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Microbiome engineering: enhancing climate resilience in corals

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Running heads:
HE Epstein *et al*.
Microbiome engineering for coral resilience

The world's coral reefs are under unparalleled pressure due to climate change, stimulating research focused on preventing further damage and loss in these ecosystems. The coral microbiome has been widely acknowledged as crucial to coral health and function, playing multiple roles in key biological processes. Recent empirical studies suggest that microbes may contribute to coral host tolerance of thermal stress, and harnessing these benefits through microbiome engineering (ME) may provide a mechanism for enhancing climate resilience in corals. Although coral ME is in its infancy, similar and successful ME approaches that are already underway in other fields – including agriculture, medicine, and wastewater treatment – may serve to guide and improve ME techniques in corals. We discuss current applications of ME, identify three key research priorities that

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will help elucidate the viability of ME for corals, and consider the implications of using these approaches for reef restoration.

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In a nutshell:

- The development of innovative restoration and conservation tools is urgently needed to combat the global climate-change-driven decline in coral reefs
- A growing body of evidence indicates that microorganisms play critical roles in coral climate resilience
- Examples from systems such as the human gut microbiome provide evidence that microbial manipulation can be used as a tool to improve host (eg human) health and resilience, and could therefore provide guidance for experimentation in corals
- Microbial manipulation may be able to enhance climate resilience in corals in the face of rapid climate change, which could have implications for coral reef restoration and management

The health and function of all multicellular life on Earth relies on the consortium of microbial partners – including prokaryotes (ie bacteria and archaea), single-celled and filamentous eukaryotes (eg fungi and algae), and acellular viruses – that make up an organism's microbiome and play a vital role in many biological processes (Zilber-Rosenberg and Rosenberg 2008). Microbes that live within and on the tissues of host organisms are at the interface between a host and its environment, and can contribute to immunity (Thaiss *et al.* 2016), nutrition (Flint *et al.* 2012), metabolic function (Thaiss *et al.* 2016), early development (Fraune and Bosch 2010), and host gene expression (Larsson *et al.* 2012), and can even regulate life span (Smith *et al.* 2017). The immense complexity of host–microbiome interactions warrants an expanded ecological and evolutionary view of an organism that incorporates the entire "holobiont": that is, a collective biological entity including the host, its microbiome, and other associated symbionts (note: for definitions of selected specialist terms used in this review, see WebTable 1).

A healthy microbiome is a diverse and highly structured community that is essential to a host's ability to survive changes in environmental conditions (Mueller and Sachs 2015). A robust microbiome is also dynamic, and changes in microbiome composition may be required for a healthy holobiont under varying environmental conditions. However, when an organism is exposed to persistent environmental perturbations, host–microbiome symbiosis can become compromised, leading to an unpredictable shift in microbial community structure, termed dysbiosis (Roder *et al.* 2014). Dysbiosis can interrupt the services that the microbiome provides to the host, often resulting in a loss of host resilience and the onset of disease (Teplitski *et al.* 2016). Given that environmental conditions are being rapidly altered by anthropogenic climate change, dysbiosis may become more common, with major implications for all multicellular life.

Reef-building corals are host to one of the most taxonomically and functionally diverse microbiomes known, greatly exceeding the microbial diversity of the human gut and rivaling that of sponges (Blackall *et al.* 2015). The success of corals in colonizing the vast majority of shallow tropical oceans is often credited to their affiliated microbes, specifically endosymbiotic dinoflagellate microalgae of the family Symbiodiniaceae (Figure 1) and associated prokaryotes. These endosymbiotic microalgae support coral growth and health by contributing to both carbon fixation and carbon translocation to the coral host, which can meet or even exceed its respiratory requirements (Muscatine and Porter 1977). Coral-associated prokaryotes have diverse metabolic roles and contribute substantially to nitrogen (Lesser *et al.* 2007), carbon (Neave *et al.* 2017), and sulfur (Raina *et al*. 2013) cycling. Hundreds to thousands of operational taxonomic units (OTUs), or putative species, have been identified within single coral species (Hernandez-Agreda *et al.* 2017). The number of prokaryotic cells alone can be as high as 1×10^6 per square centimeter of coral surface area (Blackall *et al.* 2015), which is between 100 and 1000 times the number of prokaryotic cells found per square centimeter on human skin (Whitman *et al.* 1998). Despite the high microbial diversity and variability in corals, researchers are now working on identifying the core microbiome of corals (ie the microbes that are commonly present among conspecifics or even across species; Hernandez-Agreda *et al.* 2017). It has been proposed that the coral microbiome is partitioned into a stable and persistent core component, a species or location-specific component, and a large and highly variable component that is influenced by both biotic and abiotic factors (Hernandez-Agreda *et al.* 2016).

Rapid climate change as a result of anthropogenic greenhouse-gas (GHG) emissions is putting unprecedented stress on coral reef ecosystems (Hoegh-Guldberg 1999; Hughes *et al*. 2018). Corals already live close to their physiological and thermal limits, and there is widespread concern that climatic changes will outpace the rate at which corals can adapt or acclimatize (van Oppen *et al.* 2015, 2017). Although microbes have been proposed as one route for rapid acclimatization/adaptation (eg due to their shorter generation times; Zilber-Rosenberg and Rosenberg 2008), a combination of stressors, including elevated seawater temperatures, ocean acidification, and eutrophication, can have direct effects on coral microbial community structure (Vega-Thurber *et al.* 2009; Ziegler *et al.* 2017). Anthropogenic environmental changes may drive alterations to or losses in members of both the Symbiodiniaceae and prokaryote communities (or their activities) that can result in coral bleaching or disease, and even coral death. This process may, in part, drive reef loss on a global scale.

As ocean temperatures rise and mass-scale coral bleaching events decimate coral reefs worldwide (Hughes *et al.* 2018), it has become necessary to focus efforts on preventing further damage to and loss of these systems. Although this ultimately involves tackling climate change and curbing GHG emissions, targeted efforts are being made toward applying microbiome research to inform the development of conservation tools that may buy corals more time (Figure 2). One such approach is microbiome engineering (ME), which we define here as the experimental manipulation of individual microbes, microbial communities, or their hosts (specifically, host-based mechanisms of microbial recognition) – all of which can be used to improve host performance and fitness. ME can be achieved through exerting artificial selection on the host–microbiome association, inoculating the host with beneficial microbes, genetically engineering specific microbial strains, or a combination of these approaches. ME could be a powerful tool for boosting coral resilience to climate change (van Oppen *et al*. 2015, 2017; Damjanovic *et al.* 2017). Industries such as agriculture and human medicine now use ME approaches regularly to improve crop yields and human health, respectively. Although only a few studies have implemented an experimental approach to shaping coral microbiomes (eg dos Santos *et al.* 2015; Chakravarti *et al.* 2017; Damjanovic *et al.* 2017), theoretical work – including the Coral Probiotic Hypothesis (Reshef *et al.* 2006) and the Beneficial Microorganisms for Corals (BMC) concept (Peixoto *et al.* 2017; Panel 1) – and empirical studies from other systems suggest that artificial selection on a microbiome can improve host fitness over relatively short time frames; as such, ME could prove to be an important mechanism for mitigating disease and increasing stress tolerance or climate resilience in corals.

We outline current applications of ME, discuss how these approaches may be applied to corals, and consider the implications they may have for coral reef restoration and conservation.

Current applications of ME

ME in plants and soil

Plants live in close association with a complex and diverse microbial community that inhabits both their tissues and the soil in which they grow. Fungal communities living within plant tissues provide protection for the host plant against salinity (Waller *et al.* 2005) and heat (Redman *et al.* 2002) stress, as well as drought conditions (Rodriguez and Redman 2008); Redman *et al.* (2002), for instance, reported that the thermal tolerance of the grass species *Dichanthelium lanuginosum* was dependent on the presence of a specific *Curvularia* fungus. The region of soil directly adjacent to plant roots – the rhizosphere – also hosts microbiota that play active roles in mediating nutrient cycling, pathogen resistance, host immunity/adaptation, and stress tolerance in plants (reviewed in Berendsen *et al.* 2012).

Exploitation of soil and plant microbiota through ME approaches has been implemented to modify specific traits to improve the quality and productivity of plants, including crops. For example, through the application of a combination of ME approaches, researchers were able to alter rhizosphere bacteria to modify the flowering time of plants; over 10 generations, rhizosphere bacterial communities were selected for their ability to induce shifts in the timing (either early or late) of flowering in *Arabidopsis thaliana*, and these bacterial communities were then introduced to the rhizosphere of four new host plants, where they were able to reproduce a shift in their respective host’s flowering time (Panke-Buisse *et al.* 2015). Alteration of specific host traits through microbiome manipulation provides a platform for improving agricultural production and food security, or for increasing plant host resilience to disease and environmental stressors. Other implementations of ME approaches that use a plant–soil feedback include crop rotations that reshape soil microbiota to increase the yield of subsequent crops (Deguchi *et al.* 2007), inoculations with specific rhizosphere bacterial strains to stimulate plant growth (Rojas-Solís *et al.* 2018), and inoculations with rhizosphere bacterial mixtures to promote systemic resistance against pests and disease (Ramamoorthy *et al.* 2001). It has recently been discovered that direct tissue inoculations can also confer pathogen resistance; for instance, endophytic bacteria isolated from manuka (*Leptospermum scoparium*), which naturally produces antimicrobial oils, have been used to treat bacterial canker in kiwifruit (*Actinidia* spp) through direct wound inoculation (Wicaksono *et al.* 2018).

ME in disease mitigation

It has become increasingly evident that many human diseases are strongly linked to the breakdown of the symbiotic microbial community (eg obesity [Turnbaugh *et al.* 2009], inflammatory bowel disease [IBD; Gupta *et al.* 2016]), and therefore mitigation of some diseases could be achieved through manipulations of the human microbiome. For instance, it is suggested that dysbiosis of the intestinal microbial community in humans promotes inflammation and cause IBD, which is characterized by the inflammation of the gastrointestinal tract and includes such disorders as ulcerative colitis and Crohn’s disease. One potential treatment of IBD is the use of fecal microbiota transplants, in which fecal matter from a healthy donor is introduced to the intestinal tract of a diseased recipient as a means of “resetting” the disrupted microbiome (reviewed in Gupta *et al.* 2016).

ME has been identified as a particularly important tool for mitigating outbreaks of vector-borne human diseases, but in such cases the microbiome of the pathogen vector (eg the mosquito; Culicidae) is targeted for manipulation instead of the human microbiome. Mosquitoes can carry pathogens that cause serious human diseases, such as Zika virus, yellow fever, and dengue fever (Benelli 2015). Dengue is an especially common disease in tropical and subtropical areas across the globe, traditional management of which has focused on eliminating mosquitoes through insecticidal regimes. These programs have been insufficient, as evidenced by the increase in contracted cases of dengue (Schmidt *et al.* 2017) but recent work has shown that inoculating dengue-carrying mosquitoes with the bacterium *Wolbachia* shortens the lifespan of mosquitoes and consequently reduces the potential for disease transmission to humans (McMeniman *et al.* 2009). *Wolbachia* has also been found to reduce the susceptibility of mosquitoes to initial dengue infection (Moreira *et al.* 2009) and can limit the replication of dengue virus within the mosquito (Frentiu *et al.* 2014).

Inoculation of *Wolbachia* into mosquitoes will possibly become an important control measure for the suppression of dengue fever in humans, and research is now focused on how to scale up the spread of *Wolbachia*-inoculated mosquitoes to cover large urban areas (eg The World Mosquito Program, www.eliminatedengue.com/program; Schmidt *et al.* 2017).

Inoculation with certain microbes can also prevent disease in non-human hosts. For example, bacteria recovered from coral mucus and Symbiodiniaceae symbionts can disrupt biofilm formation of the necrotizing coral pathogen *Serratia marcescens* through the inhibition of quorum sensing (Alagely *et al.* 2011), which is the cell-to-cell communication used by bacteria to control collective behavior and organize themselves spatially, particularly into biofilms. This behavior can allow these biofilms to resist antimicrobial or antibacterial compounds and can also help in the regulation of pathogenicity (Pasmore and Costerton 2003). To experimentally test the antimicrobial effect of the bacteria recovered from coral mucus and Symbiodiniaceae, Alagely *et al.* (2011) inoculated the anemone *Exaiptasia pallida* with the pathogen *S marcescens*, after first introducing the anemone to a cocktail of bacteria. They found that use of the beneficial bacteria as a probiotic inhibited progression of the disease caused by *S marcescens* by blocking its quorum sensing and thus interfering with biofilm formation (Alagely *et al.* 2011). In addition to demonstrating the antimicrobial properties of coral mucus and Symbiodiniaceae, as well as highlighting the success of inhibiting quorum sensing, this was also one of the first studies to test the feasibility and viability of probiotics in a cnidarian model.

ME in enhancing stress tolerance in corals

Research and development of ME applications to corals is still in its early stages. One of the first successful empirical studies of coral microbiome manipulation explored the possibility of using microbial inoculations to increase the resistance of corals to oil pollution; in an aquarium experiment, dos Santos *et al.* (2015) inoculated coral with a consortium of bacteria, which had been selected for its collective ability to degrade water-soluble oil (ie a BMC consortium), and thereby reduced the negative health impacts of oil exposure to the coral host. Other researchers have begun to investigate the possibility of inoculating corals with heat-selected members of the Symbiodiniaceae (Chakravarti *et al.* 2017) or communities of bacteria from "donor" heat-resistant corals (Damjanovic *et al.* 2017), with the goal of increasing coral resilience to thermal stress. Although both of those studies reported successful inoculation, the extent to which the manipulations conferred thermal tolerance to the coral host and the long-term stability of the introduced symbiosis remain uncertain. Coral ME is clearly still in the "proof of concept" stage, but empirical studies such as these form a promising foundation for future experimental work.

The way forward: research priorities for ME in corals

Like other host–microbiome systems, harnessing the benefits of the coral microbiome is a challenge due to the high diversity and spatiotemporal variability of microbial partners in corals. Advancing the field of coral ME requires a focus on specific research topics that will help address major knowledge gaps. We propose three major research priorities that will help elucidate the viability of using ME as a restoration tool for corals: (1) identifying beneficial microbial functions, (2) identifying and maintaining stable associations with microbial partners, and (3) performing trial experimental manipulations of coral microbiomes.

Identifying beneficial microbial functions

Understanding and identifying the functional roles of the microbial consortia and/or individual microbes is essential for determining which services provided by the microbiome can be harnessed to increase coral host climate resilience. Specific microbes that assert control over a host phenotypic trait will be key targets for ME (eg *Wolbachia* in mosquitoes [McMeniman *et al.* 2009], *Curvularia* in grass [Redman *et al*. 2002]). For corals, as described above, this was recently proposed as the BMC concept (Peixoto *et al*. 2017), and could be used to increase thermal tolerance. Functional analysis can be performed through traditional culturing methods in combination with phenotypic assays, metabarcoding (biodiversity assessment through targeting of specific microbial genes), metagenomics (study and analysis of microbial genomes), metatranscriptomics (study and analysis of microbial gene expression), and metaproteomics (assessment of proteins contained within a targeted microbiome) to decipher the functional properties of either the entire microbiome or specific microbial partners within it (Marx 2017). Metabarcoding has already been widely applied to corals (Blackall *et al.* 2015), but the functional potential of the microbiome cannot be predicted from barcode data, such as the barcode region of the 16S ribosomal RNA (rRNA) gene, and requires genome sequences. Metatranscriptomics and metaproteomics reveal which microbial genes are active at a given time point. This approach has been implemented in more recent studies, such as an examination of active microbial players in coral disease (eg Daniels *et al.* 2015) and identification of key proteins of healthy and diseased corals, which provided insights into how the transition occurs between these two states (Garcia *et al.* 2016). These “meta’omics” techniques, in addition to traditional culturing methods, may offer further elucidation of active and important microbial partners during climate-based stress events (eg coral bleaching), which can then be targeted for ME.

Identifying and maintaining stable associations with microbial partners

Deciphering which microbes are stably associated with corals is important for the development of microbial manipulations to enhance coral climate resilience. For instance, if probiotic inocula are composed of microbes that form a temporally stable association, they are more likely to provide long-term benefits to the coral host; if they are not, then the benefits will likely be only short term. Members of coral microbial communities are susceptible to changes throughout their development due to variation in environmental conditions (Figure 3). Nevertheless, a growing body of evidence suggests that despite these temporal and spatial changes in microbial communities, a subset of taxa is stably associated with coral species or genera (ie the core microbiome; Hernandez-Agreda *et al.* 2017). Improving the current understanding of how and when temporal and spatial shifts occur, such as the natural winnowing of the coral microbiome (eg Lema *et al.* 2014), and whether these shifts represent active host-controlled processes (eg Sweet *et al.* 2011), will allow scientists to determine a possible developmental stage or time at which introduced manipulations would be more likely to remain stable over time.

The stability of associations between microbes and hosts has been suggested to relate to the mode of transmission, where maternally transmitted (ie inherited) symbionts are typically more stably associated with their host than those transmitted (ie acquired) from the environment (Moran *et al.* 2008). However, this is not always the case. In corals, reproductive strategy is highly correlated with the mode of transmission of the dinoflagellate endosymbionts, where most broadcast spawning corals exhibit horizontal transmission (environmental transmission) and most brooders (corals that develop their larvae within the body cavity of polyps) exhibit vertical transmission (parental transmission) (Lesser *et al.* 2013). Although the long-term stability of Symbiodiniaceae symbionts in brooding corals exceeds the stability in broadcast spawners (Thornhill *et al.* 2006), some brooders acquire additional symbionts from the environment, which can also be maternally transmitted to the next generation (Quigley *et al.* 2018). In addition, other invertebrates have been found to form stable relationships with horizontally transmitted microbes. For example, the Hawaiian bobtail squid (*Euprymna scolopes*) initially acquires the bacterium *Vibrio fischeri* from the environment and harnesses the bacterium's bioluminescence ability for use in an organ that emits the bacterially produced light. This symbiosis is stable throughout the life of the squid, and is maintained through the expulsion of a majority of the bacteria during the day (time of quiescence) and subsequent regrowth of the bacterial population prior to night (time of activity) (Nyholm and McFall-Ngai 2004).

The mode of transmission of prokaryotes in corals appears to be less strictly correlated to reproductive strategy. For instance, horizontal transmission of prokaryotes has been detected in the broadcast spawning coral species *Pocillopora meandrina* (Apprill *et al*. 2009), where it is suggested that external bacteria are incorporated into the ectodermal tissues of late stage planulae via phagocytosis. Conversely, *Mussismilia hispida*, also a broadcast spawner, has been found to vertically transfer prokaryotes from parental mucus to gametes prior to spawning (Leite *et al.* 2017). Gaining a better understanding of prokaryote transmission in a range of coral species, along with assessing the maintenance of these prokaryotic associations (eg whether any host or microbe-controlled cellular mechanism is at work) through time or across generations, may help to elucidate microbiome stability and identify better BMC targets for ME trials.

Performing trial experimental manipulations of coral microbiomes

Lessons learned from other host systems, such as plants and humans, can provide a foundation for developing more effective experimental manipulations in corals that, if proven successful, can subsequently be implemented as a tool for improving the success of coral restoration initiatives. Two main experimental approaches to manipulating microbiomes, through direct and indirect selection (Mueller and Sachs 2015), may be particularly useful for structuring coral ME research (Panel 2). Direct selection on the microbiome can be used to identify and target specific microbes responsible for thermal or other stress tolerance (eg dos Santos *et al.* 2015; Chakravarti *et al.* 2017). Advances in coral ME for addressing climate-change impacts may also focus on indirect selection. For instance, large multigenerational experiments that examine the effects of current and future climate change on corals (eg Evolution 21; www.aims.gov.au/evolution-21) provide opportunities to obtain critical information on how microbial communities respond to climate change. As corals acclimatize to these future conditions either within or across generations, it may be possible to identify key microbial partners that aid in acclimatization of the coral holobiont to future conditions.

Experimental manipulations, whether based on direct or indirect selection, provide an important means to address many of the knowledge gaps already identified. For instance, if a manipulation experiment is successful in achieving a desired trait without researchers having prior knowledge of the underlying biological/microbial mechanisms, it is possible for them to work backward to hypothesize how and why observed shifts in the microbiome occurred. The treatment of wastewater began in this way; since the early 1900s, the nutrient cycling capabilities of microorganisms have been harnessed to remove excess nutrients from industrial or municipal wastewater, but these were "black-box" treatments, for which the chemical composition of influent and effluent was known, while the biochemical pathways were only hypothesized and the microbial species involved were unknown. For example, the enhanced biological phosphorus removal (EBPR) method was first employed in the 1970s (Barnard 1974), but it was not until the 1990s that polyphosphate-accumulating microbes were confirmed to be responsible for phosphate removal from wastewater (Jenkins and Tandoi 1991). EBPR works by cycling influent wastewater and its associated microbial biomass first through an anaerobic zone and then through an aerobic zone in order to place selection pressure on the microbial biomass. Cycling in this way results in a selection of microorganisms with a higher capacity for intracellular accumulation of polyphosphates (Blackall *et al*. 2002). The discovery of microorganisms as the main drivers for wastewater treatment was a revelation, and modern methods are now informed by more rigorous microbiological and microbial ecological studies to enhance treatment efficiency (Barnard *et al.* 2017). Although wastewater treatment is a closed system, as opposed to the natural system in which corals are found, this built-environment example clearly demonstrates that working backward from experimental manipulations can result in important advances in ME techniques, and provides a unique view on using indirect selection pressure to manipulate a microbial community to select a targeted function.

Conclusions

The broad spectrum of ME approaches provides a powerful basis for the application of ME to enhance climate resilience in corals. However, the field of coral ME is in its infancy. Current progress is focused primarily on proof of concept, which will provide the foundations for future management options but requires further empirical and applied studies to advance toward successful implementation. Success in other fields, such as agriculture, medicine, and wastewater treatment, can provide direction and guidance for applying ME approaches in corals to improve coral health, increase the number and/or quality of ecosystem services, and enhance resilience to the stressors of climate change. Here, we have identified three key research priorities that will foster successful development of ME approaches in corals, addressed crucial knowledge gaps, and offered insight into the biological challenges of implementing these approaches in a naturally complex environment and on large geographic scales. With climate stressors causing unprecedented changes in coral reef systems, effective conservation, restoration, and management are more critical than ever, and we propose that ME can be a valuable tool for coral reef restoration. Such interventions should be combined with other practices to help resolve the coral reef crisis, the most important of which is the curbing of GHG emissions.

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Supporting Information

Additional, web-only material may be found in the online version of this article at

Figure 1. A 5-day-old coral recruit with algal symbionts (species within the Symbiodiniaceae) clearly seen within its tissues.

Figure 2. Members of the coral microbiome and potential applications for their use in coral reef management or restoration. BMC: Beneficial Microorganisms for Corals concept (Panel 1; Peixoto *et al*. 2017).

Figure 3. Schematic of the transmission of coral-associated prokaryotes throughout the developmental stages, as well as shifts in community composition resulting from an environmental stress event such as bleaching. Although some core members remain the

same (represented by the blue and gold dots), prokaryote community composition can be highly affected by changes in environmental conditions.

SPS – please embed Figure 4 in Panel 2

Figure 4. (a) Implementation of direct selection of beneficial microbes for inoculation. (b) Implementation of a selection pressure on the coral host to indirectly select beneficial microbes for inoculation.

Panel 1. Theoretical concepts in coral ME

The Coral Probiotic Hypothesis

The Coral Probiotic Hypothesis (Reshef *et al.* 2006) was originally proposed to help explain the evolutionary success of corals. Conceptually, this hypothesis is straightforward: the coral is host to a diverse and metabolically active population of symbiotic microorganisms (the microbiome), the abundance and diversity of which can change in response to variation in environmental conditions in a manner that may allow for holobiont acclimatization or adaptation to those new conditions. Under high selection pressure, these microbiome changes can occur within days to weeks, and may enable host adaptation at a much faster rate than natural selection on the host genome.

The Coral Probiotic Hypothesis makes several predictions that can be tested. As outlined in Reshef *et al.* (2006), these include (but are not limited to):

- in corals that become resistant to a particular pathogenic bacterium, there should be a visible increase in the abundance of a counteractive bacterial strain that can prevent or inhibit the pathogen;
- in corals treated with antibiotic compounds that remove beneficial bacteria, there should be evidence of infection and reduced metabolic activity;
- when exposed to slowly increasing temperatures, corals should adapt or acclimatize more readily to temperature stress than when temperatures are increased at a more rapid pace; and
- a coral treated with an inoculum of bacteria taken from a stress-adapted conspecific should adapt or acclimatize to that stress more rapidly.

The Beneficial Microorganisms for Corals (BMC) concept

Piexoto *et al.* (2017) recently expanded on the Coral Probiotic Hypothesis by proposing the BMC concept. While the main theoretical components of this concept are similar to those of the Coral Probiotic Hypothesis, the BMC concept provides a method for further applying the Coral Probiotic Hypothesis to coral microbial research through two procedures:

- first, by proposing potentially beneficial mechanisms provided by the microbiome and isolating any potentially beneficial microbial players, or BMC; and
- second, by empirically testing these BMC for their role in coral resilience to environmental perturbations, both in situ and in controlled aquarium systems.

Panel 2. Experimental approaches to ME in corals

Two main experimental approaches, originally identified by Mueller and Sachs (2015) for plants, can be applied to structuring coral ME research: namely, the direct and indirect selection of the microbiome.

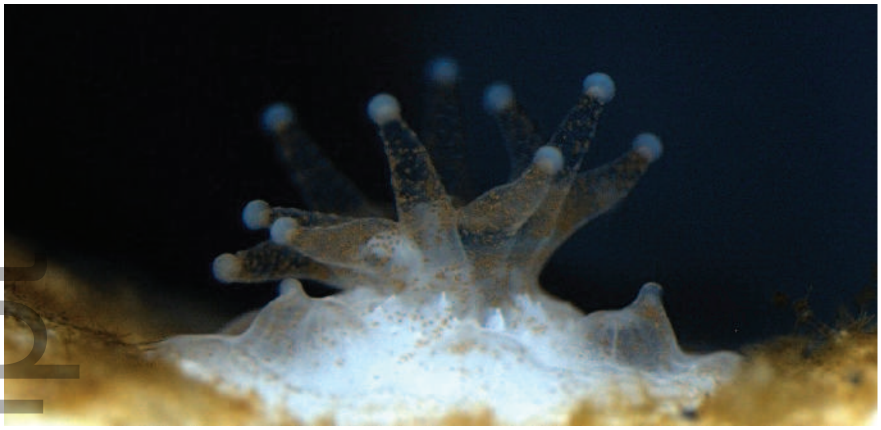
Direct selection: selection of a specific beneficial microbe or community of microbes for improved host performance or fitness (Figure 4a).

Advantages: greater control over the manipulation of individual taxa or communities to target beneficial microbial roles. *Disadvantages*: it is necessary to have prior knowledge of microbial function through meta'omics or culturing methods, or by characterizing holobiont or microbial phenotypes, to choose the appropriate beneficial microbe or microbial consortium. Furthermore, direct selection can only be done for culturable microbes. *Example*: Redman *et al.* (2011) found that inoculating rice (*Oryza sativa*) plants with a specific endophytic fungus increased rice growth rate, biomass, and reproductive yield while at the same time reducing water consumption by 20–30%, allowing rice plants to thrive in drought conditions. *Application to corals*: similar to the BMC concept (Peixoto *et al.* 2017), direct selection on the microbiome can be performed in corals by isolating microbes with certain traits and using these isolates to create an inoculum. Experiments of this sort should be carried out using replicate but known coral genotypes to reveal the effects of the host genotype on the microbiome.

Indirect selection: selection of a specific host trait (phenotype), allowing for an indirect selection of microbiome function (Figure 4b).

Advantages: prior knowledge of microbiome function is not necessary, making indirect selection a more cost-effective method of ME. *Disadvantages*: experiments may be time-consuming, as they can span multiple generations of the host, and there is little to no control over which microbial taxa are being manipulated, which affects the reproducibility of results. It must also be demonstrated that the microbiome did in fact change and play a role in selection of the host trait. *Example*: by selectively breeding the best performing wheat plants (*Triticum* spp) that were growing in acidic soils, de Sousa (1998) was able to develop aluminum resistance via indirect selection, a process controlled by the microbiome. *Application to corals*: this approach, which has been previously proposed as a mechanism of assisted evolution for corals (van Oppen *et al*. 2015), will involve the use of a selection pressure, such as elevated temperature, to select for a specific phenotypic trait (eg reduced susceptibility to bleaching). From that point, it will be possible either to continue down a selective breeding pathway of the host or to create an inoculum with the selected host's tissues and/or mucus to inoculate other corals.

For either approach to be successful, ME must be optimized to target key coral host traits that will enhance climate resilience, such as reduced susceptibility to bleaching, increased disease resistance, enhanced calcification or growth, and/or increased fecundity.



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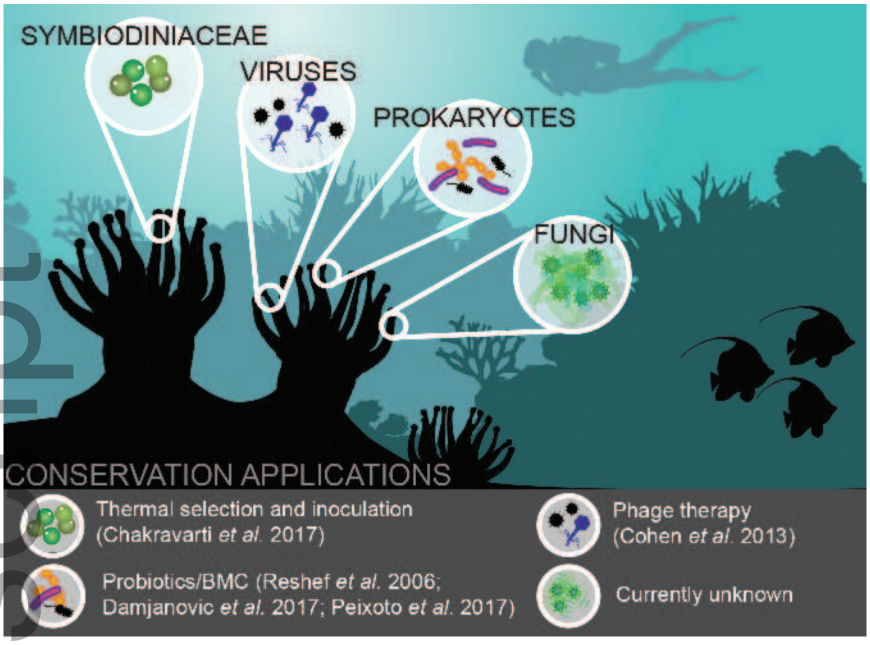

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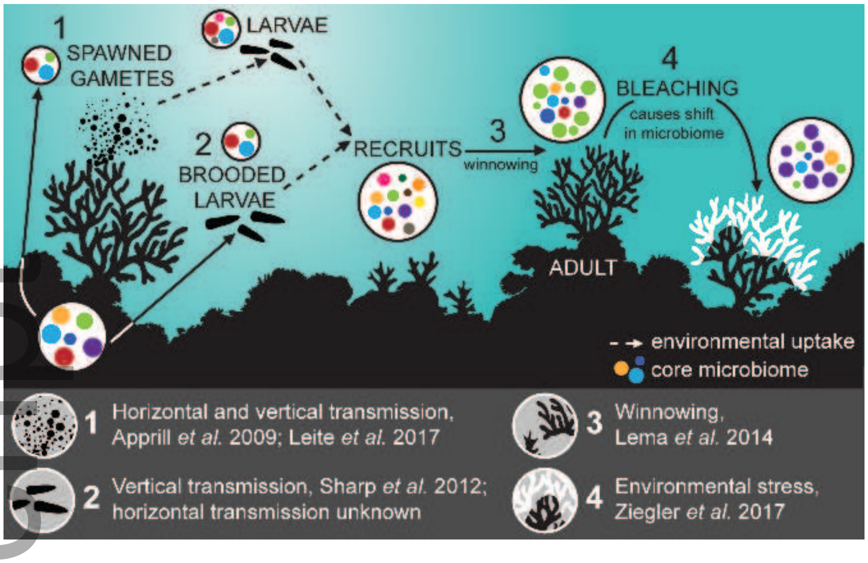

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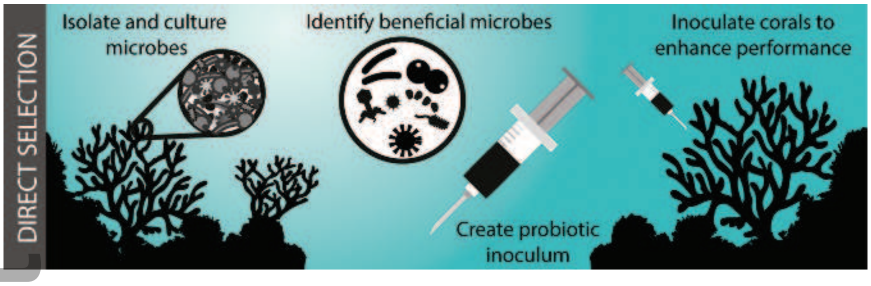

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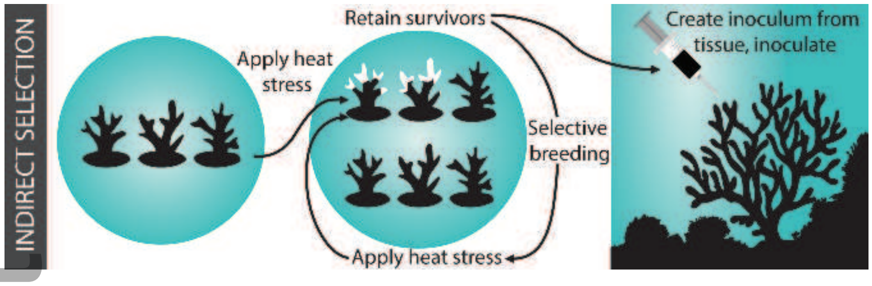

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