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Multi-Partner Interactions in Corals in the Face of Climate Change

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Abstract. Recent research has explored the possibility that increased sea-surface temperatures and decreasing pH (ocean acidification) contribute to the ongoing decline of coral reef ecosystems. Within corals, a diverse microbiome exerts significant influence on biogeochemical and ecological processes, including food webs, organismal life cycles, and chemical and nutrient cycling. Microbes on coral reefs play a critical role in regulating larval recruitment, bacterial colonization, and pathogen abundance under ambient conditions, ultimately governing the overall resilience of coral reef systems. As a result, microbial processes may be involved in reef ecosystem-level responses to climate change. Developments of new molecular technologies, in addition to multidisciplinary collaborative research on coral reefs, have led to the rapid advancement in our understanding of bacterially mediated reef responses to environmental change. Here we review new discoveries regarding (1) the onset of coral-bacterial associations; (2) the functional roles that bacteria play in healthy corals; and (3) how bacteria influence coral reef response to environmental change, leading to a model describing how reef microbiota direct ecosystem-level response to a changing global climate.

Introduction

The health of coral reefs is declining on a global scale and continues to be threatened by overfishing and habitat destruction. Anthropogenically induced global climate change has been identified as a significant threat to these sensitive ecosystems. As temperatures rise, bleaching and diseases are increasing, and excess atmospheric carbon dioxide is

greatly altering reef ecosystems by changing seawater chemistry through decreases in pH (Anthony *et al.*, 2011).

In a recent review, Bosch and McFall-Ngai (2011) highlight the significance of viewing animals as “metaorganisms”—multicellular organisms consisting of a macroscopic host and multiple microorganisms that interact synergistically to shape the ecology and evolution of the entire association. In this sense, the term metaorganism can be applied to a broad range of animal-microbe symbioses, ranging from humans to sponges (Bosch and McFall-Ngai, 2011). Coral research within this perspective has revolutionized the way that researchers study corals. In scleractinian (hard) corals, the term “holobiont” (Knowlton and Rohwer, 2003) was adapted to indicate that corals are dynamic, multi-domain assemblages consisting of an animal host, symbiotic dinoflagellates in the genus *Symbiodinium*, bacteria, archaea, fungi, and viruses (Rohwer *et al.*, 2001, 2002; Stat *et al.*, 2006; Wegley *et al.*, 2007; Thurber *et al.*, 2009). The term metaorganism is especially useful for describing corals and reflecting that corals’ response to environmental change is driven by physiological interactions among the various microorganisms associated with the tissue, skeleton, and mucous layer. Corals harbor *Symbiodinium*, which provides fixed carbon to the host *via* photosynthesis, serving as the trophic foundation for coral reef ecosystems. It has been proposed that corals have additionally evolved to exploit specific bacterial metabolic capabilities that, in turn, directly modulate the survival of the coral holobiont in the marine environment (Zilber-Rosenberg and Rosenberg, 2008). An extensive characterization of the diverse microorganisms in corals will guide our understanding of the ecology of corals and coral reef ecosystems in response to a changing global climate.

Coral microbiology is a rapidly growing area of study. Early culture-based studies of coral-associated bacteria provided a foundation from which genomics, metagenomics,

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Abbreviations: CCA, crustose coralline algae; QS, quorum sensing.

and transcriptomics approaches were established in corals, leading to exciting new advances in our current understanding of the diversity and dynamics of coral-associated bacterial communities. Evidence is accumulating that bacteria have an enormous influence on coral health and resilience, particularly with respect to changing reef environments (Azam and Worden, 2004; Rosenberg *et al.*, 2007; Bourne *et al.*, 2009; Ainsworth *et al.*, 2010; Garren and Azam, 2012). The field of marine microbial ecology underwent a revolution in the 1990s, when culture-independent molecular techniques revealed that bacterial diversity from culture-based assessments was largely underestimated (Azam, 1998). Studies of persistent associations between corals and bacteria, both beneficial and pathogenic, were enhanced by new methods and approaches from this revolution. Those techniques were adopted by coral microbiologists, resulting in the discovery that particular components of bacterial communities are specific to some coral host species (Rohwer *et al.*, 2002).

The cost and time associated with characterizing these complex bacterial assemblages initially posed a challenge to scientists attempting to identify patterns of diversity across a large scale. However, the gradually decreasing cost and increasing efficiency of high-throughput methods, including 454 pyrosequencing technology, allowed researchers to perform community 16S rRNA gene profiling and metagenome sequencing in a broad range of coral specimens. Recent applications of 16S pyrosequencing in corals have produced hundreds of thousands of 16S sequences—in contrast to hundreds of sequences from cloning methods. Results from pyrosequencing-based studies provide evidence of the presence of “coral-specific” groups of bacterial ribotypes (Reis *et al.*, 2009; Kvennefors *et al.*, 2010; Sunagawa *et al.*, 2010; Ceh *et al.*, 2011). Experiments investigating the bacterial component of coral surface mucous layers suggest that the composition of bacterial communities in coral mucus is distinct from other surface-associated biofilms and is influenced by the physical and biochemical properties of the mucus (Barott *et al.*, 2011; Sweet *et al.*, 2011b). Although corals maintain specific groups of bacteria, variation among individuals of a coral species may occur according to location (Guppy and Bythell, 2006; Littman *et al.*, 2009; Kvennefors *et al.*, 2010; Ceh *et al.*, 2011).

Bacterial communities are maintained in microhabitats within an individual coral host, spatially structured within chemical micro-niches, or compartments, in the skeleton, tissues, and surface mucous layer of corals (Rohwer *et al.*, 2001, 2002; Daniels *et al.*, 2011; Sweet *et al.*, 2011a). This spatial microheterogeneity is similar to previously described trends in the speciation of the dinoflagellate *Symbiodinium* in branching acroporid corals (Rowan and Knowlton, 1995). With that in mind, new collection techniques and apparatuses have recently been developed to enable collection from specific compartments of the coral, with minimized

contamination by bacteria from other compartments (Sweet *et al.*, 2011a).

Recent research surveying bacterial communities in a large number of marine sponges suggests that bacteria detected in sponges can be classified in three categories (Schmitt *et al.*, 2011): *core* (groups of bacteria that are shared across many sponges), *species-specific* (groups of bacteria that are specific to certain sponge hosts), and *variable* (groups of bacteria that are transiently associated with the host, probably due to passive attachment from seawater). The recent composition analyses of bacterial assemblages in corals indicate that a similar classification scheme can be applied to coral-associated bacteria. An interesting difference between corals and sponges is that while many sponges have been documented to transmit diverse, specific bacterial communities in their gametes or larvae (Schmitt *et al.*, 2007; Sharp *et al.*, 2007), most corals appear to acquire specific bacteria from the seawater each generation (Sharp *et al.*, 2010; Apprill *et al.*, 2009). The mechanisms by which corals selectively and specifically recruit their core and specific bacterial components are largely undescribed, but they likely involve the physical properties and the chemical structure of the mucous layer, which is thought to be unique in specific coral species (Bythell and Wild, 2011). Bacteria that successfully colonize the mucus are, in turn, involved in cycling nutrients and organic compounds in corals and on the reefs, and the resident microbes have the potential to modulate the bacterial community structure in coral mucus and tissue.

Here we review recent advances in the study of the coral metaorganism and specifically address (1) the onset of coral-bacterial associations; (2) the functional roles that bacteria play in healthy corals; and (3) how bacteria influence coral reef response to environmental change. These new discoveries are the basis for a model of how coral-associated and reef-inhabiting microbiota influence ecosystem-level responses to global climate change.

Onset of Coral-Bacterial Associations

The Caribbean coral *Porites astreoides* has been shown to transmit a bacterial component to its offspring (Sharp *et al.*, 2012). However, this seems to be an exception to the rule in scleractinian corals. In eight other coral species that have been examined (Apprill *et al.*, 2009; Sharp *et al.*, 2010), corals do not appear to inherit bacteria from parents; rather, bacterial colonization occurs in planula larvae or post-settlement stages. Many bacterial phylotypes detected in planulae and post-settlement stages of *P. astreoides* have also been documented in the adult (Wegley *et al.*, 2007), suggesting that corals acquire specific bacterial phylotypes.

Exploration of bacterial communities in early life stages of corals has not only provided new information about bacterial infection in corals, but it has also simplified anal-

ysis of diversity and dynamics of bacterial communities in corals across spatiotemporal scales. In contrast to their adult counterparts, swimming planula larvae of most corals have not yet accumulated a high bacterial load from the surrounding environment or by feeding (Apprill *et al.*, 2009; Sharp *et al.*, 2010); as a result, it is more tractable to characterize and quantify the associated bacterial component in these larvae. Similar phylogenetic clades of bacteria were detected in 16S rRNA gene sequence clone libraries from multiple larval specimens of the Caribbean coral *Porites astreoides* (Sharp *et al.*, 2012) and in the Pacific coral *Pocillopora meandrina* (Apprill *et al.*, 2009), suggesting that some groups of bacteria are common across different coral species. A number of bacterial types have been commonly detected in multiple species of corals, but of particular interest are those belonging to the phylum α -proteobacteria (Apprill *et al.*, 2009; Raina *et al.*, 2009; Sharp *et al.*, 2012). The α -proteobacteria (particularly the Roseobacteriales) are abundant in the oceans, often constituting a third of the bacterioplankton (Wagner-Dobler and Biebl, 2006). This same group of bacteria is also closely associated with phytoplankton, including the dinoflagellate coral endosymbiont *Symbiodinium* (Webster *et al.*, 2004). Many of these bacteria, now classified as *Ruegeria* spp., were originally designated *Silicibacter* spp. (Yi *et al.*, 2007). It is unknown whether these bacteria play a functional role in corals, but their consistent detection in early life stages of corals and in seawater during coral spawning may be an indication that they are significant to the health of larvae, or even to adult colonies (Apprill *et al.*, 2009; Apprill and Rappe, 2011; Sharp *et al.*, 2012).

New research focusing on the molecular basis of bacterial colonization of the coral tissues or surface mucous layer indicates that coral mucous biofilm communities are a result of selection processes driven by the coral holobiont rather than by incidental attachment by bacteria in the seawater (Sweet *et al.*, 2011b). This is consistent with recent findings from studies in the cnidarian *Hydra*, in which researchers found that the composition of the surface-associated bacterial community is driven directly by host metabolism and production of compounds in the surface layer of *Hydra* (Augustin *et al.*, 2010). It is likely that there are specific molecules that influence colonization in the coral mucous layer. Lectin-mediated uptake of *Symbiodinium* has been demonstrated in corals (Wood-Charlson *et al.*, 2006), but very little is known about bacterial uptake or invasion in corals.

Functional immunological molecules with bacterial binding capacity have been found in corals, describing a means by which the host may control associated microbial composition (Kvennefors *et al.*, 2008; Kvennefors and Roff, 2009). Molecules that control the activities of other coral-associated microbes are thought to be derived from the coral host and in some cases from the associated bacteria (Ritchie,

2006; Teplitski and Ritchie, 2009; Vidal-Dupiol *et al.*, 2011a, b). As previously described in a broad range of other animal-microbe systems (McFall-Ngai *et al.*, 2012), molecules that direct bacterial infection of animal tissue-associated bacteria may be conserved, regardless of whether the bacteria are beneficial, commensal, or pathogenic.

Role of Bacteria in Health of Coral and Coral Reefs

Recent coral microbiology research has described how bacterial communities contribute to the overall physiology and ecology of apparently healthy corals. These discoveries were made possible both by new molecular technologies and by novel fieldwork-based approaches. Bacteria within corals govern the biogeochemical cycling within coral tissues. In addition, bacteria on surfaces in the reef environment influence and facilitate settlement of coral larval, and resident microbes in corals play a role in defining the composition of the bacterial community in corals.

Studies over the past several years indicate that coral-associated bacteria influence biogeochemical cycling within corals and on reefs. Metagenomic data from the bacterial fraction of DNA from the coral *Porites astreoides* indicate the presence of numerous genes capable of degrading diverse aromatic compounds (Wegley *et al.*, 2007). Coral-associated bacteria have been shown to be involved in cycling mucous-derived particulate and dissolved organic compounds in the reef environment (Wild *et al.*, 2004, 2009; Huettel *et al.*, 2006). In addition, the bacterial metagenome of *P. astreoides* consists of genes encoding enzymes involved in cycling nitrogen *via* nitrogen fixation, ammonification, nitrification, and denitrification (Wegley *et al.*, 2007). The detection of bacterial nitrogen fixation genes is consistent with previous biochemical research in which cyanobacterial nitrogen fixation was detected (Lesser *et al.*, 2007). Further research focusing on *nifH* gene diversity in two species of *Montipora* (Olson *et al.*, 2009) suggests that nitrogen-fixing bacteria in corals are not limited to cyanobacteria but also belong to taxa representing the α -, β -, γ -, and δ -proteobacterial classes (Olson *et al.*, 2009). Bacteria have been shown to be significant players in transforming nitrogen (Fiore *et al.*, 2010) as well as sulfur and carbon compounds (Ferrier-Pages *et al.*, 2001; Raina *et al.*, 2009; Kimes *et al.*, 2010) in corals and on coral reefs.

Bacteria outside of the coral animal also exert influence on the behavior of corals during their early life stages. Particular species of crustose coralline algae (CCAs) have been shown to facilitate larval settlement of the threatened coral species *Acropora cervicornis* and *A. palmata* in the Florida Keys and the Caribbean (Ritson-Williams *et al.*, 2010). The integration of microbiological and chemical ecology approaches suggests that the facilitation of larval settlement by CCAs may be regulated by bacteria growing in biofilms on the surface of CCAs (Negri *et al.*, 2001;

Webster *et al.*, 2004; Tebben *et al.*, 2011). To date, all of the CCA-associated bacteria implicated in inducing coral metamorphosis and settlement belong to the γ -proteobacteria. A strain of the γ -proteobacterium *Pseudoalteromonas* sp. isolated from the surface of the CCA species *Hydrolithon onkodes* induces significant levels of larval metamorphosis in the corals *Acropora willisae* and *A. millepora* in laboratory experiments (Negri *et al.*, 2001). Researchers have recently shown that exposure to *Pseudoalteromonas* isolates cultured from *Negoniolithon fosliei* and *Hydrolithon onkodes* significantly increases rates of metamorphosis on the Pacific coral *Acropora millepora* (Tebben *et al.*, 2011). Bioassay-guided isolation identified the inductive molecule as tetrabromopyrrole (Tebben *et al.*, 2011). Other strains of *Pseudoalteromonas* and *Thalassomonas* have also been shown to induce larval settlement and metamorphosis in the coral *Pocillopora damicornis* (Tran and Hadfield, 2011). Not all tested isolates of *Pseudoalteromonas* and *Thalassomonas* were inductive in that study, indicating that the ability to induce settlement is taxon-specific. In addition, the isolation source of the bacteria (algal surface *vs.* coral surface) was not linked to the strains' inductive properties (Tran and Hadfield, 2011). Together, these studies indicate that coral recruitment and successful larval attachment and metamorphosis (which is crucial for continued repopulation of coral reef ecosystems) is strongly governed by the activity of specific bacteria in reef environments.

Recent research has focused on the role of bacteria native to the coral surface mucous layer that control bacterial colonization within the mucus, ultimately regulating resistance to disease. Corals have been shown to protect themselves against pathogen infection *via* the presence of allelopathic properties in the mucus (Geffen and Rosenberg, 2005; Ritchie, 2006) or the coral tissue (Koh, 1997; Kelman *et al.*, 2006; Gochfeld and Aeby, 2008). However, antimicrobial assays with numerous Red Sea corals reveal that the capabilities of coral species for antibiotic production are highly variable (Kelman *et al.*, 2006). Bacteria isolated from corals are able to inhibit the colonization and growth of many other types of bacteria, including potentially invasive coral pathogens (Reshef *et al.*, 2006; Ritchie, 2006; Wegley *et al.*, 2007; Gochfeld and Aeby, 2008; Nissimov *et al.*, 2009; Shnit-Orland and Kushmaro, 2009; Sharon and Rosenberg, 2010; Kvennefors *et al.*, 2012). In addition, the presence of a high number of genes involved in antibacterial compound biosynthesis have been detected in metagenomes from multiple corals (Wegley *et al.*, 2007; Thurber *et al.*, 2009). It is not clear to what extent these bacteria and the metabolites they produce play a role in community structure. *In situ* antibiotic production by bacteria is known to be a means of securing a niche by controlling microbial populations competing for the same resources (Nielsen *et al.*, 2000; Rao *et al.*, 2005). It is therefore likely that bacteria in

and on the coral host govern the dynamics of coral microbiota.

Although the mechanisms by which mucous-associated bacteria prevent pathogenic infection are still unknown, the data indicate that a sophisticated system of bacterial cell-cell chemical signaling known as quorum sensing (QS) may be involved in microbial pathogenesis in corals. QS is modulated by small diffusible compounds called autoinducers, which are molecules that, when accumulated to a threshold concentration within a diffusion-limited environment, result in synchronized group behaviors. This density-dependent regulation allows bacterial populations to act in unison, effectively magnifying their ecological impact. Though the cell-cell communication systems differ among bacterial species, QS has been demonstrated to regulate many bacterial behaviors, including biofilm formation, antibiotic production, bioluminescence, and pathogenesis (Ng and Bassler, 2009), and it commonly drives important interactions between bacterial communities and their hosts (Rasmussen and Givskov, 2006; Dobretsov *et al.*, 2009).

Quorum sensing in bacterial pathogens is the mechanism by which virulence genes are expressed relative to pathogen density in the host, thereby initiating a coordinated attack once bacterial cell numbers reach a critical mass (Dobretsov *et al.*, 2009). Both eukaryotes and prokaryotes have evolved to recognize and counter QS in pathogens, and there is evidence that eukaryotic signal-mimics can stimulate QS responses in bacteria (Teplitski *et al.*, 2011). Other bacteria can counter-attack by producing quorum-quenching acylases or lactonases that break down signaling molecules (Teplitski *et al.*, 2011). In addition to the signal-degrading enzymes, eukaryotes can inhibit or activate bacterial QS by producing compounds that mimic QS signals. For example, Rajamani *et al.* (2008) demonstrated that lumichrome, a derivative of the vitamin riboflavin that is produced by the unicellular alga *Chlamydomonas reinhardtii* (as well as other prokaryotes and eukaryotes) can interact with the bacterial receptor for QS signals and elicit QS responses.

Quorum sensing may inhibit or activate pathogenesis, antibiotic production, exoenzyme production, and attachment by beneficial bacteria within coral tissues and on surfaces. Coral extracts contain compounds capable of interfering with QS activities (Skindersoe *et al.*, 2008; Alagely *et al.*, 2011) that may be involved in regulating the colonization of coral mucus by pathogens, commensal bacteria, or beneficial bacteria. The source of this activity is difficult to pinpoint and could originate from the coral, the dominant endosymbiont, or any associated bacteria. Alagely *et al.* (2011) recently showed that both coral- and *Symbiodinium*-associated bacteria alter swarming and biofilm formation in the coral pathogen *Serratia marcescens*. These phenotypes are typically controlled by QS, although inhibition of QS by these isolates remains to be demonstrated. There are few studies on the *in situ* roles of QS in corals, but

this process is likely to be used in both pathogenesis and mutualistic interactions (Krediet *et al.*, 2009a, b; Teplitski and Ritchie, 2009; Tait *et al.*, 2010). While it is clear that at least some coral-associated commensals and pathogens produce QS signals under laboratory conditions (Tait *et al.*, 2010; Alagely *et al.*, 2011), it is not clear whether these signals accumulate to threshold concentrations in natural environments.

It is feasible that *Symbiodinium* spp. also produce signaling molecules that control bacterial cell-cell communication, which would influence the specific complement of bacteria that associate with corals. Perhaps bacterial species-specificity in corals is, in part, driven by *Symbiodinium* within the coral, but this has yet to be tested. The potential for *Symbiodinium* to be a source of antibacterial compounds in corals represents an aspect of bioactive compound production that is not yet described. It is likely that the source of antibacterial activity in corals is a combination of allelopathic chemicals produced by the coral, by associated bacteria, or by endosymbiotic dinoflagellates. In a study conducted by Marquis *et al.* (2005), eggs from 11 coral species were tested for antibacterial activity, and the only species exhibiting antibiotic activity was the one coral species in the study that incorporates *Symbiodinium* into the egg before the egg is released, suggesting a potential allelopathic contribution of *Symbiodinium*. It is also possible that coral-associated bioactive compounds are derived from bacteria whose presence or activity is influenced by *Symbiodinium*, but this has yet to be tested.

Role of Bacteria in Reef Ecosystem Responses to Environmental Change

The latest research on how coral-associated bacterial communities mediate responses of corals and coral reef ecosystems to environmental change addresses shifts in both the phylogenetic structure and metabolic capabilities of bacterial assemblages in corals. Multiple approaches and tools from microbiology, molecular biology, microscopy, and chemical ecology have been used to identify the role of bacterial communities in response to threats such as increased sea-surface temperature, increased organic carbon and nutrient levels in seawater, increased macroalgal and cyanobacterial cover on reefs, and decreased seawater pH.

Rising sea-surface temperatures are linked to increases in coral diseases worldwide. However, the study of microbial coral diseases has been challenging due to many factors including microbial dynamics in the marine environment, the complications of proving unequivocal disease causation, and insufficient diagnostic tools (Pollock *et al.*, 2011; Weil and Rogers, 2011). Some bacteria identified as coral pathogens include *Serratia marcescens* (Sutherland *et al.*, 2011), *Aurantimonas corallicida* (Denner *et al.*, 2003), and a consortium of bacterial and cyanobacteria phylotypes that make

up what is known as Black Band Disease (Sekar *et al.*, 2008). The most common bacteria present and problematic for corals are members of the Vibrionaceae that have been implicated in coral bleaching (Kushmaro *et al.*, 1997; Ben-Haim and Rosenberg, 2002) and a myriad of coral diseases (Patterson *et al.*, 2002; Frias-Lopez *et al.*, 2003, 2004; Kline *et al.*, 2006; Cervino *et al.*, 2008). The Vibrionaceae are a common but diverse group of heterotrophic marine bacteria, collectively referred to as vibrios. Vibrios have been shown to be present in higher abundance on coral surfaces before obvious signs of distress (Ritchie, 2006; Mao-Jones *et al.*, 2010). This group includes human pathogens and benign planktonic and animal-associated marine bacteria. Bleaching of the scleractinian coral *Oculina patagonica* in the eastern Mediterranean Sea was shown to be caused by *Vibrio shiloi* (Kushmaro *et al.*, 1997). *Vibrio coralliilyticus* was isolated from bleached corals of the genus *Pocillopora damicornis* and shown to cause coral bleaching and tissue sloughing (Ben-Haim and Rosenberg, 2002). In these pathogens, toxin production and the ability to infect coral tissue have a strong temperature dependence (Kushmaro *et al.*, 1997; Ben-Haim and Rosenberg, 2002). *Vibrio* dynamics are affected by water temperature and salinity, yet little else is known about environmental drivers of their abundance and distribution in the marine environment (Johnson *et al.*, 2010). These organisms are often cultured rapidly and are able to utilize a wide range of carbon sources, suggesting that the biogeochemical significance of vibrios may vary with the nutrient state of the environment (Thompson *et al.*, 2004). Some reef organisms are thought to be vectors for coral disease agents, specifically vibrios. These include organisms that come into contact with, or feed on, corals such as fireworms, snails, and corallivorous fishes (Weil and Rogers, 2011). Several recent reviews offer a comprehensive summary of the occurrence and possible environmental determinants of coral diseases (Rosenberg *et al.*, 2009; Pollock *et al.*, 2011; Weil and Rogers, 2011). Research on processes governing pathogen dynamics, abundance, and pathogenesis has informed us on coral defense mechanisms.

The coral surface mucous layer and its resident microbes appear to be significant in defending corals from microbial diseases. Mucus harvested from the coral *Acropora palmata* during a period of increased seawater temperatures does not exhibit significant antibiotic activity compared to mucus sampled at lower temperatures (Ritchie, 2006). This suggests that the protective capacity of some corals may be lost when temperatures increase, providing a mechanism to explain how increased temperatures lower coral resistance and increase susceptibility to diseases. In addition, when temperatures increase, the dominant bacterial flora in coral mucus shifts from antibiotic-producing bacteria to pathogens (Ritchie, 2006). This finding indicates that a balance of potentially beneficial microbes may be important for the overall physiological health of reef corals. Rising sea-sur-

face temperatures can cause a breakdown of coral-*Symbiodinium* symbiosis. In addition, shifting seawater temperatures can simultaneously affect interactions among other microbes, particularly bacteria present in or on the coral, rendering the host susceptible to opportunistic or secondary infection by certain bacteria (Ritchie, 2006; Lesser *et al.*, 2007). Research on the Pacific coral *Acropora millepora* indicates that after bleaching (the loss of *Symbiodinium*) there is a dramatic shift to a *Vibrio*-dominated community (Bourne *et al.*, 2007), but it is unclear whether the bacterial communities are responding to the absence of the *Symbiodinium*, to physiological changes in the coral host, or to the increased light and sea-surface temperature. Following bleaching-induced coral mortality, nitrogen-fixing bacteria increase in abundance on coral skeletons (Holmes and Johnstone, 2010). The resulting increase in available nitrogen in the seawater has the potential to affect the growth of macroalgae and other nitrogen-limited primary producers, including benthic cyanobacteria (Holmes and Johnstone, 2010). Taken together, these results demonstrate that temperature stress and coral bleaching have the potential to alter the composition and metabolism of coral-associated bacterial assemblages, with significant impacts on the health of corals and coral reef communities.

As a result of heightened fishing pressure, decline in herbivore populations, and increased nutrient levels, reefs are undergoing a “phase shift” from coral-dominated ecosystems to algal-dominated ecosystems (Pandolfi *et al.*, 2003). Overgrowth by turf macroalgae and benthic cyanobacteria has been documented on adult coral colonies on reefs (Ritson-Williams *et al.*, 2005). Concern is growing for how this shift in ecosystems affects bacterial communities within coral reefs (Dinsdale *et al.*, 2008). Recent research demonstrates that allelochemicals from macroalgae and benthic cyanobacteria have the potential to mediate shifts in abundance and community composition of microbiota associated with adult corals (Morrow *et al.*, 2011). When tested against a library of strains isolated from algal surfaces, from mucus of the Caribbean corals *Montastraea faveolata* and *Porites astreoides* in direct contact with algal surfaces, and from the mucus without direct contact of algae, chemical extracts from six species of macroalgae and two species of benthic cyanobacteria stimulated the growth of some strains but inhibited the growth of other strains (Morrow *et al.*, 2011). While some of the algal extracts had broad-spectrum activity against the collection of test isolates from phylogenetically diverse environmental bacteria, other extracts specifically increased the growth rates of the bacterial genus *Vibrio* (Morrow *et al.*, 2011). Many of the active compounds in the study were hydrophilic, indicating that the bioactive compounds from algae or cyanobacteria may be readily solubilized and transported throughout seawater, providing a potential mechanism for algae to regulate microbial activity without direct contact, especially in low-

flow benthic systems (Morrow *et al.*, 2011). Allelopathic interactions among algae and corals have been shown to have detrimental effects on coral larval behavior, recruitment, and survival (Kuffner and Paul, 2004; Kuffner *et al.*, 2006; Ritson-Williams *et al.*, 2009). It is unknown how the bioactive compounds influence health of the early life stages, but it is feasible that the observed effects are linked to shifting bacterial communities associated with the coral planulae and recruits.

Smith *et al.* (2006) explored the effects of macroalgae on bacterial growth in the coral surface mucopolysaccharide layer. The results of that research, together with prior work on controlled exposure of coral fragments to seawater with increased dissolved organic carbon (DOC) levels (Kline *et al.*, 2006), suggest that an excess of DOC, exuded from macroalgae, leads to coral mortality (Smith *et al.*, 2006). In addition, Barott *et al.* (2011) found that the community composition of bacteria on surfaces of multiple reef macroalgal species is distinct from those found on coral surface mucous layers.

On the basis of these studies, it is clear that macroalgae have the potential to act as reservoirs of specific bacteria (beneficial, commensal, or pathogenic) not usually native to the coral mucous layer. Macroalgae also release compounds into the surrounding seawater that can have direct inhibitory or stimulatory effects on the coral-associated microbiota and, hence, on the health of the coral host.

Ocean acidification is a major concern for marine ecosystems in general—particularly those dependent on calcifying organisms, as secretion of calcium carbonate skeletons depends directly on carbonate saturation state in seawater (Caldeira *et al.*, 2007). Recent research suggests that a decrease in seawater pH can alter marine bacterial communities, but very little is known about the large-scale impacts of those changes (Joint *et al.*, 2011). Laboratory manipulations of seawater pH have shown that acidification can result in loss of *Symbiodinium* endosymbionts, decrease in calcification, depression of overall net productivity in corals (Anthony *et al.*, 2008), and dissolution or slowed deposition of coral skeletons (Fine and Tchernov, 2007). In addition, decreased seawater pH levels have been attributed to a decline in overall abundance of crustose coralline algae (Kuffner *et al.*, 2008), some of which have been shown to facilitate coral recruitment in reefs (Ritson-Williams *et al.*, 2010). Experiments demonstrate that lower P_{CO_2} levels in seawater result in significant detrimental effects on early life stages of the coral *Porites astreoides*, including fertilization success, larval settlement rates, post-settlement growth, and post-settlement skeleton deposition (Albright *et al.*, 2008, 2010).

Several laboratory-based studies have focused specifically on the impacts of ocean acidification on coral microbiota. Meron *et al.* (2011) explored shifts in microbial assemblages associated with the coral *Acropora eurystoma*

exposed to ambient seawater and seawater with pH 7.3 over a period of 2 mon using denaturing gradient gel electrophoresis profiles and 16S rRNA gene clone libraries. According to the resulting cluster analysis, a decrease in pH results in an increase in detection of Rhodobacteraceae and a decrease in detection of Bacteroidetes and Deltaproteobacteria (Meron *et al.*, 2011). Relative to libraries from corals exposed to ambient seawater, clone libraries from *A. eurytoma* exposed to pH 7.3 conditions exhibited a higher percentage of clones representing bacteria closely related to those detected in stressed, injured, or diseased invertebrates (Meron *et al.*, 2011). In another study with the Pacific coral *Porites compressa*, individuals exposed to an extremely low pH (6.7) exhibited shifts in bacterial community diversity (Thurber *et al.*, 2009). Though the mechanism by which this occurs is not yet clear, it has been suggested that the altered seawater pH indirectly causes a shift in the bacterial diversity by impacting host metabolism, which results in a shift of nutrients and carbon available to the associated microbiota (Meron *et al.*, 2011).

Metagenomic analysis of *P. compressa* mucus revealed potential functional shifts in the associated microbiota as a result of decreased pH and increased temperature (Thurber *et al.*, 2009), most notably an increase in the number of detected genes for antibiotic and toxin production. Mucus from corals exposed to a decreased pH exhibits low antimicrobial activity (Meron *et al.*, 2011), and mucus of *Acropora palmata* exhibits lower antibacterial activity after prolonged warm periods (Ritchie, 2006). Together, these results warn that even slight changes in seawater pH and temperature can have ecologically significant effects on coral-associated microbiota and, hence, on coral's susceptibility to bacterial pathogens. The shift in the coral microbiome phylogenetic profile has been proposed as a potential indicator for declining coral health before the corals exhibit more obvious signs of stress or disease (Thurber *et al.*, 2009; Ainsworth *et al.*, 2010; Garren and Azam, 2012).

A Model for Climate-Change-Induced Shifts in the Coral Metaorganism

The research reviewed here suggests that alterations in sea surface temperature, algal and cyanobacterial abundance on reefs, and seawater pH can have detrimental effects on corals by decreasing protective qualities of the coral mucous layer, *via* inhibition of growth or compound production in beneficial bacteria or by alteration of host-associated compound biosynthesis. Another aspect of coral-bacterial interactions that has garnered much attention is the ability of bacteria on reef substrates to influence successful larval recruitment. These surfaces include crustose coralline algae (CCAs), which are coated with microbial biofilms and are thought to be involved in mediating coral larval settle-

ment (Webster *et al.*, 2001; Ritson-Williams *et al.*, 2009, 2010; Tebben *et al.*, 2011; Webster *et al.*, 2011).

Figure 1 represents the current model of corals and their interdependence on associated microbes. Both coral tissue and coral mucus contain abundant and diverse microbial communities (Fig. 1a). When sea-surface temperatures increase, antibacterial compounds in the coral mucus disappear. Simultaneously, antibacterial-producing bacteria normally associated with healthy corals decrease while bacteria with pathogenic capabilities increase (Fig. 1b). Mathematical modeling of this system suggests that once this shift to pathogen dominance is established, this state persists long after conditions return to those favorable for the reestablishment of beneficial microbes (Mao-Jones *et al.*, 2010). Recent data from coral mucus bacterial metagenomes exposed to decreased pH (Thurber *et al.*, 2009; Meron *et al.*, 2011) indicate that ocean acidification may also result in a similar shift in the protective properties of coral mucus.

On the basis of this model and the data reviewed in this paper, we present a second model of coral-bacterial interactions in which environmental changes lead to shifts in bacterial communities on reef surfaces (Fig. 1c and d). It has been shown that increased temperatures change the phylogenetic composition of CCA-associated bacterial communities and the success of larval recruitment (Webster *et al.*, 2011). In addition, it was recently shown that decreased pH inhibits settlement of the coral *Porites astreoides* (Albright *et al.*, 2008, 2010). Temperature may affect the growth, abundance, or bioactive metabolite biosynthesis of beneficial bacteria, particularly *Pseudoalteromonas* spp., on reef surfaces that are important for successful recruitment, which can ultimately result in a decline of new recruitment on reefs. Though the effects of decreased pH on surface biofilms have not been well described, this condition may alter the bacterial biofilm community and influence larval settlement success. Figure 1c and d shows a schematic model of reef surface-associated microbes before (c) and after (d) increased sea-surface temperature or ocean acidification. In ambient conditions on the reef, CCAs, or bacteria growing on CCA surfaces, produce compounds that facilitate larval settlement (Fig. 1c). When sea-surface temperatures increase, bacterial communities on CCAs change, resulting in lower larval recruitment rates (Fig. 1d). Similarly, as pH decreases, larval settlement decreases (Albright *et al.*, 2008, 2010). It is hypothesized that the inductive properties of CCAs, whether they are due to compounds released by bacterial biofilms on CCAs or by the CCAs themselves, decrease (Fig. 1d). As in the coral mucus (Fig. 1a and b), there is a shift in the bacterial community of the reef surfaces. In this case, under increased sea-surface temperatures, the bacterial community dominated by inductive bacteria, such as *Pseudoalteromonas* and *Thalassomonas*, moves to a community dominated by bacteria that may not have inductive properties.

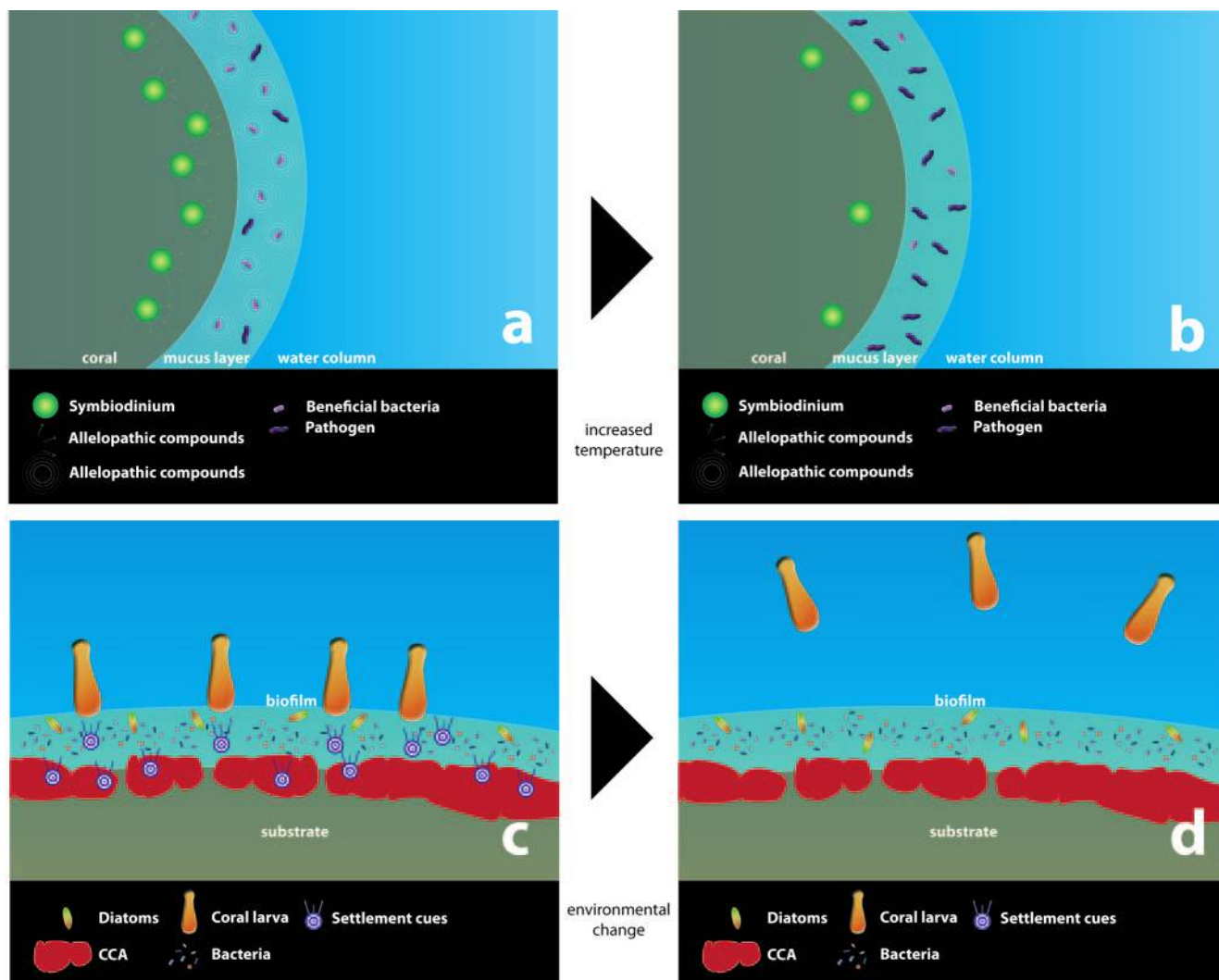


Figure 1. Schematic of coral surfaces and associated microbes. (a) Under normal conditions, the coral animal, associated endosymbiotic algae, or native bacteria may produce allelopathic compounds that regulate the abundance and activities of other microbes that come into contact with the coral. (b) Under conditions of coral stress (such as increased temperature or decreased pH), production of allelopathic compounds may be altered within the coral holobiont, either by affecting production by the coral host or by the associated microbes. Simultaneously, native beneficial bacteria are replaced by pathogenic bacteria on the coral surfaces. (c) Crustose coralline algae (CCA) and biofilm microbial communities facilitate attachment and settlement of coral larvae *via* inductive compounds (settlement cues) produced by the CCA or by recruiting specific bacteria that release these cues. (d) Certain types of environmental change (decreased pH, lower PCO_2 , increased temperature) may alter the abundance of the inductive bacteria or their production of settlement cue compounds, resulting in reduction of attachment, metamorphosis, and successful settlement of coral larvae.

Next Questions: Microbe-Microbe Interactions in Corals

One of the next steps in increasing our understanding of coral fitness is a comprehensive characterization of coral-associated microbial interactions. For example, it is unclear if *Symbiodinium* plays a role in selectively recruiting bacteria to corals, if *Symbiodinium* affects bacterial physiology or secondary metabolite biosynthesis, or if bacterial metabolism influences *Symbiodinium* activity.

Little is known about the nature of free-living *Symbiodinium*, including what bacterial mutualisms may be present before coral acquisition of *Symbiodinium*, in the case that the algal symbiont is not transmitted vertically. Members of the Roseobacteriales group are specifically present in association with *Symbiodinium* cultures and are able to increase *Symbiodinium* growth rates *in vivo* (Ritchie, 2011). This observed association between α -proteobacteria and dinoflagellates may be a true mutualism with benefits for both the

bacteria and the algal host. The bacteria may benefit by having a readily available source of organic compounds such as dimethylsulfoniopropionate (DMSP), a preferred source of reduced sulfur (Miller and Belas, 2004; Raina *et al.*, 2010). The algae may derive benefits from the bacterial production of antimicrobials such as tropodithietic acid (Geng and Belas, 2010) and bioactive compounds such as vitamin B-12 (Geng and Belas, 2010). A genomic comparison of the *Roseobacter* clade of α -proteobacteria indicates that some type of surface-associated lifestyle is central to the ecology of all members of the group (Slightom and Buchan, 2009).

Very little is known about how *Symbiodinium* affects bacterial communities in corals (or *vice versa*) or how these interactions impact the fitness of the coral host. Recent studies suggest that bacterial communities in juvenile corals differ significantly if they were initially colonized by different strains of *Symbiodinium* (Littman *et al.*, 2009) with different photosynthetic efficiencies (Littman *et al.*, 2010). It has been hypothesized that DMSP production by *Symbiodinium* plays a role in structuring bacterial communities in corals by attracting certain bacteria to the surface mucous layer of corals (Raina *et al.*, 2009, 2010).

An important adaptive property of many α -proteobacteria is the presence of a bacterial system for diversity generation facilitated by gene transfer agents (GTAs) (Paul, 2008). GTAs are defective bacteriophages that are able to randomly package bacterial host DNA and transfer DNA to other α -proteobacteria (Paul, 2008). It has recently been shown that *Symbiodinium*-associated α -proteobacteria produce GTAs and are able to transfer genes to a range of bacteria in the marine environment (McDaniel *et al.*, 2010). Furthermore, gene transfer *via* this mechanism is much higher in the coral reef environment than in other marine environments, suggesting an alternate mode of adaptation *via* swapping of potentially beneficial genes among marine bacteria (McDaniel *et al.*, 2010) and possibly the coral holobiont.

A fundamental requirement of model systems is that they address interspecies interactions in a metaorganism. Research on host-microbe interactions can greatly benefit from a well-documented host-microbe study that spans the spectrum from pathogenicity to mutualism. Much work has been done on the basal metazoan *Hydra* to illustrate the value of a model systems approach (Weis *et al.*, 2008; Bosch *et al.*, 2009). Because *Hydra* is associated with a limited number of bacteria, it has provided valuable insight into the molecular basis of immunity and symbiosis in simple animals. Cnidarian and dinoflagellate models can also be used to elucidate roles of bacteria in both coral and *Symbiodinium* biology. Ideally, these models require cultured symbionts (bacterial and dinoflagellate) and an easily maintained cnidarian host (Weis *et al.*, 2008). Our ability to culture many of these bacterial symbionts will aid in exploring functions that are otherwise impossible to study due to the complex nature of the coral holobiont. Generation of genome se-

quence data from animal hosts and their associated microorganisms will exponentially enhance our basic understanding of symbiotic associations at the molecular level. This includes reconstruction of host-symbiont phylogenies, analysis of genes important in specific interactions, comparative genomics, and advanced technologies. The sea anemone *Aiptasia pallida* has recently been proposed as a model for coral biology for a number of reasons (Weis *et al.*, 2008). While corals are difficult to grow in captivity, this species is hardy to laboratory manipulation and grows quickly in aquaria. Many protocols have been developed to manipulate *Symbiodinium* density in *A. pallida* without lethal effects on the host, and as a result, this organism has successfully been used to describe mechanisms of coral bleaching (Dunn *et al.*, 2007) and disease (Alagely *et al.*, 2011). *Aiptasia pallida* represents an opportunity to integrate a model systems approach with novel technologies from the “omics age” to learn more about multipartner interactions in corals in a moment of great environmental change.

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