Coral Probiotics: Premise, Promise, Prospects

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Abstract

The use of Beneficial Microorganisms for Corals (BMCs) has been proposed recently as a tool for the improvement of coral health, with knowledge in this research topic advancing rapidly. BMCs are defined as consortia of microorganisms that contribute to coral health through mechanisms that include (*a*) promoting coral nutrition and growth, (*b*) mitigating stress and impacts of toxic compounds, (*c*) deterring pathogens, and (*d*) benefiting early life-stage development. Here, we review the current proposed BMC approach and outline the studies that have proven its potential to increase coral resilience to stress. We revisit and expand the list of putative beneficial microorganisms associated with corals and their proposed mechanisms

that facilitate improved host performance. Further, we discuss the caveats and bottlenecks affecting the efficacy of BMCs and close by focusing on the next steps to facilitate application at larger scales that can improve outcomes for corals and reefs globally.

INTRODUCTION

The synergistic and interdependent relationship between a macroscopic host organism and its associated microorganisms forms a metaorganism, or holobiont (1). In this relationship, the host and its microbiome act as a biological unit to constitute the resulting phenotype and maintain the fitness of the metaorganism. Corals depend on a diverse group of symbionts (2), including endosymbiotic dinoflagellates of the family Symbiodiniaceae, which reside within the gastrodermis of most shallow-water coral species (3). These photosynthetic endosymbionts largely supply the corals' energy requirements through provision of photosynthates (4). The symbiosis between corals and their Symbiodiniaceae is the "engine of the reef" (5), facilitating construction of the coral calcium–carbonate skeleton, which constitutes the three-dimensional landscape of the reef and thus the complex habitat for thousands of other marine species (5). This delicately balanced symbiosis is threatened by ocean warming, which can trigger the loss or expulsion of Symbiodiniaceae cells, also known as bleaching (6), and, when severe, extensive coral mortality (6, 7). Mass bleaching events driven by marine heatwaves are the main driver of global reef loss (6), exacerbated by local impacts such as sewage, overfishing, and disease outbreaks (8, 9).

In addition to the Symbiodiniaceae, corals harbor other groups of microorganisms that inhabit the wide variety of microhabitats provided by the host mucus, tissues, and skeleton (10–13) (**Figure 1**). These microbes include bacteria, viruses (eukaryotic and bacteriophages), microeukaryotes, and archaea (14, 15), which may be beneficial, neutral, or detrimental to coral health (16–18). Whereas the association between corals and their Symbiodiniaceae is generally specific (19), the relationship between corals and their bacteria is potentially more flexible and may shift in response to changing environmental conditions (5, 18, 20). This flexibility provides a potential avenue for the metaorganism to acquire new traits through association with novel microbes or changes in the relative abundance of established microorganisms (21, 22), a characteristic exploited in coral-probiotics approaches.

There are currently very few known coral pathogens. Although 28 coral diseases have now been described affecting a wide variety of corals across the globe, the causative agents of only six have been described (14, 23). Despite more than 40 years of research in the field, several significant knowledge gaps remain concerning disease in corals in general (16, 23). The six known coral pathogens include *Vibrio coralliilyticus*, described as the most well-characterized coral pathogen and associated with coral bleaching and white syndromes (24, 25); *Serratia marcescens* [cause of white pox disease; also known as acroporid serratiosis (26)]; *Pseudoalteromonas pirati* [cause of *Montipora* white syndrome (27)]; *Vibrio tubiashii* [cause of *Porites* white patch syndrome (28)]; *Vibrio shiloi* [cause of bleaching in the coral *Oculina patagonica* (29)]; and *Thalassotalea loyana* [formally *Thalassomonas loyana* and cause of white plague disease (30)]. Nonetheless, even for these well-described coral pathogens there remain some inconsistencies and conflicting results among various studies, as coral disease may be the outcome of microbial dysbiosis (31, 32).

Conversely, coral-associated microbes are also vital for metaorganism function and health (10). Members of the microbiome play roles in energy and nutrient acquisition, reproduction, mitigation of toxic compounds, and pathogen control (12). The increase in frequency of bleaching events and emerging coral diseases shows that the disruption of the relationship between the coral host and its associated microbes is a major cause of reef deterioration (31, 32), contributing to the
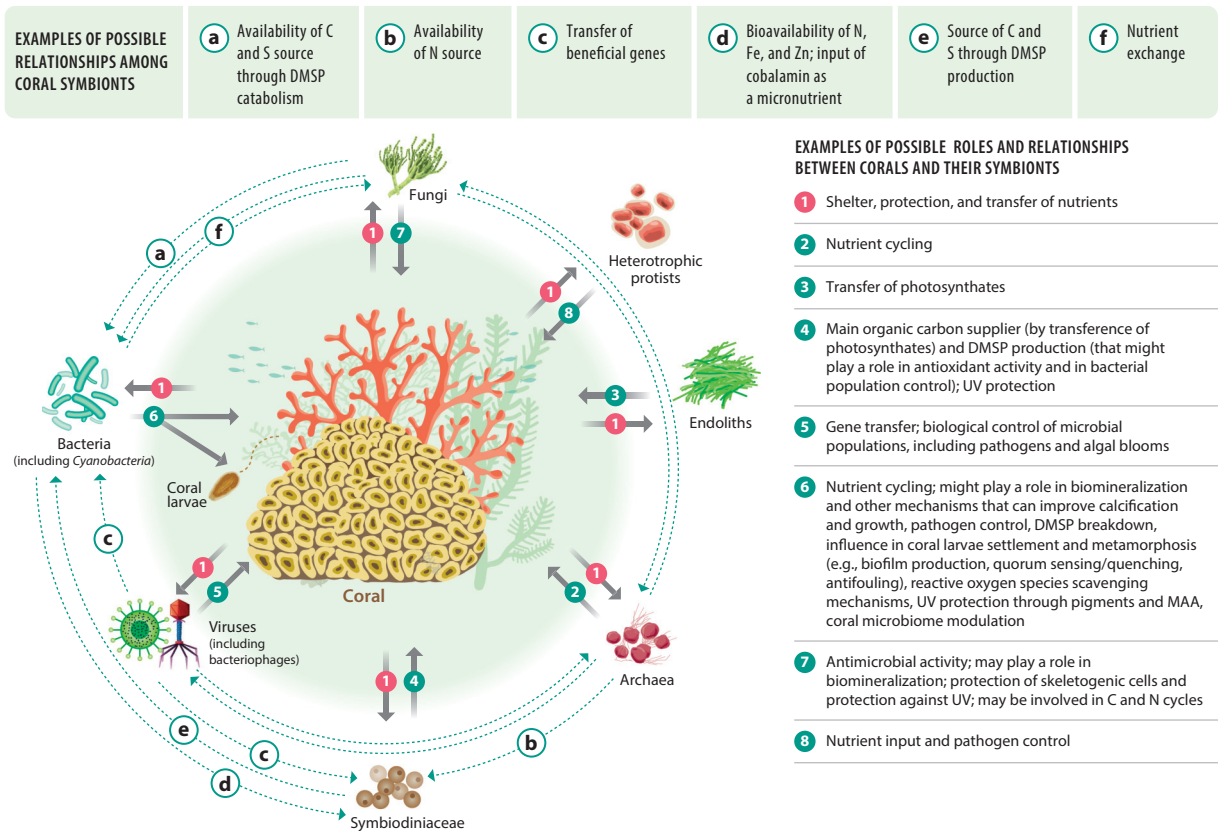
EXAMPLES OF POSSIBLE RELATIONSHIPS AMONG CORAL SYMBIONTS

a Availability of C and S source through DMSP catabolism

b Availability of N source

c Transfer of beneficial genes

d Bioavailability of N, Fe, and Zn; input of cobalamin as a micronutrient

e Source of C and S through DMSP production

f Nutrient exchange

Fungi

Heterotrophic protists

Endoliths

Bacteria (including *Cyanobacteria*)

Coral larvae

Coral

Viruses (including bacteriophages)

Archaea

Symbiodiniaceae

EXAMPLES OF POSSIBLE ROLES AND RELATIONSHIPS BETWEEN CORALS AND THEIR SYMBIONTS

1 Shelter, protection, and transfer of nutrients

2 Nutrient cycling

3 Transfer of photosynthates

4 Main organic carbon supplier (by transference of photosynthates) and DMSP production (that might play a role in antioxidant activity and in bacterial population control); UV protection

5 Gene transfer; biological control of microbial populations, including pathogens and algal blooms

6 Nutrient cycling; might play a role in biomineralization and other mechanisms that can improve calcification and growth, pathogen control, DMSP breakdown, influence in coral larvae settlement and metamorphosis (e.g., biofilm production, quorum sensing/quenching, antifouling), reactive oxygen species scavenging mechanisms, UV protection through pigments and MAA, coral microbiome modulation

7 Antimicrobial activity; may play a role in biomineralization; protection of skeletogenic cells and protection against UV; may be involved in C and N cycles

8 Nutrient input and pathogen control

Figure 1

Possible relationships among coral symbionts and between corals and their symbionts. Abbreviations: DMSP, dimethylsulfoniopropionate; MAA, mycosporine-like amino acid.

massive loss of reef ecosystems worldwide. Hence, coral microbiology can be considered for the development of interventions for reef conservation and restoration purposes. An example of intervention is microbiome manipulation through the use of Beneficial Microorganisms for Corals (BMCs) (12). Approaches for microbiome manipulation using beneficial native microbes consist of identifying microorganisms exhibiting putatively beneficial roles for the hosts and increasing their abundances in the metaorganism, an approach that has already been widely applied in other scientific fields (33–35). This type of intervention can be used to actively and specifically modulate the coral microbiome or, alternatively, to restore the microbiome to a natural/undisturbed state (18) and therefore avoid microbial dysbiosis. BMCs are, in fact, a promising strategy presenting the potential to protect natural populations of corals in the reef both before (prevention) and after (bioremediation) environmental impacts occur (12).

In this review, we present the premise and promise of the use of BMCs, discussing its original outline and summarizing the recent results obtained from BMC application in laboratory trials (see the section titled Coral Microbiome Manipulation Using BMCs). We update the framework of the selective screening of BMCs (see the section titled Moving Forward), including new putative beneficial mechanisms and tools to track coral symbionts, based on emerging knowledge of the processes of microbial community assembly. Finally, we discuss the prospects and next steps

Symbiosis: biological interaction between two different biological entities; the ecological relationship of a symbiotic interaction can be mutualistic (the two organisms benefit from the relationship), commensalistic (one organism benefits while the other neither benefits nor is harmed), or parasitic (one benefits while the other is harmed)

for overcoming limitations to the application of BMCs to corals and reefs (see the section titled Moving Beyond: Challenges and Potential Solutions). In Current and Future Challenges, we introduce and summarize the main questions addressed in the review.

BMC: STATE OF AFFAIRS

Microbiome manipulation or engineering is an emerging approach to treat diseases and potentially increase the adaptability of host macroorganisms (36). Altering the host microbiome has proven effective in the treatment of several diseases in humans and wild animals and may therefore be an alternative to antibiotics and chemical therapy (37). For example, the administration of live microorganisms to benefit host health (i.e., probiotics) has been demonstrated for amphibians (34), bats (38), bees (35), and more recently, corals (39). Specifically, probiotics increased protection of amphibians against the fungal pathogen *Batrachochytrium dendrobatidis* (34) and increased the survival of bats fivefold, even in field trials, in their fight against the devastating white-nose syndrome, which is also caused by a fungal pathogen (40). All of these probiotics are native strains, i.e., isolated from the target hosts, and selected considering their putative antagonisms against specific pathogens (e.g., frogs, bats) or potential to support overall metaorganism health (e.g. corals, bees, frogs).

The Coral Probiotics Hypothesis (CPH) was initially postulated to explain observations that coral health varied with changes in the microbial community composition, as well as the resistance to pathogens acquired by certain corals (22). The CPH postulates that alterations in the abundance of microbial community members could help the coral adapt to new environmental conditions (22). An extension of this hypothesis was put forward recently, noting that the propensity for change can be denoted as microbiome flexibility, with the important caveat that the capacity for restructuring of the host-associated microbiome in light of prevailing environmental change is host specific (18, 21). Hence, the extent to which hosts can adjust their microbiomes may differ, which is likely to have consequences for environmental adaptation (21). In accordance with the CPH and microbiome flexibility postulates, and the discussion about the biological control of coral pathogens (41), the term Beneficial Microorganisms for Corals (BMCs) was coined to describe specific microorganisms exhibiting functions that have beneficial effects on the coral metaorganism, as well as a first attempt to define strategies for their application (12). The putative beneficial functions need to be tested in vitro to develop probiotics with in vivo health benefits, including coral protection and aid in recovery from diseases and bleaching. This approach, which is focused not only on the mitigation of a single impact itself but also on the organisms' overall health, is based on the premise that a healthier organism will more efficiently cope with any impact. This improvement of the coral's overall health through the BMC approach can be extended by adding specific members of the probiotic consortia that can mitigate specific and local impacts. Alternatively, the use of BMCs can also be combined with other approaches, through so-called customized medicine for corals (42).

Although the initial survey proposing BMC mechanisms was published relatively recently (12), the study of coral-associated microbiomes and their mechanisms of interaction has advanced greatly, and new insights have been gained. Here, we discuss the proof of concept of BMC efficiency and update the list of microorganisms and their functions that potentially enhance coral fitness, along with further research priorities.

Coral Microbiome Manipulation Using BMCs

Santos and colleagues (43) proposed to protect corals from the specific environmental impact of oil via inoculation with probiotic bacteria. They demonstrated the mitigation of the effects of this

contaminant via its breakdown and the consequent improvement in the health of exposed corals. Rosado et al. (39), using a holistic probiotic approach, next demonstrated in laboratory trials that BMCs can increase coral resistance to thermal stress (30°C) and pathogen challenge (*V. coralliilyticus*). The study used a BMC consortium containing seven bacterial strains belonging to the genera *Halomonas*, *Cobetia*, and *Pseudoalteromonas* (39). The bacteria were isolated from the coral *Pocillopora damicornis* and selected according to functions postulated as being beneficial, according to the original list of BMC mechanisms (see 16), which included several traits, such as antagonism toward *V. coralliilyticus*, mitigation of reactive oxygen species (ROS), dimethylsulfoniopropionate (DMSP) breakdown, and nitrogen cycling. These traits were identified in the BMC candidates through biochemical and molecular screening and then assembled into a consortium. This approach was based not on a single impact or contaminant but on the concept of restoring metaorganism homeostasis by actively removing stress factors (e.g., ROS) and reintroducing and facilitating growth of microbial strains associated with coral health. During the study, *P. damicornis* nubbins treated with the BMC consortium showed a modified microbiome, as well as reduced bleaching compared with the control groups, demonstrating that addition of the BMC consortium mitigated the signs of bleaching caused by high temperatures and pathogen challenge (39).

Morgans et al. (44) recently tested the application of Symbiodiniaceae as a probiotic to corals subjected to experimental bleaching; they observed significant differences in bleaching recovery and mortality when *Acropora millepora* fragments were inoculated with *Durusdinium trenchii* and *Cladocopium goreaui*. Inoculation with *C. goreaui* resulted in significantly less bleaching than seen in control fragments and fragments inoculated with *D. trenchii* subjected to heat stress, though the inoculated Symbiodiniaceae strains were not detected in the coral tissues by ITS2 amplicon sequencing at the end of the experiment. This led the authors to suggest that these probiotics may not establish symbiosis with the corals, but instead indirectly supplement heterotrophic nutrition. However, the higher resilience to heat stress provided by *C. goreaui* compared with controls and *D. trenchii*–inoculated corals suggests that unless the corals had different feeding profiles for the two Symbiodiniaceae strains, addition of *C. goreaui* may provide some yet-unidentified benefit to the host.

Microbial manipulation seems to be particularly promising in early life-history stages, when environmental microorganisms are often recruited as part of microbiome establishment and development. Damjanovic et al. (45) explored this concept recently by inoculating and tracking specific microorganisms associated with larvae of different coral species. The authors showed that it is feasible to change the microbiome of corals in early life stages by inoculating recruits with a bacterial consortium prepared in the laboratory.

Together, these attempts indicate the potential of inoculation with specific microorganisms or consortia thereof to manipulate the microbiome in different stages of coral development to improve holobiont health and resilience. Still, probiotic experiments have used microorganisms with potentially beneficial traits countering specific stress conditions, but their roles in promoting the long-term establishment of a more resistant metaorganism remain unclear. The exact mechanisms that underlie the observed effects have also not yet been elucidated and remain a research priority. Identifying the mechanisms for a metaorganism's recovery, and facilitating the growth of a more resilient microbiome, will help determine the next steps for implementing probiotics in damaged coral reefs.

Finally, understanding how natural variations and environmental factors affect coral-associated microbes is a key step to successfully applying BMCs to reef ecosystems. It is known that microbiomes naturally vary depending on factors such as coral species (46) and geographic locations (13, 18, 46). Elucidating how these factors will affect the beneficial strains (re)introduced to the coral colonies is going to be crucial to determine whether the microbial strains will succeed in

colonizing the corals and promote benefits to their health. This way, although all these previously mentioned studies succeeded in introducing BMC strains to the coral holobiont, long-term experiments are still necessary to investigate how stable BMC–coral associations are (or are not).

Moving Forward

Studies of the symbiotic relationships within coral metaorganisms have highlighted additional mechanisms that should be investigated for BMC-type applications. Here, we present an updated perspective on the use of BMCs, including new mechanisms to be explored and strategies to scale up their application. The microbial functions proposed as beneficial for corals fall into four general categories: promoting coral nutrition and growth, mitigating stress and impacts of toxic compounds, providing pathogen control, and benefiting early life-stage development (**Table 1**).

Nutrition and growth. Corals acquire essential macro- and micronutrients from the environment (heterotrophy) or from translocated compounds provided by their symbiotic algae or microorganisms (4). In shallow-water corals, the translocation of photosynthetic products by the associated microalgae can satisfy up to 90% of their energy demands (4), in addition to having direct effects on coral homeostasis. Additional symbionts containing photosynthesis-related genes are also found in the coral microbiome. Chromerids, also known as corallicolids (47, 48), have been found to be associated with corals. These microbes are the closest free-living relatives to the Apicomplexa, a group of obligate intracellular parasites that includes causative agents of diseases in humans and livestock. Most apicomplexans have been shown to harbor a nonphotosynthetic plastid, called an apicoplast (49). However, the coral-associated corallicolid species *Chromera velia* and *Vitrella brassicaformis* contain chlorophyll-biosynthesis genes (48), and these organisms are the second most abundant coral-associated microeukaryotes, comprising approximately 2% of retrieved 18S ribosomal RNA gene sequence reads in surveys of 38 coral species (48). The capacity of these symbionts to perform photosynthesis is debated, with speculation that corallicolids are evolutionary intermediates transitioning from phototrophy to parasitism, and further work is needed to understand their function in corals (46, 48, 50). Carbon may be supplied to the coral holobiont through other metabolic pathways. For example, Robbins et al. (51) undertook deep sequencing of the *Porites lutea* holobiont and identified carbon-fixation metabolic pathways in nonphototrophic Alphaproteobacteria, Nitrospirota, and Thaumarchaeota. In addition, a bacterial strain isolated from corals has been reported to host a bacteriochlorophyll gene, suggesting the potential ability for chlorophyll biosynthesis and subsequent carbon fixation through photosynthesis (52). The extent to which these other microbiome community members contribute to the total fixed carbon pool of the coral holobiont must be tested empirically.

Microbial partners likely provide other essential nutrients to corals. Both Symbiodiniaceae and coral cells lack the ability to synthesize certain vitamins, including vitamin B12 (cobalamin), which is important for many functions, such as amino acid synthesis. In corals, the concomitant high concentrations of cobalamin and bacterial cells in the gastric-cavity fluid (53) provide evidence that symbionts are delivering this essential compound to both coral cells and their dinoflagellate symbionts (54). Recent studies have shown that some coral symbionts possess genes for the production of vitamin B12, biotin, thiamin, and riboflavin (51, 52). For example, Robbins et al. (51) identified 23 of 52 metagenome-assembled genomes derived from *P. lutea* that encode genes for the biosynthesis of biotin and thiamine, 41 for riboflavin, and 3 for cobalamin, further indicating that these microbes potentially underpin the holobiont function.

Several host-associated bacteria have also been shown to sequester and produce important micronutrients and trace metals, such as iron (55). Experiments exposing corals to low iron

Table 1   Proposed beneficial characteristics and their mechanisms

Proposed beneficial characteristic (BMC characteristic)	Beneficial mechanism	Examples of references supporting the putative beneficial mechanism
Nutrition and growth		
Photosynthesis	Input of organic compounds to the holobiont, photosynthetic facilitation of calcification	Burriesci et al. (151) Davy et al. (152), Tremblay et al. (153)
Nitrogen fixation	Input of fixed nitrogen to the holobiont	Olson et al. (154), Lema et al. (155)
Organic nitrogen and carbon cycling and regulation	Control of organic compound distribution	Kimes et al. (156)
Breakdown of DMSP	Increase carbon and sulfur availability to coral-associated microbes	Raina et al. (95), Vanwonterghem et al. (157)
Production of siderophores	Increase bioavailability of iron to Symbiodiniaceae	Lawson et al. (67)
Cobalamin synthesis	Input of cobalamin as a micronutrient for Symbiodiniaceae	Matthews et al. (69)
Production of urease enzyme	Urea hydrolysis may enhance coral calcification by stimulating precipitation of $CaCO_3$ and by supplying inorganic carbon for the calcification reaction	Biscéré et al. (60)
Mechanisms affecting the protection of skeletogenic cells	Enhance the survival of skeletogenic cell types	Domart-Coulon et al. (63)
Mitigation of toxic compounds or stress		
Production of antioxidant molecules and mechanisms (e.g., catalases, superoxide dismutase, glutathione, ascorbate peroxidase, and monodehydroascorbate reductase)	Minimize the concentration of reactive oxygen species	Lesser (78), Diaz et al. (79)
Production of mycosporine-like amino acids and carotenoids	Protect coral tissue against ultraviolet radiation	Dunlap & Shick (85), Osman et al. (89)
Synthesis and exportation of compatible solutes (e.g., betaines, floridoside, DMSP)	Increase coral thermotolerance or tolerance of reactive oxygen species, photoprotection	Ochsenkühn et al. (80), Ngugi et al. (82), Yost et al. (90)
Breakdown of toxic compounds, such as oil hydrocarbons	Mitigation of toxic contaminants in seawater that are in direct contact with the mucus layer	Santos et al. (43)
Early life development		
Production of signals for larval settlement	Contribute to larval settlement modulation or regulation	Webster et al. (158), Heyward & Negri (159), Shikuma et al. (160)
Production of biofilm	Facilitate larvae settlement	Sneed et al. (119)
Quorum quenching, inhibition or breakdown of QS molecules	Disrupt cell–cell signaling of beneficial microbes for other species competing for space	Modolon et al. (103)
Pathogen control		
Production of antibiotics	Biological control of pathogens	Ritchie (92), Kvennefors et al. (93)
Production of DMSP	Control of bacterial populations on the coral surface	Raina et al. (95)

Table 1 *(Continued)*

Proposed beneficial characteristic (BMC characteristic)	Beneficial mechanism	Examples of references supporting the putative beneficial mechanism
Quorum quenching, inhibition or breakdown of QS molecules*	Disrupt cell–cell signaling of pathogens, potentially inhibiting virulence mechanisms	Teplitski & Ritchie (41), Sharp & Ritchie (98), Tait et al. (99), Zimmer et al. (100), Certner & Vollmer (101), Zhou et al. (102)
Top-down control of coral pathogens (e.g., bacterivory and phage control)	Selective foraging may shape bacterial communities in corals and control pathogen populations	Welsh et al. (107, 110), Pernthaler (108)
Breakdown of DMSP	Remove chemoattractant for potential pathogens	Garren et al. (97)

Abbreviations: BMC, Beneficial Microorganisms for Corals; DMSP, dimethylsulfoniopropionate; QS, quorum sensing.

availability increased their vulnerability to thermal stress (56). Reich et al. (55) observed that low iron concentrations caused changes in the concentrations of other trace metals (e.g., zinc, copper, cobalt, manganese, nickel, molybdenum, and vanadium) within corals. Symbiodiniaceae require relatively high concentrations of iron for cell division and growth, though different clades respond differently to iron limitation, which may influence their response to environmental stresses. Certain bacterial strains produce siderophores to take up iron from the environment, forming complexes that can reenter the cells via specific receptors (57). The potential production of siderophores by these bacterial symbionts should therefore be considered a putative BMC mechanism, as it may be a source of bioavailable iron in the coral metaorganism, because this element is usually insoluble in seawater (54).

Exploring the processes associated with calcification is another key area of research. Calcification is the process through which corals and other calcifying organisms precipitate aragonitic calcium carbonate ($CaCO_3$) to build their skeletons (58). Light indirectly enhances calcification (59) through the increase of Symbiodiniaceae photosynthesis rates. Indeed, the concomitant diversification of Symbiodiniaceae and radiation of reef-building corals (3) strongly support this photosynthetic facilitation of calcification hypothesis. Even though calcification processes in corals have been studied extensively, the mechanisms involved in the enhancement of $CaCO_3$ deposition are not well understood. The equation summarizing the calcification process is $Ca^{2+} + HCO_3^- \leftrightarrow CaCO_3 + H^+$, which means that any process that increases Ca^{2+} and HCO_3^- and/or removes H^+ locally will favor the formation of $CaCO_3$ and consequent skeleton mineralization (60). One enzyme that has the potential to contribute indirectly to the calcification process is urease. Urease catalyzes the nickel-dependent hydrolysis of urea, producing ammonia and CO_2, which may direct the calcification equation toward product formation. The influence of urease on the calcification of scleractinian corals was suggested almost 45 years ago, when urease activity was detected in coral tissues with and without Symbiodiniaceae (61). Recent experimental evidence supports this hypothesis, with corals exposed to nickel- and urea-enriched environments showing higher rates of urea uptake and hydrolysis, which coincided with a significant enhancement of long-term calcification rates, as measured by differences in coral buoyant weight and estimations using the alkalinity anomaly technique in *Acropora muricata* and *P. damicornis* corals (60). Even though the factors that significantly affect urease production at the calcification site remain unclear, some coral-associated bacteria can hydrolyze urea (62). Hence, enrichment of microorganisms with urease activity at the site of calcification may promote skeletal growth and mitigate effects such as ocean acidification.

Other microbial processes may also influence the structural integrity of the coral skeleton. For example, a peculiar interaction between symbionts residing in the coral skeleton has been

reported, in which *P. damicornis* was able to associate with an inoculated fungal strain belonging to the genus *Cryptococcus* and which seemed to promote the survival of skeletogenic cell types (63). Although the authors suggest that this effect was not necessarily beneficial, but a way to compensate for the erosion caused by the fungus, we suggest that this microbial-mediated mechanism of supporting and recovering the coral skeleton should be investigated further in other microbial groups. Endolithic microorganisms are prevalent in the coral skeletons, structured by defined light, pH, and nutrient and oxygen boundaries and characterized by microalgal (*Osteobium* spp.), fungal, and heterotrophic bacterial communities (64). These endoliths play a role in reef erosion by dissolving calcium carbonate and weakening coral skeletons (64), though some studies have also indicated a potential for nutrient cycling and metabolite transfer, which may underpin the health of the overlying coral tissues. For example, Fine & Loya (65) reported increases in photoassimilates transferred from endoliths to bleached coral tissues, highlighting the potential of this alternative energy source to support corals through thermal stress events. Finding ways to promote the growth of endolithic communities that support coral energy demands during periods of stress is another area of research that should be explored.

Numerous microbial-mediated mechanisms can potentially support not only the coral host but also Symbiodiniaceae growth and metabolism, and BMCs should target both. Even though Symbiodiniaceae–bacteria interactions have begun to be studied only recently, it was previously suggested that free-living bacteria can support Symbiodiniaceae nutrition and survival (66, 67). The reported co-occurrence of bacteria and Symbiodiniaceae taxa (68) in coral holobiont compartments reinforces the hypothesis that these interactions can be specific and conserved. These observations have led some authors to suggest that Symbiodiniaceae may have an innate dependence on specific bacteria for exchange of nutritional resources, forming an intimate relationship that may promote optimum metabolic fitness of the coral metaorganism (69). This is further supported by the description of aragonitic spherulites that are formed by free-living Symbiodiniaceae in association with bacterial communities (70) and thus form a dinoflagellate–bacterial calcifying community. This aspect remains unexplored with regard to its BMC potential.

Mitigation of toxic compounds and stress. Corals are constantly under threat of exposure to toxic compounds, including those produced directly by anthropogenic activity, such as sewage pollution and oil spills (71, 72), and those produced by the coral metaorganism itself during episodes of stress (73). A wide range of microorganisms can use toxic xenobiotics as substrates (62, 74) or to break down toxic metabolic products (62, 75). It is therefore clear that coral-associated microorganisms have the potential to mitigate coral stress in different environmental conditions.

ROS produced by corals and their endosymbiotic algae under high temperatures seem to be capable of inducing coral bleaching (73). Consequently, the production of antioxidant molecules and microbial antioxidant activity may be a beneficial function to protect heat-stressed corals (12, 76), and this function includes enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase, which are found in many organisms (77). Although the mechanisms through which these microbial enzymes actually protect corals against oxidative stress remain to be ascertained, an exogenous addition of ROS scavengers has been shown to decrease the extent of coral bleaching (78), and corals (or their associated microbes) seem to be able to control superoxides in their immediate surroundings (79). Similarly, an increase of floridoside, a metabolite that counters ROS, as a consequence of elevated salinity levels has been demonstrated to effectively lower bleaching susceptibility (76, 80, 81). The osmolytes floridoside and betaine, which are also produced by coral symbionts, may act as ROS scavengers and photoprotectors, respectively (80, 82), putatively contributing to coral thermal tolerance. The production of compatible solutes commonly used for osmotic protection by coral-associated microorganisms is especially interesting in

the context of heat stress, owing to similarities between ROS-producing mechanisms in photoautotrophic organisms under stressful salinity and heat conditions (80, 83).

In addition to enzymatic defenses against oxidative stress, the damage from ROS can also be controlled in photosynthetic organisms through the use of photoprotective compounds (84). These compounds could mitigate the damage done to the photosystems of coral-associated algae and consequently reduce ROS production. Mycosporine-like amino acids have been identified in taxonomically diverse coral species and have been dubbed "natural sunscreens" owing to their capacity to absorb UV radiation, whereas others are better known for their antioxidant activities (85). They have also been detected in marine heterotrophic bacteria, cyanobacteria, and microalgae (85), and coral symbionts can produce these molecules (86). Carotenoids are another group of molecules with well-documented antioxidant and photoprotective properties in photoautotrophs (87), including Symbiodiniaceae algae (88). Coral-associated bacteria such as *Erythrobacter* spp. have been found to contain large amounts of carotenoids and have been proposed to mitigate heat stress–related production of ROS in the coral metaorganism (89).

Other solutes have also been associated with the mitigation of heat and oxidative stress in corals. DMSP, for example, is an osmolyte found in many species of marine algae, including members of the Symbiodiniaceae (51, 90). DMSP and its breakdown products [dimethylsulfide (DMS), acrylate, dimethyl sulfoxide, and methanesulfinic acid] have been found to scavenge different types of ROS in marine algae (91). The changes in DMSP levels in corals owing to copper exposure may be linked to the antioxidant function of the solute (90). DMSP produced by coral-associated algae may therefore benefit corals because of its antioxidant role. However, an excess of DMSP can also trigger (or be involved in) dysbiotic processes, as discussed in more detail below. The activities of coral-associated microorganisms can also mitigate stress from contaminants generated by human activity, such as oil (43), through breakdown of these compounds within corals or in the surrounding environment in direct contact with the metaorganism.

Pathogen control. Microorganisms found in corals can produce antimicrobial compounds, which can be important for structuring coral microbial communities and are potentially beneficial to host health (92, 93). Competition between pathogens and symbiotic microorganisms occurs in corals (93, 94), and selection of microorganisms antagonistic to known coral pathogens can be useful in applying BMCs to corals at risk of diseases. Some examples of possible roles and relationships between corals and their symbionts are summarized in **Figure 1** and **Table 1** and were discussed by Peixoto et al. (12). Rosado et al. (39) showed that a probiotic consortium tested in aquarium experiments successfully protected coral nubbins against infection by the pathogen *V. coralliilyticus*, and different mechanisms inherent to native microorganisms have been suggested to promote the biological control of this pathogenic organism.

Production of DMSP by coral endosymbiotic algae can structure coral-associated microbial communities by supporting bacteria that can metabolize DMSP, DMS, and acrylic acid (95). This metabolic potential includes bacteria from the genus *Roseobacter*; many of these bacteria possess antimicrobial properties (e.g., production of thiotropocin) that may aid the corals in resisting pathogens (96). Breakdown of DMSP can therefore be viewed as a possible BMC characteristic. Further, differences in pH found between the coral mucus (~7.7) and the surrounding seawater (~8.3) have been hypothesized to result from the presence of high concentrations of DMSP breakdown products (DMS and acrylate), which might also prevent initial colonization by potential pathogens (95). Furthermore, DMSP has been discovered to drive chemotaxis and chemokinesis in the coral pathogen *V. coralliilyticus*, and attraction of this pathogen to *P. damicornis* has been demonstrated, with a stronger chemical cue directing the bacteria to heat-stressed corals (97).

DMS, however, has been shown to provoke only a very weak chemotaxis in *V. coralliilyticus* (97), and the other products of DMSP breakdown have not yet been explored in relation to chemotaxis.

Quorum sensing (QS) cell–cell signaling enables bacteria to communicate with one another via autoinducers and to control the expression of characteristics associated with antibiotic production, biofilm formation, and pathogenesis (98). This type of communication has been suggested to be used in pathogenic and mutualistic interactions within coral-associated microbial communities (98), and study of QS in corals has revealed that it may participate in different coral diseases. Among the autoinducer substances known to participate in QS are the acyl-homoserine lactones (AHLs), which are produced by different coral-associated vibrios, including the coral pathogens *V. coralliilyticus*, *V. shiloi*, and *Vibrio harveyi* (99). AHL signaling has been related to coral diseases and is linked to the structuring of bacterial communities and disease progression in *Diploria strigosa* corals infected with black-band disease (100). In *Acropora cervicornis*, the addition of exogenous AHLs produced symptoms similar to those of white-band disease and, in conjunction with a healthy coral homogenate, was able to act as a disease-causing agent (101). Aquarium experiments with *P. damicornis* also found evidence for a relationship between AHL regulation and coral-bleaching progression, because corals treated with exogenous AHLs and AHL-producing bacteria were more susceptible to bleaching and displayed more significant changes in their bacterial community compositions than control groups treated with the same substances with the addition of a QS inhibitor (102). This suggests that the inhibition of QS or the breakdown of QS autoinducers such as AHLs may be a potential BMC characteristic that protects corals from pathogens and bleaching (12, 98, 103).

Top-down control of coral pathogens may also benefit the host. This biological control can be performed by viruses capable of infecting disease-causing agents or by natural predators. Phages associated with the coral mucus layer have been posited to provide a first line of immunity against bacterial pathogenesis and are also suggested to regulate coral-associated microbial communities (104). The use of phage therapy as a method to control coral diseases has been increasingly discussed in recent years, and the positive aspects of this method of biological control, such as target specificity, replication, and safety, have been highlighted previously (41, 105). Treatments using phages able to infect the coral pathogens *V. coralliilyticus* and *Thalassomonas loyana* (105, 106) could ameliorate disease symptoms or stop disease transmission in aquarium experiments, and manipulation of bacteriophages can be regarded as a potentially useful tool against coral disease.

Pathogen predators are also a self-replicating potential solution for coral infections, capable of mediating microbial communities associated with the host (107). Bacterivory by microeukaryotes and predatory bacteria is postulated to regulate microbial communities in different aquatic ecosystems via selective foraging (108, 109). In corals, studies of microbial predation found that the bacteria *Halobacteriovorax* could prey on the coral pathogens *V. coralliilyticus* and *V. harveyi* in plaque assays (110). Furthermore, inoculation of *Halobacteriovorax* in aquarium experiments prevented the increase of *V. coralliilyticus* in corals and stabilized the rest of the host's microbiome, also preventing blooming of opportunistic bacteria in stressful temperatures (107). These studies highlight the importance of predatory bacteria for the structuring of coral microbiomes and also demonstrate the potential to manipulate pathogen predators to protect against disease.

Early life development. Sexual reproduction in coral results in a swimming larva or planula that acquires microbial communities either horizontally from the environment (111) or inherited vertically from the parental gametes (112). Apprill et al. (113) investigated the bacterial colonization of planulae derived from the spawning species *Pocillopora meandrina* and observed internalized bacteria after 79 h, indicating likely horizontal transfer. These bacteria were affiliated with the *Roseobacter* clade, many members of which possess antimicrobial traits, which may benefit the early

coral life stages by excluding pathogens. Investigation of the broadcast-spawning coral *Acropora tenuis* also showed a lack of internalized bacteria in the offspring, though mucus surrounding these early life stages was colonized by members of *Rhodobacteraceae*, *Alteromonadaceae*, and *Oceanospirillaceae* (45). In contrast, the brooding coral *Porites astreoides* was demonstrated to vertically transmit members of the *Roseobacter* clade to their planulae, and these associates were maintained in settled juveniles (114). Similarly, Damjanovic et al. (115) found that larvae and recruits of the brooding coral *Pocillopora acuta* inherited not only Symbiodiniaceae cells from the parents but also bacteria from the family *Rhodobacteraceae* and the genus *Endozoicomonas* (both phylum *Proteobacteria*). Ceh et al. (116) investigated the seawater surrounding broadcast and brooding coral species after release of their gametes and planulae, found increases of members of *Alteromonas* and *Roseobacter*, and suggested that this increase may aid in transfer of beneficial microorganisms to the offspring. Diazotrophs have also been reported associated with the early life stage of *A. millepora* corals, potentially supporting nitrogen demand in developmental stages prior to the uptake and establishment of Symbiodiniaceae populations (117). The early life stages of corals are influenced by their symbionts; for example, the nutrition provided by Symbiodiniaceae is essential to the success of newly settled recruits. However, the influence of other members of the microbiome, such as the apicomplexan-like *C. velia* and bacterial and archaeal communities, on the biology of early life stages is still unclear.

Bacterial surface biofilms have been found to facilitate the settlement of some coral larvae. For example, a *Pseudoalteromonas* strain (a genus that includes members able to produce a biofilm) was isolated from crustose coralline algae (CCA) and shown to induce metamorphosis of coral larvae of the genus *Acropora* (118, 119). Sneed et al. (119) also found that a strain of *Pseudoalteromonas* isolated from marine biofilms induced metamorphosis of *P. astreoides*, *Orbicella franksi*, and *Acropora palmata* larvae; they attributed this effect to the derived bacterial compound tetrabromopyrrole. However, it is unlikely that tetrabromopyrrole is the only compound produced by strains of *Pseudoalteromonas* able to induce metamorphosis. *Pseudoalteromonas luteoviolacea*, for example, produces ordered arrays of phage tail–like metamorphosis-associated contractile structures that induce metamorphosis in larvae of the tubeworm *Hydroides elegans* (120). Though several reports have corroborated the importance of biofilm-forming microbes for coral settlement, metamorphosis, and recruitment, the signaling steps that underpin these processes remain elusive. Many *Pseudoalteromonas* and other biofilm-forming species have also been found to live in association with adult corals (39), suggesting that adult corals act as a reservoir of biofilm-forming microbial species, which are constantly exchanged with the reef environment to support coral recruitment. Non-biofilm-forming bacteria that support the growth of biofilm-forming strains have been shown to increase settlement of other marine invertebrate species (121). The processes that promote coral settlement are complex, though the roles of microbial biofilms, chemical cues, and cell–cell communication require further investigation and may be part of the BMC framework.

The four categories of beneficial mechanisms—coral nutrition and growth, mitigation of stress and toxic compounds, pathogen control, and early life development—can act synergistically to benefit corals. For example, microbial nitrogen fixation serves as a nitrogen input for the metaorganism, alongside heterotrophy, and is an important source of nutrients for both corals and Symbiodiniaceae (122, 123). The activity of microbial enzymes that participate in other phases of nitrogen cycling can help to control its distribution in the metaorganism, limiting nitrogen entry into the Symbiodiniaceae and controlling pathogens and the symbiosis between algae and corals (122, 123). In parallel, corals living in waters with excess dissolved inorganic nitrogen have shown greater susceptibility to thermal stress (124), indicating the potential importance of microorganisms that cycle this element for protection against bleaching (mitigation of stress or toxic

compounds). Nutritional balance within the coral holobiont is important, as both an excess and a limitation of essential nutrients can destabilize the delicate symbiosis and ultimately harm the metaorganism.

Identifying Putative BMC Molecular Markers

A wide range of coral-associated and free-living marine bacteria possess putative beneficial traits. This raises the question of how to discriminate a symbiont from opportunistic organisms found associated with coral, in particular, compounded by the notion that the metaorganism assemblage by default is fluid, i.e., dynamic across space and time [sensu Jaspers et al. (125)]. The molecular signatures of symbionts might be helpful in screening. For example, in insects, symbionts can be sequestered in bacteria-bearing host cells, called bacteriocytes, which in some species cluster together to form a bacteriome (126). Similar clusters, termed coral-associated microbial aggregates (CAMAs), are observed in corals (13, 127). In insects, antimicrobial peptides, such as coleopticin-A from weevils, also selectively target endosymbionts within the bacteriocytes and inhibit their cell division (128). If the synthesis of coleopticin-A is blocked, the bacteria escape from the bacteriocytes and spread into surrounding tissues, which is detrimental to the host. In this example, it seems that the coevolution of host and symbiont has enabled the immune system to facilitate the endosymbiotic relationship. In corals, we still do not know if the CAMAs are beneficial or detrimental to the coral metaorganisms, though many are dominated by *Endozoicomonas*-affiliated taxa (13). How the coral regulates the composition and abundance of these CAMAs needs investigation, with a view to determining whether they can be modulated to benefit host health.

Bacterial symbionts of corals have recently been found to be enriched in genes encoding for eukaryotic-like proteins (ELPs), compared with planktonic bacteria (51). ELPs often contain characteristic repeat domains, such as the ankyrin repeat (ANK) or the WD40-repeat, which are involved in mediating protein–protein interactions (51, 129). These proteins and domains, as the name suggests, are frequently found in eukaryotes but are also increasingly found and recognized in bacteria and archaea, in particular those with a pathogenic or symbiotic lifestyle (130). ELPs can modulate cellular processes in their host; recent work has shown that ANK-type ELPs found in sponge symbionts can interfere with the phagocytosis of eukaryotic cells (131, 132). This likely helps symbionts to escape phagocytosis in filter-feeding sponges or allows bacteria to persist intracellularly (e.g., in bacteriocytes), similarly to what has been shown for ANK proteins secreted by *Legionella pneumophila*, which allow for intracellular persistence in macrophages (133, 134). Genes for ANK and WD40-type proteins were present and often abundant in all major bacterial symbiont clades found in the coral *P. lutea* (51), indicating that these genes may be generally important for mediating symbiotic interactions with corals. We therefore suggest that ELPs could be used as molecular markers to distinguish a BMC candidate from allochthonous bacteria.

Moving Beyond: Challenges and Potential Solutions

BMC studies performed to date have relied upon delivery of free cells into the water column or onto coral nubbins held in small-scale laboratory trials or aquaria systems (39, 43, 45). In these smaller systems, free cells may become trapped in coral mucus and possibly transferred to other compartments directly via ingestion of suspended cells or the mucus-entrapped BMCs, although this still needs to be documented directly. The current lack of strategies to effectively scale up the application of BMCs from laboratory trials to the field is a crucial bottleneck to be addressed. Nonetheless, we believe that comparisons to the successful development and deployment of microorganisms in other aquatic systems can offer invaluable insights that can be tested and extrapolated to coral reefs.

In practice, delivery of microorganisms in aquatic systems is a major challenge, owing to direct dilution and water currents, as well as interactions with a wide range of naturally occurring micro- and macroorganisms. In aquaria and aquaculture tanks with limited water volume and controlled flow, the dilution issue can be resolved; however, in open aquatic systems such as oceans, innovative strategies need to be developed. One of the approaches that might be adopted to address this problem is immobilization of the cells.

Immobilization of microbial cells to enhance delivery efficiency is used in several industries, such as wine production (135), biofuel production (136), and pollutant/waste remediation (137, 138). Immobilization of cells offers numerous advantages, including maintenance of their metabolic activity owing to better-controlled chemical conditions and physical protection. Also, slow release of cells from a solid support can reduce the need for constant reinoculation. Additionally, immobilized cells will disperse less widely compared with free-living cells, thereby effectively reducing potential issues concerning escaped microorganisms affecting or interacting with non-target organisms (139).

The selection of a carrier is a crucial step in immobilization strategies. Desirable characteristics include low cost, nontoxicity, high carrying capacity, permeability to growth substrates, and ease of preparation and handling (140). In the case of BMCs, it should be possible to break down the carrier naturally or to regenerate it. Microorganisms can be immobilized in carriers made from three types of material: inorganic, organic, or a composite of both (137). Organic carriers include naturally occurring products (e.g., coconut husk, chitin, alginate, agar, and guar gum) and synthetic polymers (e.g., polypropylene, polyurethane, and acrylamide). Inorganic carriers include charcoal, clay, silica, ceramics, and celite, among others (141). Whereas inorganic carriers are usually more resistant, less costly, and easier to handle and reuse, organic carriers are more adaptable and typically biocompatible and therefore safer for other organisms (142). Composite carriers, which combine the advantages of both organic and inorganic carriers, provide some level of compromise between these respective (dis)advantages and include substances such as polyvinyl alcohol, sodium alginate, and polyvinyl alcohol–guar gum (137).

Cells can attach strongly or loosely to the carrier's surfaces or internal pores via mechanisms such as adsorption, entrapment, and encapsulation. The mechanisms that microorganisms use to attach to surfaces are well studied (143) and can thus be used to select suitable surfaces. Entrapment, where cells are trapped within a support matrix, has the advantage that cells are confined inside a solid matrix, which provides resistance to physical, chemical, or biological impacts. This method also allows the entrapment of high densities of cells that can be released slowly and continuously. The matrix most often used for entrapment immobilization is the polymer alginate (137). Microencapsulation of living cells is a type of entrapment in which the cells are enclosed by a semipermeable membrane (137). The main advantage of microencapsulation systems is that the entrapment fluid can offer optimal conditions for cell function.

Another option is bioencapsulation, where living carriers, such as the live feed organisms *Artemia* spp., rotifers, or copepods, are used to successfully deliver nutrients, immune stimulants, and probiotics to several aquatic organisms (144, 145). Live feeds have high palatability and digestibility and can stimulate a natural feeding response in animals (146). Nevertheless, for many established aquaculture finfish species, coating of formulated feeds with probiotics is the most common delivery strategy, as they offer precise control of nutritional components and ease of preparation and application (146, 147).

Application of BMCs in aquaculture facilities and the field has been proposed to produce healthy and resilient coral larvae, juveniles, or adults for reef-restoration purposes. Adapting the immobilization methods discussed above (or developing novel ones) for use with coral probiotics not only is possible (**Figure 2**) but is a gap that urgently needs to be filled. Among the carrier
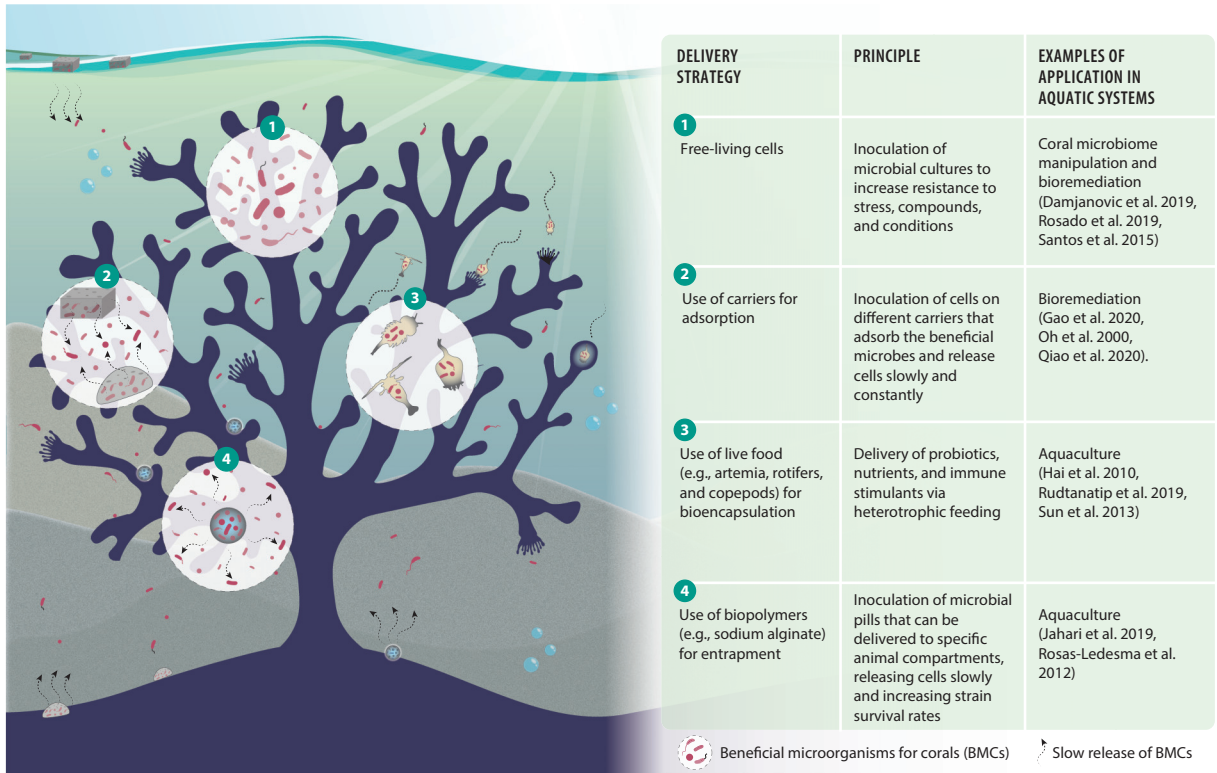
DELIVERY STRATEGY	PRINCIPLE	EXAMPLES OF APPLICATION IN AQUATIC SYSTEMS
1 Free-living cells	Inoculation of microbial cultures to increase resistance to stress, compounds, and conditions	Coral microbiome manipulation and bioremediation (Damjanovic et al. 2019, Rosado et al. 2019, Santos et al. 2015)
2 Use of carriers for adsorption	Inoculation of cells on different carriers that adsorb the beneficial microbes and release cells slowly and constantly	Bioremediation (Gao et al. 2020, Oh et al. 2000, Qiao et al. 2020).
3 Use of live food (e.g., artemia, rotifers, and copepods) for bioencapsulation	Delivery of probiotics, nutrients, and immune stimulants via heterotrophic feeding	Aquaculture (Hai et al. 2010, Rudtanatip et al. 2019, Sun et al. 2013)
4 Use of biopolymers (e.g., sodium alginate) for entrapment	Inoculation of microbial pills that can be delivered to specific animal compartments, releasing cells slowly and increasing strain survival rates	Aquaculture (Jahari et al. 2019, Rosas-Ledesma et al. 2012)

Beneficial microorganisms for corals (BMCs)   Slow release of BMCs

Figure 2

Examples of delivery strategies to be explored for the deployment of Beneficial Microorganisms for Corals (BMCs) in the field and examples of their applications in aquatic systems.

materials discussed above, biocompatible polymers and live feed organisms seem to possess the required characteristics to deliver coral probiotics both in aquaria and in the field. The development of customized carriers that specifically attach to coral structures is desirable for successful application on the reef, but this would require detailed understanding of the chemical composition and biological characteristics of such structures. Aquaria-based experiments will be helpful during initial development and testing of delivery strategies appropriate for different coral species and life stages. Application in aquaria and aquaculture facilities is inherently easier than field application, given that water volumes and flow rates can be controlled, the costs and logistical requirements for repeat applications are lower, and there is no risk of delivery to nontarget organisms. A transition to field applications will likely have to occur via controlled testing in large mesocosm systems that aim to replicate processes on coral reefs.

CONCLUSIONS

The manipulation of coral microbiomes through the administration of BMCs, i.e., coral-specific probiotics specifically selected for corals, has emerged recently as a promising tool to harness and explore coral–microbiome relationships and, ultimately, to ameliorate impacts on corals and reefs. Importantly, the new knowledge being generated in the field of coral probiotics can be further extrapolated to support the development of probiotics for other threatened wildlife. The ability

to manipulate/tailor the microbiome will allow us to explore the location of symbionts within the host, as well as the mechanisms of symbiotic interaction that can help to elucidate key questions in coral biology, such as the importance of partner fidelity and the transmission of symbionts (19). The recent advancements in aquarium methods and facilities that allow research laboratories to predictably spawn scleractinian corals (148) will greatly facilitate studies of microbial uptake and establishment in early life stages of corals. The use of fluorescence in situ hybridization and advanced imaging approaches linked with stable isotope labeling techniques may facilitate the understanding of BMC interactions within the host (149). An alternative approach to be explored is the use of fluorescently labeled bacterial cells or fluorescent latex beads (150) to visualize and track the uptake and establishment of BMCs delivered to early and late life stages. This approach has the advantage of enabling visualization in live specimens with intact mucus layers, so that live bacteria can be directly enumerated based on their fluorescent signal complementing agar plating and microscopy observations. Because of the genetic manipulation involved, such approaches are not field deployable and should be restricted to experiments studying underlying BMC mechanisms or optimizing delivery strategies. Solutions to the above can, in turn, provide novel or complement existing tools to improve BMC uptake and stability. In a nutshell, the ability to successfully manipulate host–microbial associations also requires an intricate knowledge of the processes that govern these interactions. Therefore, the field has a component of knowledge generation and knowledge application. Regarding the application of BMCs in reefs, delivery of free cells is probably unrealistic, and an optimized immobilization approach will be required. Possible delivery strategies include the deployment of slow-release devices and targeted underwater or surface release of BMCs immobilized in feed items (live feeds, feed aggregates or pellets, encapsulated feeds), adsorbed to a solid carrier or entrapped in a supporting matrix. Discussion of such strategies should involve an environmental risk assessment with local government and community involvement and should also consider the costs versus benefits of applying these measures to protect coral reefs. Finally, an understanding and admission that many, if not all, reef ecosystems have already changed in response to local and global impacts needs to be taken to heart and the goal of any restoration measure clearly stated from the onset.

CURRENT AND FUTURE CHALLENGES

Challenge 1: Understanding the role of individual microbial symbionts in coral metaorganism fitness.

- What functions do microorganisms perform to underpin coral health, how are these functions contributed (e.g., through provision of essential nutrients, metabolites, proteins, vitamins, trace metals or antioxidants), and what are the quantitative effects?
- How does environmental stress impact these symbiotic relationships, and what are the consequences for the coral metaorganism?
- Which microbial taxa are essential for metaorganism functioning, and which are functionally redundant?
- Why is understanding the responses of coral microbiomes to natural variation, such as among coral species, geographic locations, and time, a critical step to develop successful coral probiotics approaches?

Challenge 2: Identifying, tracking, and testing beneficial characteristics of coral-associated microorganisms.

- What are the known and putative beneficial characteristics of these coral-associated microbes? What are the main groups (taxonomic and functional) of beneficial microbes associated with corals?

- What are the best methods to track the location of microbes in the coral mucus, tissue, and skeletal microhabitats?

Challenge 3: Developing effective BMC delivery approaches for corals.

- What are the best strategies to deliver BMCs to corals?
- What are the best immobilization approaches to reach the target, with minimum inoculum loss and environmental impact?
- What is the best choice for large-scale delivery? How often would they need to be reinoculated?

Challenge 4: Testing strategies to apply BMCs in realistic mesocosms and ultimately on the reef.

- Are there realistic mesocosms for testing BMC delivery strategies before field inoculation?
- Will reefs be monitored after BMC inoculation?
- What are the best coral health parameters to determine whether BMCs are benefiting corals in mesocosms and in the field?

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