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Cite this article: Krediet CJ, Ritchie KB, Paul VJ, Teplitski M. 2013 Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. Proc R Soc B 280: 20122328.
http://dx.doi.org/10.1098/rspb.2012.2328

Received: 1 October 2012
Accepted: 9 January 2013

Subject Areas:
ecology, microbiology

Keywords:
coral microbiology, commensal bacteria, microbiota

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2012.2328 or via http://rspb.royalsocietypublishing.org.

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Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases

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Over the last decade, significant advances have been made in characterization of the coral microbiota. Shifts in its composition often correlate with the appearance of signs of diseases and/or bleaching, thus suggesting a link between microbes, coral health and stability of reef ecosystems. The understanding of interactions in coral-associated microbiota is informed by the on-going characterization of other microbiomes, which suggest that metabolic pathways and functional capabilities define the 'core' microbiota more accurately than the taxonomic diversity of its members. Consistent with this hypothesis, there does not appear to be a consensus on the specificity in the interactions of corals with microbial commensals, even though recent studies report potentially beneficial functions of the coral-associated bacteria. They cycle sulphur, fix nitrogen, produce antimicrobial compounds, inhibit cell-to-cell signalling and disrupt virulence in opportunistic pathogens. While their beneficial functions have been documented, it is not certain whether or how these microbes are selected by the hosts. Therefore, understanding the role of innate immunity, signal and nutrient exchange in the establishment of coral microbiota and in controlling its functions will probably reveal ancient, evolutionarily conserved mechanisms that dictate the outcomes of host–microbial interactions, and impact the resilience of the host.

1. Introduction

Healthy corals are crucial to the productivity and sustainability of reef ecosystems and the surrounding human communities [1]. Aside from their role in reef ecosystems, corals are fascinating models of host–microbe interactions. Corals are 'holobionts'—multi-partite symbiotic organisms formed by polyp animals, endosymbiotic dinoflagellates, bacterial and viral associates of polyps and dinoflagellates [2]. Impressive progress has been made in understanding the establishment, maintenance and sanctions in the polyp-dinoflagellate symbiosis [3]. However, precious little is still known about the mechanisms that govern native coral-associated microbial populations. The impetus for addressing these uncertainties comes from the ever-expanding appreciation of the role of the commensal bacteria in other eukaryote–bacterial interactions, and their demonstrated roles in coral nutrition, larval metamorphosis and resistance to pathogens [4]. The urgency for defining functions of healthy coral microbiota is being driven by the reports of the rapid decline in coral reefs worldwide.

A number of studies have documented the composition of coral-associated microbial communities (see the electronic supplementary material, table S1). Different studies either support or disprove the hypotheses on the host specificity of coral-associated microbiota. It is noteworthy that meta-analyses of sequence-based surveys are complicated by the differences in the sampling schemes, approaches and the inherent biases of the technologies used to

define the composition of microbiomes (see the electronic supplementary material, table S1). However, when complementary techniques were used to define coral microbiota over time and geographical locations, more specific associations (e.g. larvae of the brooding coral *Porites astreoides* with *Roseobacter* and *Marinobacter*, dominance of *Oceanospirillae* in adult *P. astreoides*) were documented [5,6]. To what extent sequence-based surveys of coral-associated microbiota should be refined is an open question, which will not be addressed in this review.

To date, no single genus of bacteria appears to be an obligate symbiont of corals. Should we expect to find such a tight association between a coral host and a bacterial symbiont? Even though our understanding of host–bacterial partnerships was shaped by the decades of research in tightly coevolved bipartite symbioses (e.g. *Vibrio fischeri*–bobtail squid; rhizobia–legumes, etc.), the vast majority of host–bacterial associations characterized recently involve more than two partners. In this respect, a synopsis of the Human Microbiome Project is informative: the analysis of over 3.5 Tb of high-throughput data revealed that individuals share few common members of the microbiota and that the 'core' human microbiome should be interpreted not in terms of the diversity of the bacterial ribosomal RNA genes (or even the diversity of bacterial genes altogether), but should rather be defined based on the functionality of metabolic pathways within the microbiome [7]. Therefore, we focus this review on functions of coral-associated microbiota and mechanisms of microbe–microbe, coral–microbe and dinoflagellate–microbe interactions within the coral holobionts' microbial communities. Addressing these uncertainties will facilitate progress in understanding coral health, nutrition (and, more globally, nutrient cycling in reef ecosystems) and coral development. With this review, we will consider the following questions.

(i) How are the composition and functions of the coral-associated microbial communities controlled by the host? Can innate immunity discriminate between 'beneficial' and 'pathogenic' microbes? Or do corals enrich their microbiota for those with beneficial functions by releasing specific nutrients and signals?
(ii) If functional metabolic pathways are key to maintaining the 'core' microbiota, what are the potential roles for horizontally-transferred genes in coral-associated microbial communities?
(iii) What are the functions of each of the holobiont partners in disease and interactions with pathogens? Do corals have mechanisms to recruit beneficial micro-organisms and do they rely on them for protection against pathogens? Conversely, is it possible that the observed disease symptoms are caused by the commensals that escape restrictions—yet uncharacterized—which are imposed by the hosts on the associated microbiota?
(iv) If the assumption that opportunistic pathogens cause coral diseases is correct, how do pathogens establish within robust coral-associated microbial communities? Do they interfere with signalling and metabolic exchange within the native microbiota? Or do they rely on a more efficient use of the coral mucopolysaccharides and other nutrients?
(v) As coral populations continue to decline, is there a realistic hope of devising pro-active approaches to manipulate the associated microbiota to manage coral health?

2. The coral holobiont: a multi-partite symbiotic organism

Corals are intimately coevolved symbioses formed by polyps, unicellular algae and associated microbes. This complex symbiotic assemblage was termed a 'holobiont' [2]. The use of this term in reference to corals expands the original definition meant to describe eukaryotic organisms, which were themselves a product of reticulate evolution resulting from a merger (rather than hybridization) of organisms of different lineages, with each holobiont partner maintaining their own genomes [8]. Within the coral holobiont, the symbiotic photosynthetic dinoflagellates from the genus *Symbiodinium* reside inside membrane-bound vacuoles within specialized cells of the polyp; their photosynthate is assimilated by the polyp and is the main source of carbon nutrition [9]. The dinoflagellates translocate approximately 60–80% of their photosynthate to the coral host, allowing the holobiont to thrive in otherwise nutrient-poor waters [10]. These associations are both dynamic and flexible in that throughout their lifetime, corals can expel their dinoflagellate symbionts and acquire new strains (or even clades) of *Symbiodinum*, and thus the dinoflagellate endosymbionts can contribute significantly to the physiological attributes of the coral holobiont [11]. This flexibility allows for associations with clades that may be more effective under an array of environmental conditions, which may aid in the holobiont response to environmental stressors.

The flexibility of the associations between corals and dinoflagellates prompted scientists to investigate the flexibility in the interactions between corals and other microbes. A field report that indicated that the same species of corals harvested in the same geographical locations was no longer susceptible to infections with *Vibrio shiloi* [12] led to the Hologenome Theory of Evolution, which postulates that in multi-partite symbiotic organisms, the combined 'hologenome' (a consortium of the genetic material of all the members of the holobiont) acts as a single unit of evolution, with faster evolving micro-organisms providing the plasticity needed to adapt to the rapidly changing environment [4,13]. This offers a more nuanced interpretation of evolutionary processes. Since the original formulation of this hypothesis, another field study reported that the Caribbean coral *Acropora palmata*—while still susceptible to the white pox—can no longer be infected by *Serratia marcescens* PDL100, which was associated with an outbreak of white pox in *A. palmata* only a decade earlier [14,15]. Recent reports of the succession of the microbial communities associated with the developmental stages of *P. astreoides* across temporal and geographical scales [5,6] and the discovery of the potentially beneficial functions in α-proteobacteria and strains of *Marinobacter* [16] lend further support to the hologenome evolution hypothesis, although alternate explanations of these observations exist. The hologenome evolution hypothesis will be significantly strengthened by the characterization of the host factors, which in response to a specific stress actively manipulate functions and/or composition of the associated microbiota. Below, we review some of the possible

mechanisms that could be involved in the assembly and function of the holobiont.

(a) Host genetic and epigenetic factors that control coral-associated microbiota

If corals depend (at least in part) on their microbial partners for the overall health and nutrient acquisition, how do they influence the composition and/or functions of the associated microbiota? To effectively structure the associated microbiota, hosts must either (i) be able to detect specific micro-organism-associated molecular patterns (MAMPs) and trigger defence responses to exclude undesirable community members and/or select for the symbionts, (ii) excrete broadly active antimicrobial compounds to select against general environmental organisms, (iii) release chemical cues and/or nutrients that would attract micro-organisms with potentially beneficial functions, or (iv) attract and maintain keystone microbes that would—in turn—shape the microbiota, which is resistant to invasions by potential pathogens. Strong evidence for scenarios (i) and (ii) would indicate that the composition of the associated microbiota is more important, whereas evidence in support of scenarios (iii) and (iv) would argue that the function, rather than composition of the microbiota is more consequential to the holobiont's health and stability.

The ability to discriminate amongst potential symbionts and other micro-organisms based on their surface structures (lipopolysaccharide, peptidoglycan, flagellin, etc.), and/or timing or place of their presentation has been documented during the establishment of two-partner symbioses, such as *Vibrio fischeri*–bobtail squid and rhizobium–legume [17–19]. Genomes of Cnidarians (including Hydrozoa and Anthozoa) encode homologues of proteins capable of recognizing micro-organisms and their associated molecular patterns: C-type and other lectins, membrane-associated Toll-like receptors and intracellular nucleotide-binding and oligomerization-like receptors [20–26]. In fact, *Acropora* CEL-III lectin is among its fast-evolving genes and is under positive selective pressure with the highest sequence divergence found within the domain predicted to recognize carbohydrate ligands [21], thus potentially underlying flexibility in recognizing a broad range of potential pathogens and/or symbionts. Such flexibility is consistent with the demonstrated ability of a purified lectin from *Acropora millepora*, Millectin, to bind and coagulate vibrios, Gram-positive bacteria as well as cells of *Symbiodinium* [22]. Two other lectins (PdC and concanavalin) were strongly upregulated in *Pocillopora damicornis* following challenge with a virulent strain of *V. coralliilyticus* [27]. Bacterial and dinoflagellate surface structures recognized by the cnidarian pattern recognition receptors are not yet known.

Because bacterial lipopolysaccharide (LPS) is a common MAMP, the ability of the commercially available LPS from *Escherichia coli* O127 : B8 to elicit defence-related physiological responses in three corals was tested [28]. The prophenoloxidase activity of *Stephanocoenia intercepta* and *P. astreoides* (but not of *Montastraea faveolata*) was modestly but statistically significantly increased in response to *E. coli* LPS when corals also experienced heat stress, but not at ambient temperature [28]. Even though prophenoloxidase activity (typically leading to melanization) was increased, no associated increase in melanin accumulation within treated samples was detected [28]. Therefore, while corals and other cnidarians have the capability to recognize MAMPs under some experimental conditions, it is not yet known what MAMPs are detected and how this affects the ability of the polyps to structure the associated microbial communities.

Antimicrobial compounds produced by corals probably function in controlling the associated microbiota. For example, the antimicrobial peptide damicornin was most active against a fungus and some (but not all) Gram-positive bacteria, and had no effect on the four tested vibrios [29]. Organic extracts of the coral *Siderastrea siderea* showed selective antimicrobial activity against two of four strains of Gram-positive bacteria isolated from coral surfaces [30]. Antibiotic activity against nine strains of marine bacteria, including known coral pathogens and bacteria related to those from coral surfaces, was found in the crude aqueous extracts of three common Hawaiian corals, *Montipora capitata*, *Porites lobata* and *Pocillopora meandrina* [31]. Extracts of *M. capitata* displayed the most antimicrobial activity, which might be related to the presence of montiporic acids A and B, which are cytotoxic and antimicrobial polyacetylene carboxylic acids found in *Montipora* spp. [32]. Otherwise, the chemical structures of antimicrobial compounds in corals are not known.

Exposure of corals to pathogens also induces production of enzymes with predicted defence functions: phenoloxidase, peroxidases and chitinases, as well as melanin, which is the end product of phenoloxidase [33,34]. Genome-mining projects identified a number of homologues of the genes with predicted functions in chemical defence [20,35], products of which are probably involved in the interactions with microbes.

The possibility that corals (or animals in other holobionts) somehow establish and maintain relationships only with keystone microbes and in turn rely on them to structure the rest of the associated microbiota is potentially intriguing. Such interactions have been recently modelled [36]. The on-going sequencing and metagenomics projects focusing on taxonomic and functional diversity of coral microbiota will offer data to further parametrize and validate this model.

(b) The coral holobiont and disease

Much of our understanding of coral diseases is historically dependent on field surveys. Signs of pathologies are quite general, making assignment of gross lesion morphology difficult between diseases. There are at least eighteen coral diseases that are generally recognized [13,37]. The agents responsible for some of the observed aetiologies have been identified and Koch's postulates fulfilled, however, controversies still surround this issue. The first such controversy stems from the observation that some corals are no longer susceptible to the agents that have caused diseases in the past [12,15,38]. These observations led to several intriguing hypotheses. According to one (the Hologenome Theory of Evolution, discussed above), holobionts, such as corals, can acquire beneficial partners that ward off pathogens. It is also possible that the evolutionary loss of virulence determinants can be responsible for the reduced virulence. Such loss of horizontally acquired genes with presumed virulence functions has been documented in *V. shiloi* [39], although it is not clear whether this short-term evolutionary gene loss was associated with the decreased virulence. The natural selection for disease-resistant coral genotypes [40] as well as the anecdotal evidence of priming (primitive immune memory) in corals, from which black band disease consortia were

removed [41], are also potential explanations for the loss of virulence by the historic bacterial specimens.

Alternative hypotheses suggest that coral diseases are not caused by specific pathogens, rather they are a collection of generic symptoms that could be elicited by a number of opportunistic pathogens that attack the host when its defences are compromised [42]. It is also possible that under some conditions, members of the commensal microbiota escape restrictions—yet unidentified—imposed on them by the host or other members of the host microbiota and then multiply to the numbers that exceed the carrying capacity and start to degrade host tissues. Paradoxically, observations of shifts in the composition of coral microbiota during disease outbreaks [43,44] can be interpreted as evidence to support competing hypotheses of coral diseases. It is far from clear as to whether these shifts are a cause or a consequence—or both—of the coral diseases.

Coral microbiota can also change based on environmental conditions. For example, contact between corals and macroalgae can lead to changes in microbial assemblages in corals [45–47]. Direct contact among corals and macroalgae is increasingly common, especially in the Caribbean, as reefs have been degraded by bleaching, disease, overfishing and nutrient pollution [48,49]. Macroalgae may affect coral-associated microbes by any number of mechanisms including: (i) smothering coral tissues or creating persistent hypoxic conditions [45], (ii) poisoning any member of the coral holobiont via algal secondary metabolites [46,49,50], (iii) harbouring pathogenic bacteria [51], and (iv) inhibiting or stimulating microbial growth by releasing dissolved organic carbon or antibiotic secondary metabolites [45,50,52–54]. We are only beginning to understand the mechanisms responsible for the impacts of algae and other environmental stressors on coral–microbial associations and the consequences to coral health.

(c) Nitrogen acquisition by the holobiont: unexpected roles for vibrios and rhizobia

Corals typically receive the majority of their carbon requirement from their symbiotic association with zooxanthellae [9,10]. Corals are also passive suspension feeders and trap particles and bacteria in their mucus as a nutrient source [55]. Besides serving as a direct source of nutrition to corals through bacterivory, there is new evidence that microbial members of the coral holobiont potentially contribute fixed nitrogen to either the coral polyp or the zooxanthellae. Lesser *et al*. [56] reported that large numbers of nitrogen-fixing cyanobacteria occur in the coral *Montastraea cavernosa*. The unicellular, non-heterocystis cyanobacteria express nitrogenase and have the capacity to fix nitrogen for the holobiont [56]. *Vibrio harveyi* and *Vibrio alginolyticus* are capable of nitrogen fixation in coral mucus and dominate the culturable nitrogen-fixing bacteria of the Brazilian coral *Mussismilia hispida* [57]. A comprehensive survey of nitrogen-fixing bacteria (defined as those with the *nifH* nitrogenase gene) recovered from mucus and tissues of three corals on the Great Barrier Reef revealed that the diversity of the *nifH* in mucus was generally similar to that in the surrounding seawater; however, over 70 per cent of *nifH* sequences recovered from tissues of corals were most similar to those from rhizobia [58]. Rhizobia are best known as a functionally defined group of soil α-proteobacteria that enter into symbioses with leguminous plants, and this interaction consists of a series of mutual recognition and accommodation events, eventually leading to the establishment of rhizobial bacteroids. These bacterioids are essentially intracellular organelles within differentiated plant cells [18]. Even though terrestrial rhizobia are perfectly capable of living saprophytically in soils, they do not fix nitrogen if not associated with the host. If the ability of marine rhizobia to fix nitrogen in the association with the coral holobiont is demonstrated experimentally, this will propel our understanding of evolution of nitrogen fixation and holobiont ecology.

3. Horizontal arms race: gene transfer on the coral surface

If the conclusions of the Human Microbiome Project [7] are broadly applicable to understanding the microbiota associated with other animals, including corals, then the functions of the microbiota rather than its specific composition determine the stability of the holobiont. A high frequency of horizontal gene transfer, coupled with the presence of host mechanisms for selecting the beneficial functions in the microbiota would be important pieces of evidence to support this hypothesis.

High frequency of integron- and gene transfer agent (GTA)-mediated horizontal gene transfer in coral reef bacteria has been reported recently [59–61]. GTAs are phage-like particles. They package up to 4 kb pieces of the host bacterial DNA and are capable of conferring onto coral bacteria functions that could be beneficial to their polyp hosts [61]. GTAs from coral-associated α-proteobacteria transferred genetic markers to a broad range of bacteria under ecologically relevant conditions at frequencies drastically higher than those of transformation and transduction [60,61]. These transfer elements, encoded by bacteria, facilitate mixing of genes in the reef environment, allowing selective advantage to some microbes associated with the coral holobiont.

Coral pathogens also appear to benefit from horizontal gene exchange. For example, coral mucus-associated vibrios readily exchanged integrons containing genes for antibiotic resistance, and the evolution of integrons was more rapid than the core genome [59]. While other functions could be carried on the integrons, they play a key role in the spread of antibiotic resistance in coral-associated bacteria [59]. In addition to acquiring virulence and antibiotic-resistance genes, coral bacteria could gain novel metabolic functions, such as the ability to use dimethylsulfoniopropionate (DMSP), which is produced in abundance by the symbiotic dinoflagellates [62,63].

Interestingly, *dmdA* genes involved in the use of DMSP were among over-represented sequences in the metaviriomes from ocean and coral reef environments [64]. As *dmdA* sequences were phylogenetically diverse, this suggests multiple events in which phages acquired these genes from their various hosts [64]. Their over-representation in published metaviriomes is a clear indication that these horizontally transferred genes confer a significant advantage to the bacterial hosts of the $dmdA^{+}$ phages [64]. The readiness with which coral-associated micro-organisms acquire genes that increase their ability to use host-specific nutrient sources, such as DMSP, is additional evidence in support of the hypothesis that functions of the coral-associated microbiota, rather than

their taxonomic identity, are central to the outcomes of their interactions within the coral holobiont.

4. Battlefield: slime

(a) Chemical and physical properties of coral mucus

Even though microbes have been isolated from the endolyth, digestive tracts and endosymbiotic zooxanthellae, most commonly studied coral-associated micro-organisms have been recovered from the coral surface mucopolysaccharide layer. It is within this layer that the presumed commensal microbiota interacts with potential pathogens and environmental organisms. Even though several studies have characterized functions of coral mucus in protection against desiccation and trapping particulates [55,65], it is also reasonable to hypothesize—based on the discoveries made in other animal models—that structuring of the associated microbiota is an important function of coral mucus.

Coral mucus contains sulphated glycoprotein polymers made in specialized mucocytes of the polyp from the photosynthate produced by their endosymbiotic dinoflagellates and then secreted onto the coral surface [65]. The chemical structures of coral mucus components have been determined for less than a dozen species [66–72]. Even though there are differences in the composition of mucus produced by different corals, several generalizations could be made based on these reports. The polypeptide backbone of mucus accounts for up 80 per cent of its mass, with serine, threonine, aspartate, glutamate and glycine being most common amino acids in different coral species [66,69,70]. The polypeptide backbone is decorated with sulphated oligosaccharide side chains O-linked through a mannose residue, which is different from mucins in most other animals [66,69,70]. Unlike mucins from other animals, coral mucus contains small amounts of 'plant' monosaccharides (such as arabinose and xylose), owing to its photosynthetic origin [69–71]. Although their relative amounts in mucus of different species vary, most common monosaccharides are mannose, *N*-acetyl-D-glucosamine, galactose, fucose, glucose and arabinose, with xylose and *N*-acetyl-D-galactosamine being minor components of coral mucins [66,69–72].

(b) Coral mucus use by commensal bacteria and opportunistic pathogens

Bacteria (including coral pathogens and commensals, as well as *E. coli*) can reach 10^6–10^8 cfu ml^{-1} within hours when grown on coral mucus, its low molecular weight fraction and high molecular weight mucin constituents [73–76]. *In situ*, bacterial counts in coral mucus are known to be an order of magnitude higher than those in the surrounding seawater [77]. In addition to carbon and nitrogen sources discussed above, coral mucus also contains potent antimicrobials [78]. Therefore, when crude preparations of fresh mucus are used as growth substrate, declining bacterial viability is sometimes reported [79].

To establish within presumably robust coral surface microbial communities, invading pathogens—in addition to dealing with host defence molecules present in mucus—must be able to outcompete members of native microbiota within the surface mucopolysaccharide layer, and then penetrate mucus to reach host tissues. Indeed, coral pathogens *S. marcescens* and vibrios dominate mucus microcosms set up under laboratory conditions [74,76]. When their ability to efficiently use mucus is disrupted, virulence of the pathogen is attenuated (but not abolished), probably owing to the inability of the pathogen to establish within the surface mucopolysaccharide layer [80].

Bacteria produce glycosidases, proteases and esterases when growing on coral mucus [74,81]. Coral commensals and pathogens appear to possess a similar suite of enzymatic activities, even though their metabolic capabilities estimated by Biolog Ecoplates differ [74,76]. While pathogens and commensals produce essentially the same arsenal of exoenzymes to degrade coral mucus, temporal patterns of their regulation and levels of activity are different. Unlike commensals, polysaccharide-degrading enzymes of *S. marcescens* PDL100 are strongly induced in starved cells [75]. During the early stages of mucus colonization, glycosidases in a white pox pathogen *S. marcescens* PDL100 were under strong catabolite repression by the sugars present in coral mucus, with only glucosidase, *N*-acetyl-galactosaminidase and arabinosidase—enzymes predicted to be involved in cleaving off mucin's oligosaccharide side chains—mostly free of catabolite control [75]. During the later stages (approx. 18 h) of mucus colonization, many glycosidases in commensals were downregulated in a catabolite-dependent manner, whereas in *S. marcescens* only glucose and *N*-acetyl-glucosamine had some catabolite repression effect [75]. The totality of the catabolic activities in *S. marcescens* PDL100 during the later stages of mucus colonization was more similar to that of its pathogenic conspecifics rather than environmental isolates or coral commensals [74]. These observations demonstrate that to outcompete commensals within the coral surface mucus layer, coral pathogens use strong, constitutively active glycosidases. The activities of these glycosidases provide carbon and nitrogen for the bacteria and make the polypeptide backbone of mucins available to the bacteria.

Intriguingly, we have recently discovered a novel role for the coral commensals in disrupting coral mucus colonization by pathogens. Several members of the native microbiota associated with *A. palmata* produced extracellular activities that block the induction of the glycosidases in a white pox pathogen *S. marcescens*, and thus interfere with its ability to use coral mucus [80]. It is now clear that while metabolic interactions between commensals and pathogens within coral mucus have not been studied extensively, their better characterization will reveal novel mechanisms by which coral commensals block the expansion of opportunistic pathogens.

(c) Cell-to-cell signalling and interference within the coral surface mucopolysaccharide layer

Microbes have evolved sophisticated strategies to gauge their own population densities and accordingly change global patterns of gene regulation. Such population density-dependent cell-to-cell signalling and gene regulation is often termed 'quorum sensing' (QS) [82,83]. QS is one of the mechanisms by which pathogens coordinate expression of their virulence genes [83]. Many marine bacteria, including those recovered from surfaces of marine invertebrates, are known to produce various QS signals in laboratory shake cultures [84,85] and the *in situ* production of the *N*-acyl homoserine lactone signals has been demonstrated recently in sponge-associated microbial communities [86]. The presence of compounds capable of activating or inhibiting responses of bacterial QS

reporters has been documented in the extracts of marine organisms, including corals, sponges, ascidians, algae and cyanobacteria [87–90], which suggests that QS-based signalling and signal-interference take place in natural environments.

Whether or not corals themselves or zooxanthellae can interfere with bacterial QS remains unknown, even though the ability to produce QS inhibitors and QS signal-degrading enzymes has been reported in other animals, plants and algae [91]. The ability of eukaryotes to manipulate bacterial QS is often interpreted in terms of a co-evolved strategy to control virulence and other bacterial behaviours that are consequential to the well-being of the host [91]. The ability of bacteria recovered from corals to inhibit QS in other micro-organisms has been reported in the laboratory [84,85]. Most intriguingly, *in situ* native coral bacterial isolates capable of inhibiting bacterial QS were also capable of preventing progression of a disease caused by a coral pathogen *S. marcescens* PDL100 in a model polyp *Aiptasia pallida* [16], although it is not yet entirely clear whether QS-inhibitory properties of these microbes were responsible for the observed reduction in disease signs, or just coincidental.

5. Curative functions of the native coral biota

With changing climate patterns, temperature, ocean acidification and other anthropogenic impacts, the future of coral reefs worldwide remains uncertain. In discussing the potential solutions for managing the coral reef crisis, is it reasonable to consider incorporating native beneficial micro-organisms as one of the pro-active tools for promoting stability of reef ecosystems? Such biological control strategies are widely used with some success for the management of plant pathogens. In human and veterinary medicine, formulations containing beneficial microbes are used widely as food additives ('probiotics') or—currently in limited trials—as therapeutic faecal transplants [92]. This broad popularity and reasonable success of beneficial microbes in medicine, agriculture and aquaculture invited the question of the feasibility of using beneficial coral-associated microbes for promoting coral health and potentially controlling coral diseases [93]. Clearly, there will be many logistical, ecological and ethical questions that will need to be addressed before coral 'probiotics' are widely used. Our goal here is to critically analyse and contextualize recent discoveries of the potentially beneficial functions of native coral bacteria and phages.

(a) Phage therapy

Pioneering studies demonstrated successful applications of phages (viruses of bacteria) for controlling several coral pathogens in aquaria and in reef ecosystems. These phages are specific to the coral pathogens and do not affect the resident microbiota [94–97]. What happens to the introduced phages, which are obligate parasites, in the environment in the absence of the host bacteria needs to be closely examined. Application of the GTAs [60] could be considered as a phage therapy as well; however, unlike lytic phages using for therapeutic applications, GTAs will not kill their target bacteria, rather may endow commensal coral α-proteobacteria with potentially beneficial functions. An advantage of using GTA's for these applications is that pathogenic organisms are not put under selective pressure resulting in rough (phage-resistant) variants. The ability of GTAs to transfer transposons with antibiotic-resistance genes [60,61] as well as potential virulence genes is a potential concern.

(b) Native bacteria and their potential functions in promoting coral health

Similar to well-characterized biocontrol agents, coral commensal bacteria have the potential to produce antimicrobial compounds, inhibit pathogen's catabolic enzymes and disrupt cell-to-cell communication in pathogens and competitively exclude pathogens from host surfaces [16,78,93,98]. Antibacterial, algicidal, antifouling, and cytotoxic compounds have been isolated from marine invertebrates and their microbial associates, though it is not yet clear whether any of the bioactive microbes are capable of providing the magnitude of protection typically found in successful biocontrol organisms. Culturable microbes associated with a number of corals produce antibacterial compounds against a broad spectrum of pathogens, including pathogens of corals [78,98]. Commensal bacteria from healthy corals were able to inhibit growth of known coral pathogens; however, isolates associated with and often found on diseased colonies (*Vibrio coralliilyticus* and *Pseudoalteromonas* spp.), as well as members of the Black Band Disease consortium showed strong antimicrobial activities against native coral bacteria, indicating that these strains may have a competitive advantage and may inhibit potential 'probiotic' species under favourable conditions [99,100]. It is not known whether the levels of antibiotics produced by these bacteria and accumulated *in situ* impact the coral microbiota; however, if these antibiotics get trapped within the mucus, they may very well affect the composition of coral-associated microbial communities. In addition to functioning as antimicrobials, some antibiotics are also capable of disrupting QS in pathogens, and this function of the antibiotics produced by native coral-associated bacteria remains under-explored.

6. Conclusions and future directions

Interactions among host-associated bacterial communities are critical for the overall health of the coral holobiont, but our understanding of the mechanisms and consequences of these interactions is still very much incomplete. Metagenomic sequencing projects revealed a great taxonomic diversity of coral-associated micro-organisms, with some surveys pointing at the possibility of host-specific microbial assemblages. Coral microbiology research is grossly underfunded, compared with the microbiome studies in higher organisms. Therefore, comparisons with the results from better funded, better characterized systems are invaluable. One of the main outcomes of the Human Microbiome Project is the realization that functional pathways, rather than the presence of specific taxonomic units, are what determine the stability of the host-associated microbial community. The observed high rates of horizontal gene transfer on coral surfaces and evidence of over-representation of some metabolic and antibiotic-resistance genes in the coral's microbial metagenomes also point to the fact that specific functions within the microbiota may be more important than the identity of the micro-organism carrying those genes. In the case of a brooding coral that vertically transmits bacteria [6], members of specific bacterial genera associated with the same coral (*P. astreoides*) across geographical and temporal scales [5,6,101]. What selection

mechanisms could be involved in structuring 'function-based' or 'identity-based' host-associated microbial communities? A better understanding of the mechanisms of immunity in corals and the chemical structure and function of the antibiotic and QS-inhibitory compounds produced by different members of the holobiont will help define mechanisms by which specific microbial genera may be selected by the host. A more in-depth understanding of the nutrients exchanged within the coral holobiont and their roles in selecting for microbes with specific functions will probably reveal mechanisms by which potentially beneficial micro-organisms are selected. While field observations continue to be critical to our understanding of the mechanisms governing the functions of the holobiont, understanding of the mechanisms of interactions within it will be greatly facilitated by an in-depth focus on a limited number of model systems [102].

The preparation of this manuscript was supported by grants from Protect Our Reefs program to the co-authors. Protect Our Reefs program is funded by the proceeds from the sales of 'Protect Our Reefs' specialty license plates and is managed by Mote Marine Laboratory through a peer-reviewed process. M.T. is supported by the 2012 George E. Burch Fellowship in Theoretical Medicine and Affiliated Sciences at the Smithsonian Institution. This is contribution 903 of the Smithsonian Marine Station at Fort Pierce.

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