucus contains very high concentrations of organic and inorganic matter, leading to typically rare, r-selected (i.e. fast growing and nutrient sensitive) bacteria in seawater recruiting to the mucus matrix and increasing rapidly in abundance (Allers et al. 2008). These findings suggest that the coral-associated microbiota are dynamic and selfregulating and have the capacity to prevent settlement of exogenous bacteria, including pathogens. Future studies should focus on the factors that enable pathogens to become established, as well as the additional roles (e.g. competition and niche occupation) that symbiotic bacteria play in preventing colonization by pathogens.

Coral disease and coral-associated bacteria

Over the past several decades, coral reef ecosystems have been degrading at an alarming rate (Hughes et al. 2003; Baker et al. 2008). This degradation, in part, is a consequence of coral disease (Harvell et al. 1999; Harvell 2004), for which prevalence, severity, and host and geographic range have all been increasing (Harvell 2004; Weil et al. 2006; Harvell et al. 2007). Like their terrestrial counterparts, marine epizootics cause marked declines in populations, alter community structure, and therefore threaten biodiversity (Harvell et al. 2002). To date, there are more than 20 described coral diseases (Rosenberg et al. 2007). However, due to the difficulties of isolating and culturing putative pathogens and of aseptic cultivation of host tissues, there are only six diseases for which a causative agent has been identified (Rosenberg et al. 2007; Bourne et al. 2009). Knowledge of coral disease reservoirs, transmission, and pathogenesis is limited, as is the role that coral-associated microbial assemblages play in the health and disease of coral.

To gain a better understanding of how variation in microbial assemblages associated with corals may lead to the onset of disease, numerous studies have compared bacterial assemblages between healthy and diseased coral. These studies have shown that both the composition and function of microbiota associated with healthy and diseased corals are distinct (Ritchie and Smith 1995; Cooney et al. 2002;

Frias-Lopez et al. 2002; Pantos et al. 2003; Bourne 2005; Gil-Agudelo et al. 2006; Pantos and Bythell 2006; Sekar et al. 2006; Barneah et al. 2007; Gil-Agudelo et al. 2007; Voss et al. 2007; Sekar et al. 2008; Reis et al. 2009; Sunagawa et al. 2009). Furthermore, differences between the microbiota of healthy and diseased corals appear to be systemic in some cases (Pantos et al. 2003; Breitbart et al. 2005; Pantos and Bythell 2006). That is, the bacterial assemblage of the entire diseased colony is the same and distinct from that of healthy colonies, even though only a small portion of the colony shows signs of disease. These results reinforce the idea that apparently healthy tissues of diseased colonies should not be used as control references, and that this systemic effect could be used as a diagnostic tool to identify stressed colonies susceptible to disease (Pantos et al. 2003).

There are several hypotheses for the observed variability in the structure of bacterial assemblages associated with disease. (1) Changes in environmental conditions directly or indirectly alter the microbiota of healthy coral. For instance, increases in nutrients (e.g. nitrogen, dissolved organic carbon) may ''fertilize'' nutrient-limited, r-selected, potentially pathogenic taxa, enabling them to dominate the community (Bruno et al. 2003; Kline et al. 2006; Smith et al. 2006; Voss and Richardson 2006). Nutrient increases may also play a more indirect role by compromising normal function of beneficial coral residents, thereby leading to overgrowth of pathogenic taxa (Kline et al. 2006). (2) Changes in environmental conditions alter host physiology, subsequently leading to variable microbiota. For example, because coral mucus provides an important carbon source for coral-associated bacteria (Ferrier-Pages et al. 2000; Brown and Bythell 2005; Wegley et al. 2007; Allers et al. 2008; Kimes et al. 2010), changes in production rates of mucus due to abiotic factors (e.g. temperature and/or irradiance) (Piggot et al. 2009) could also lead to variable structure of coral microbiota. (3) Colonization by pathogens directly or indirectly causes variation in the normal bacterial assemblage. For example, pathogens may directly alter community structure by outcompeting resident bacteria if they have a higher affinity for available substrates and/or are capable of producing antibiotics (Rypien et al. 2009). Pathogens may also indirectly cause variability in coral microbiota via degradation of host tissues, creating a nutrient-rich microenvironment that is readily colonized from surrounding waters by secondary r-selected invaders (Cooney et al. 2002; Frias-Lopez et al. 2002; Pantos et al. 2003; Bourne 2005; Pantos and Bythell 2006; Reis et al. 2009). In the tail of the species-distribution curve of bacterioplankton communities, there are many such r-selected taxa that maintain low abundances until prevailing conditions arise. A classical example of rare yet r-selected taxa that are present in bacterioplankton are marine *Vibrio*, which are easily cultivated on enriched solid media from seawater (Giovannoni and Stingl 2005). Given that coral reef ecosystems are defined by complex multi-partner relationships and dynamic environmental conditions, it is likely that these hypotheses are not mutually exclusive and that a combination of factors ultimately leads to the onset of coral disease. It is equally likely that coral disease is elicited by networks of interacting bacteria, and that interactions with physicochemical features of their habitat are complex and not easily disentangled by methods currently used in coral microbial ecology.

Meta-analysis of coral-associated bacterial assemblages

To gain insight into the extent to which the states of coral health are correlated with the composition of their microbial assemblages, we analyzed 16S rRNA sequence accessions to GenBank produced in 32 studies of coral microbial ecology. A summary of the studies, the methods employed, and the number and source of 16S rRNA sequences can be found in the Supplementary Material (Table S1). Our analysis is not a quantitative assessment of the composition of microbial assemblages, since different studies used distinctly different approaches (targeted 16S sequencing of DGGE amplicons to fully random sequencing of entire assemblages), with different approaches used in different compartments of the reef (e.g. coral, seawater). Note that the comparison between different habitat types is biased by variable numbers of sequences and approaches, making standardization difficult at this level of analysis. It is also important to point out that these data represent an inherent bias based on research interest. For instance, easily identifiable diseases (e.g. black band) and dominant reef species (e.g. *Montastrea* spp.) are sampled more often than are their less distinguishable, less dominant counterparts. This analysis does not include sequences derived from pyrosequencing technologies recently applied to coral microbiology (e.g. Sunagawa et al. 2010), which will likely provide extensive information on the ''tail'' of the species distribution curve not sampled by Sanger-sequenced libraries.

Coral microbiota 16S rRNA sequences were dominated mostly by bacteria (~80–90%), with both

healthy and bleached corals harboring similar dominant taxa (Fig. 1). However, in diseased corals, there were generally more *Rhodobacter*, *Clostridia*, and *Cyanobacteria* sequences, and fewer *Oceanospirillum* sequences. Interestingly, the abundance of *Rhodobacter* sequences in diseased corals is not associated with a single disease or geographic locale. Rather, *Rhodobacter* seems to be abundant under many different conditions in many different locales, including assemblages associated with black band disease from the Caribbean, Red Sea, and Great Barrier Reef (Cooney et al. 2002; Frias-Lopez et al. 2002; Bourne 2005; Sekar et al. 2006; Barneah et al. 2007; Sato et al. 2009), white plague and white band disease from the Caribbean (Pantos et al. 2003; Pantos and Bythell 2006; Sunagawa et al. 2009), and two conditions, atramentous necrosis and cyanobacterial patches, from the Great Barrier Reef (Bourne 2005; Sato et al. 2009). Surveys of bleached coral had a higher proportion of the r-selected opportunist genera, *Vibrio* and *Acidobacteria*, than did surveys of healthy coral. It is unclear whether bacterial sequence types detected in high abundance on the surface of diseased or bleached tissues represent pathogens driving the diseased state, or are merely opportunists taking advantage of shifts in the bacterial assemblage or in host physiology that are associated with bleaching and disease.

Despite these observations, it is important to note that 16S rRNA is a poor predictor of habitat or lifestyle, where closely related taxa can occupy disparate environments and carry out different functions within communities. For example, in our analysis, some sequences of α-Proteobacteria associated with disease were also closely related to those found in disparate environments, like deep-sea sediments and open-ocean plankton (Fig. 2). While other ribotypes were distinct to diseased tissues (i.e. their close relatives were found only in association with disease), our analysis emphasizes that care must be taken in interpretation of 16S rRNA surveys for identifying potential pathogens amongst complex microbial assemblages in association with corals. While informative, comparative studies of coral-associated microbial assemblages are unable to answer a key question: are shifts in community

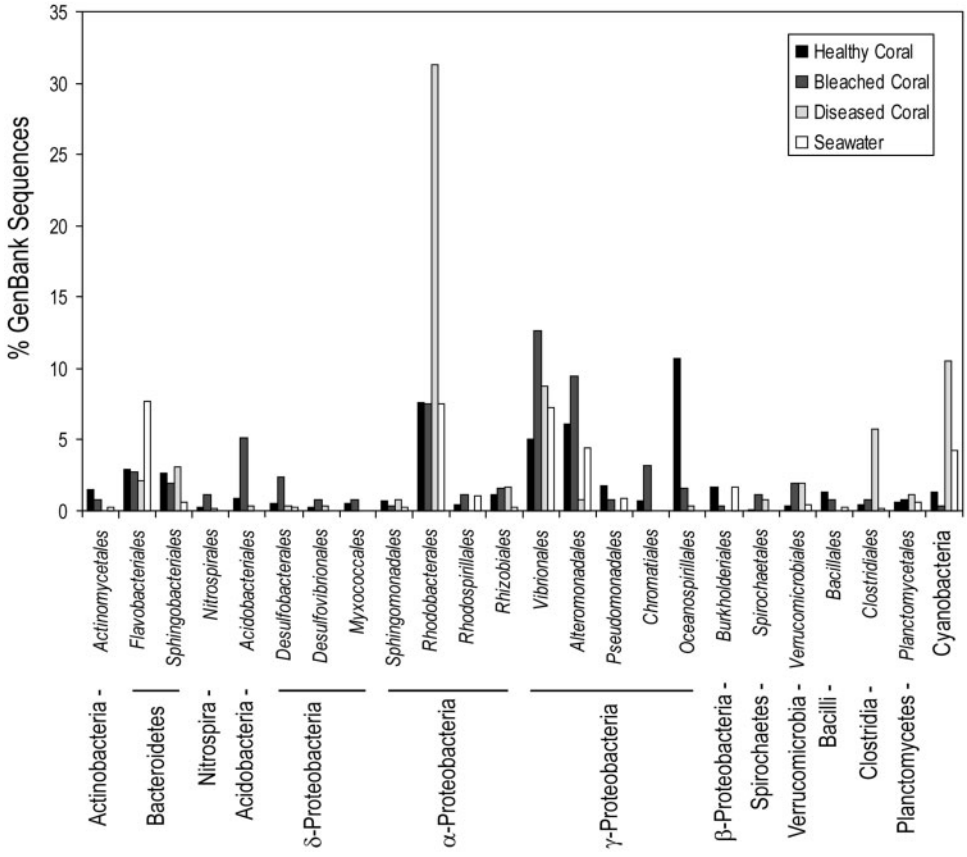

Fig. 1 Analysis of coral reef-derived 16S rRNA sequence accessions to GenBank associated with healthy ($n = 4271$ sequences), bleached ($n = 254$ sequences), and diseased ($n = 524$ sequences) coral and overlying seawater ($n = 662$ sequences). Sequence accessions were classified using the Bayesian classifier tool at the Ribosomal Database Project II. Unclassified sequences within each class are not included. Only orders representing >1% of all sequence accessions are shown.

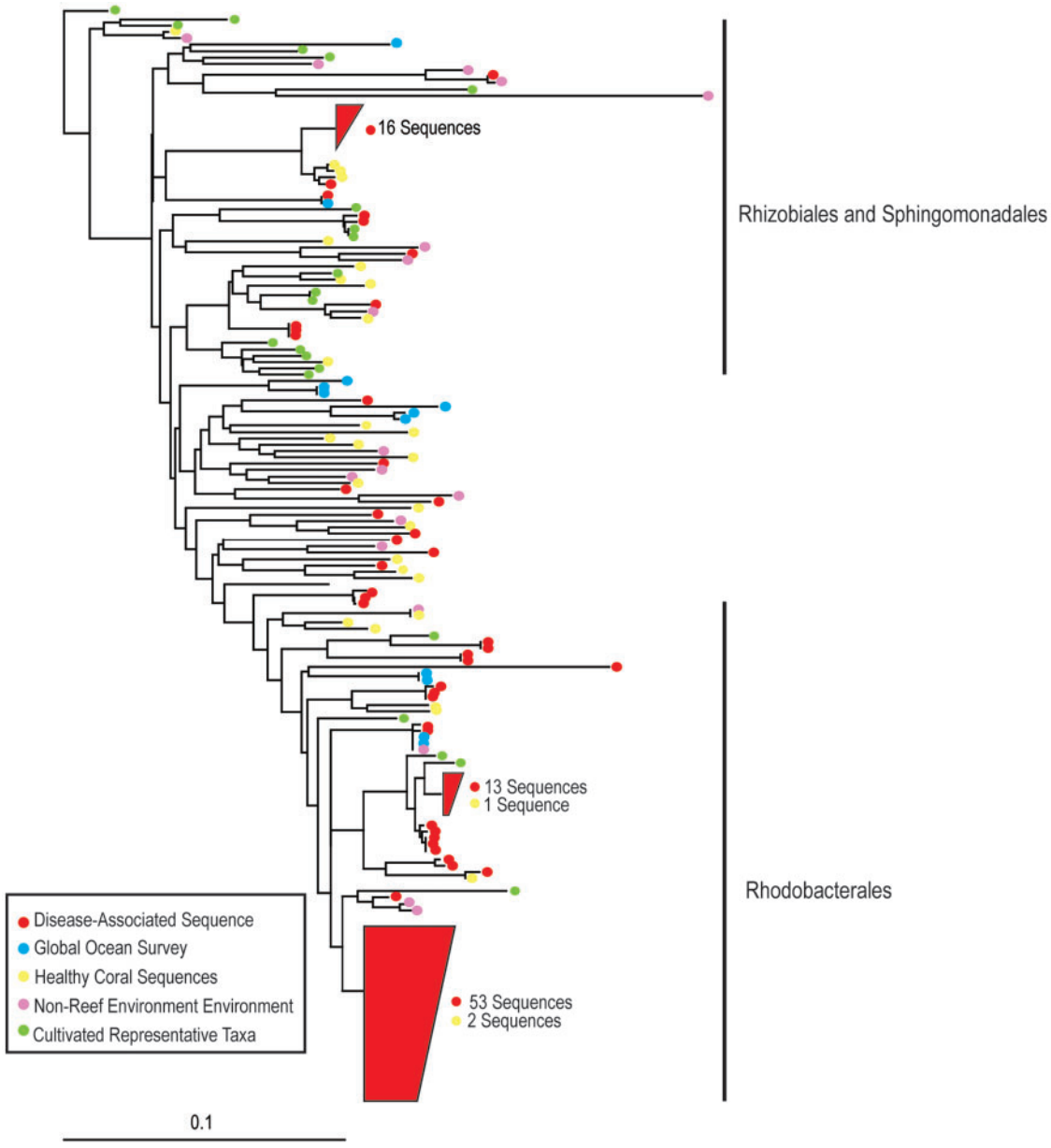

Fig. 2 Phylogenetic analysis of 93 disease-associated α-Proteobacteria and their closest matches from genome sequences, the Global Ocean Survey of bacterioplankton, and the non-redundant database at the National Center for Biotechnology Information. Branches have been collapsed where multiple sequences have been recovered. The tree was produced using neighbor-joining based on a 395 base pair alignment produced using the Ribosomal Database Project II. Scale bar = 0.1 substitutions per site. Non-reef sequences included, for example, those from deep-sea sediments, salt marsh sediments, and pelagic bacterioplankton.

structure the cause or the effect of the disease? Future comparative studies should focus on the temporal dynamics of bacterial replacement. In addition, the use of metagenomics, as opposed to 16S rRNA techniques, can provide concurrent information concerning both community function and structure, which could be useful in identifying potential pathogens through virulence genes and/or culturing conditions of putative pathogens based on physiological function (Wegley et al. 2007; Thurber et al. 2009; Ainsworth et al. 2010). Perhaps most importantly, however, these studies should follow up with active inoculations to determine the mechanisms underlying pathogen colonization and pathogenesis, and how the coral-associated microbial assemblage is altered via these mechanisms.

The potential impacts of climatic change on coral-associated bacteria

Climatic change is having measurable effects on marine and terrestrial ecosystems alike. In the

ocean, anthropogenically-driven increases in atmospheric concentrations of carbon dioxide contribute to both ocean warming and acidification (Harvell et al. 2007; Doney et al. 2009; Feely et al. 2009). Warming and acidification alone, and synergistically, have the potential to not only alter coral physiology directly (Hoegh-Guldberg et al. 2007; De'ath et al. 2009; Kleypas and Yates 2009), but also indirectly through impacts on coral-associated microorganisms, thereby potentially disrupting the normal function of the coral holobiont. This loss of function, in turn, may impact coral reef ecosystems as a whole.

The hypothesis developed to explain variability in coral–bacteria assemblages as a result of disease, namely that environmental factors can directly or indirectly affect the microbiota and/or host physiology, can also be used to predict the effects of ocean warming and acidification on the coral holobiont. While it is possible that increasing temperatures and decreasing pH of the sea surface will alter the biogeochemical role that coral microbiota potentially play, there is a paucity of research investigating this phenomenon. However, there is considerably more data concerning how climatic change, and more specifically, increasing temperatures, will affect the role that coral-associated microbiota play in disease.

Increases in seawater temperature can directly alter coral-associated bacterial structure and function, potentially leading to disease. Vega Thurber et al. (2009) demonstrated that elevated temperatures shifted the microbiome of *Porites compressa* to a more disease-associated state. That is, both the number of genes encoding virulence pathways and the abundance of ribosomal sequences associated with diseased organisms were greater in the microbial assemblage of corals exposed to elevated temperatures. Indeed, for a number of coral diseases, growth rates and/or virulence of pathogens are temperature-dependent (Alker et al. 2001; Ben-Haim et al. 2003; Cervino et al. 2004; Rosenberg and Falkovitz 2004; Remily and Richardson 2006; Ward et al. 2007). Therefore, increases in seawater temperature could potentially shift coral-associated microbial assemblages by selecting for more pathogenic taxa.

There is also evidence that increases in temperature can indirectly alter coral microbiota by compromising function of beneficial members that structure healthy communities. Several studies have demonstrated that antibacterial activity of mucus-associated bacteria is impaired under elevated temperatures (Ritchie 2006; Rypien et al. 2009; Shnit-Orland and Kushmaro 2009). For instance, Ritchie (2006) found that the antibacterial activity of apparently healthy *Acropora palmata* mucus was lost when corals were exposed to higher sea surface temperatures. Furthermore, culturable isolates from the mucus were dominated by *Vibrio*s, while this genus was far less abundant in mucus sampled prior to the thermal event. These results suggest that increased temperatures can shift coral-associated microbial assemblages away from species that regulate unaffected communities toward dominance by potential pathogens.

Temperature-driven changes in host physiology could also affect coral-associated microbiota. Perhaps one of the most striking changes in the physiology of the host is bleaching. Coral bleaching is the breakdown of the symbiotic relationship between corals and their intracellular algae, leading to the loss of the algae and/or its photosynthetic pigments. Bleaching can be caused by a variety of factors (e.g. heavy metals, sediment, pathogens) (Coles and Brown 2003), but is most commonly caused by increases in sea surface temperatures that disrupt algal photosynthesis (Hoegh-Guldberg 1999; Hughes et al. 2003). Not surprisingly, variability in the structure of bacterial assemblages also occurs during bleaching (Ritchie 2006; Bourne et al. 2008; Koren and Rosenberg 2008). It is hypothesized that bleaching leaves the coral host more susceptible to disease, presumably due to alterations in both its physiology and its coral-associated microbial assemblages. Several studies have documented a link between bleaching events and subsequent outbreaks of disease (Guzman and Guevara 1998; Harvell et al. 2001; Muller et al. 2008; Brandt and McManus 2009; Croquer and Weil 2009; McClanahan et al. 2009; Miller et al. 2009), further supporting this hypothesis.

To date, little work has been done to assess the role that ocean acidification will have on coral microbiota. The pH of the coral microenvironment is dynamic, changing in both space and in time. For instance, intracellular pH in the coral *Stylophora pistillata* ranges from 7.13 in the light to 7.41 in the dark (Venn et al. 2009), while the pH of the coral surface in *Favia* sp. varies from 7.3 in the dark to 8.5 in the light (Kuhl et al. 1995). Thus, bacteria that colonize the coral microhabitat must be able to withstand diurnal fluctuations in pH associated with algal photosynthesis.

Despite being exposed to a large range of pH, there is some evidence that increasing acidity leads to variability in coral-associated microbiota. Similar to increasing temperatures, Vega Thurber (2009) found that decreasing the pH of seawater to 7.4 shifted the microbiome of *P. compressa* to a more disease-associated state. The mechanisms driving

this shift are unknown, but like other environmental processes that drive changes in the structure of coral-associated bacterial assemblages, a complex interaction between direct and indirect effects on the coral holobiont is hypothesized. For instance, pH is an important factor regulating virulence pathways in other pathogens (Nakayama and Watanabe 1995; Li et al. 2007; Fuentes et al. 2009; Gong et al. 2009; Werbrouck et al. 2009), and while this has not been investigated in corals, it has been shown that some pathogens of corals have an optimal growth rate within the range of the pH occurring in the coral microhabitat (Remily and Richardson 2006; Rasoulouniriana et al. 2009). Furthermore, other than decreasing accretion rates, it is unknown how ocean acidification will alter the physiology and susceptibility to disease of the host. It is also possible that synergisms between increasing temperatures and decreasing pH could cause variation in coral–bacteria assemblages. For instance, Remily and Richardson (2006) found that increasing temperatures expanded the tolerance to pH of *Aurantimonas coralicida*, the causative agent of white plague II in the Caribbean. Therefore, the synergisms between the two environmental factors may enable niche expansion of potentially pathogenic bacteria.

Conclusions

Coral–microorganism interactions represent a useful model for the types of associations that are likely important for many marine invertebrates. Coral–bacteria assemblages have been relatively well studied because of the recognized role of bacteria in the biology of the coral holobiont, and the large climate-mediated stress coral reef ecosystems have suffered. From this emerging area we now know that (1) corals associate with a diverse array of bacteria, and some of these associations are species-specific; (2) bacteria can contribute both antibiotic resistance and some nutrient-cycling capabilities to their coral host; (3) there are differences in bacterial associations between healthy corals and those that are bleached and/or diseased; and (4) climate-driven temperature stress can alter coral–bacteria assemblages to become more characteristic of those found in diseased corals. However, huge gaps in knowledge remain regarding the function of coral–bacteria associations, the specificity of these associations, and the anticipated impact of climatic change. The potential exists for very small modifications in temperature or pH associated with climatic change to increase the variability of coral–bacterial populations and in turn affect the health, life history, and species composition of coral reefs.

Supplementary Data

Supplementary data are available at *ICB* online.

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