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microbial biotechnology

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The contribution of microbial biotechnology to mitigating coral reef degradation

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Reef-forming scleractinian corals are foundation species of coral reefs, as they build the three-dimensional structure of the reef, provide habitat to possibly millions of marine species and represent the most important primary producers through their association with microbial photosymbionts (*Symbiodinium* spp.) (Harrison and Booth, 2007). In addition to being extremely rich reservoirs of biodiversity, coral reefs greatly contribute to coastal protection, tourism and fisheries and their economic value is estimated in the range of billions of US dollars (Costanza *et al.*, 1997; Burke *et al.*, 2011). Coral reefs have suffered major declines over the past four decades, mainly due to anthropogenic disturbances acting on both local (e.g. overharvesting, pollution) and global (e.g. effects of climate change) scales (Bruno and Selig, 2007; Hoegh-Guldberg, 2011; De'ath *et al.*, 2012). Elevated sea surface temperature (SST) is a major driver of coral bleaching and mortality because it disrupts the critical relationship between corals and their endosymbiotic *Symbiodinium* (Hoegh-Guldberg, 1999; Lesser, 2011). The recent global mass bleaching event that lasted from 2014 to 2016 and which caused unprecedented coral bleaching and mortality (Normile, 2016; Hughes *et al.*, 2017) is reported to be the longest and most widespread on record (Cresset, 2016; NOAA Coral Reef Watch 2017).

Combatting the impacts of climate change and conserving marine resources are among the United Nations' sustainable development goals (United Nations 2017). Despite increasing awareness of the threats of climate change to biodiversity and the establishment of guidelines to preserve marine ecosystems, environmental degradation is occurring faster than the pace of coral adaptation through natural selection on standing genetic variation (i.e. genetic adaptation) (Hoegh-Guldberg, 2004; Hoegh-Guldberg *et al.*, 2007). Active interventions to help corals survive by augmenting their tolerance to and ability to recover from stress are therefore urgently required. Recently, the concept of assisted evolution has been proposed as a possible strategy for accelerating the rate of naturally occurring evolutionary processes and to develop corals capable of coping with current climate change trajectories (van Oppen *et al.*, 2015). Assisted evolution includes selective breeding of coral, preconditioning of coral to sublethal stress, laboratory evolution of *Symbiodinium* followed by inoculation of the coral with tolerant algal symbionts, and manipulation of various members of the coral microbiome (van Oppen *et al.*, 2015, 2017). This commentary focuses on the potential to mitigate coral reef degradation through the manipulation of coral-associated prokaryotes.

Corals are colonized by a huge diversity of prokaryotes (Rohwer *et al.*, 2002; Blackall *et al.*, 2015), with distinct communities occupying various microhabitats within the host, including coral tissues, the surface mucus layer, the gastric cavity and the skeleton (Sweet *et al.*, 2010; Bourne *et al.*, 2016). Bacteria scavenge limiting nutrients (Knowlton and Rohwer, 2003; Zhang *et al.*, 2015), deliver essential products to their host following carbon and nitrogen fixation (Lesser *et al.*, 2007; Kimes *et al.*, 2010) and participate in sulfur and phosphorus cycling (Raina *et al.*, 2009; Zhang *et al.*, 2015). Further, bacteria contribute to coral immune defences by occupying entry niches and by secreting antimicrobial peptides (Ritchie, 2006; Nissimov *et al.*, 2009; Shnit-Orland and Kushmaro, 2009). The composition of the coral microbiome can change with coral life stage, host health state, water temperature and acidity, nutrient levels, pollution, the presence of macroalgae, light intensity, depth or seasonal variation (Hernandez-Agreda *et al.*, 2016a; Glasl *et al.*,

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2017; Sweet and Bulling, 2017). Maintaining a healthy microbiome is thought to be essential for the well-being of corals, as destabilization in the composition and function of their associated microbial communities has been shown to take place in diseased states (Frias-Lopez *et al*., 2002; Jones *et al*., 2004; Gil-Agudelo *et al*., 2006; Sato *et al*., 2013) and under stressful environmental conditions (Zaneveld *et al*., 2016). Elevated seawater temperatures and coral bleaching are typically associated with a shift towards opportunistic and/or pathogenic bacteria, with concomitant declines in coral health (Ritchie, 2006; Bourne *et al*., 2008; Littman *et al*., 2011; Lins-de-Barros *et al*., 2013; Tout *et al*., 2015). Conversely, in some instances coral-associated microbes remain stable despite different host phenotypes (Hadaidi *et al*., 2017), changing environmental conditions (Teplitski *et al*., 2016) or in the presence of stressors (such as increased pCO_2) (Webster *et al*., 2016; Zhou *et al*., 2016).

While an optimal microbiome may help protect the host from environmental pressures or compromised health by preserving beneficial functions, its dynamic nature may also confer an adaptive potential (Webster and Reusch, 2017). Whether by alteration in the relative abundance of certain species, acquisition of new species or variants from the environment or by mutations in the genomes of the existing community, modification of the microbiome is hypothesized to provide physiological flexibility to respond to environmental disturbances (Reshef *et al*., 2006; Rosenberg *et al*., 2007). For instance, reciprocal transplantation of *Acropora hyacinthus* fragments between thermally distinct environments on the same reef resulted in an adjustment of the microbial communities to the new conditions (Ziegler *et al*., 2017). While the microbiome of cross-transplanted corals was indistinguishable from the microbiome of native corals in the same pool, it changed compared to the microbiome of the back-transplanted counterparts (Fig. 1A). Moreover, when subjected to short-term heat stress, corals that had spent the preceding 17 months in the more variable and warmer thermal regime were found to bleach less and retained a more stable microbiome. These results suggest that microbial community composition influences the response to heat stress, although host genetic adaptation and acclimatization are known to play additional roles (Barshis *et al*., 2013; Bay and Palumbi, 2014).

As the composition and function of microbial communities seem to impact the fitness of their host, manipulation of resident prokaryotes could serve as a powerful tool to increase coral tolerance to stress and assist their adaptation to a changing environment. Such approaches are increasingly used in other biological systems. For instance, in humans, faecal microbiome transplantation is now accepted as an effective treatment for *Clostridium difficile* infections and is also gaining momentum for the treatment of other bowel conditions (Borody and Khoruts, 2012; Gupta *et al*., 2016). In agriculture, inoculation of rice plants with microbes collected from other plant species growing in extreme environments can enhance the rice plants' tolerance to drought, salinity and low temperatures (Redman *et al*., 2011). In contrast to an entire microbiome transplant approach, bacterial species can also be selected and administered to the host to promote health. Probiotics are, for example, widely used in the aquaculture industry to stimulate growth, inhibit pathogens, improve water quality or augment tolerance to stress (Verschuere *et al*., 2000; Martinez Cruz *et al*., 2012; Boutin *et al*., 2013). While these approaches have not been readily applied in open marine systems, the biological control of coral diseases using phage therapy has already shown some promising outcomes in confined areas and in the laboratory for preventing and treating specific infections (Atad *et al*., 2012; Cohen *et al*., 2013).

Preliminary studies indicate that coral-associated prokaryotes can be manipulated through inoculations with specific taxa. Bacteria collected from the coral *Mussismilia hartii* were cultured on a selective medium to isolate strains capable of degrading water-soluble oil fractions (WSFs) (dos Santos *et al*., 2015). When subjected to conditions simulating an oil spill, polyps of *M. hartii* inoculated with a WSF-degrading bacterial consortium (i.e. probiotic bacteria) were less negatively affected compared to the non-inoculated polyps, as assessed by higher photosynthetic efficiencies of photosystem II of *Symbiodinium* (Fig. 1B). In this experiment, exposure to a specific microbial mixture therefore conferred health benefits to corals under environmental stress. Our preliminary experiments further support that the coral microbiome can be artificially influenced through microbiome inoculation (Data S1, S2, S3). Larvae of *Acropora tenuis* were exposed to the mucus collected from either *Acropora sarmentosa, Acropora tenuis, Diploastrea heliopora* or *Galaxea astreata*, and a no-mucus treatment was included as a control (Fig. 1C). Mucus was chosen as the inoculum as it contains a high density of coral prokaryotes (Thompson *et al*., 2014) and can be easily collected after briefly exposing coral colonies to air (Brown and Bythell, 2005). Once settled, *A. tenuis* recruits were reared in filter-sterilized and flow-through sea water for 4 months, before being sampled to assess prokaryote microbiome composition. PERMANOVA of Bray–Curtis dissimilarities indicated that the microbiomes differed significantly across treatments suggesting that a single dosage drove the microbiome of experimental corals to develop in distinct directions (Fig. 1C).

Despite these recent encouraging outcomes from artificial microbial manipulations, important challenges for

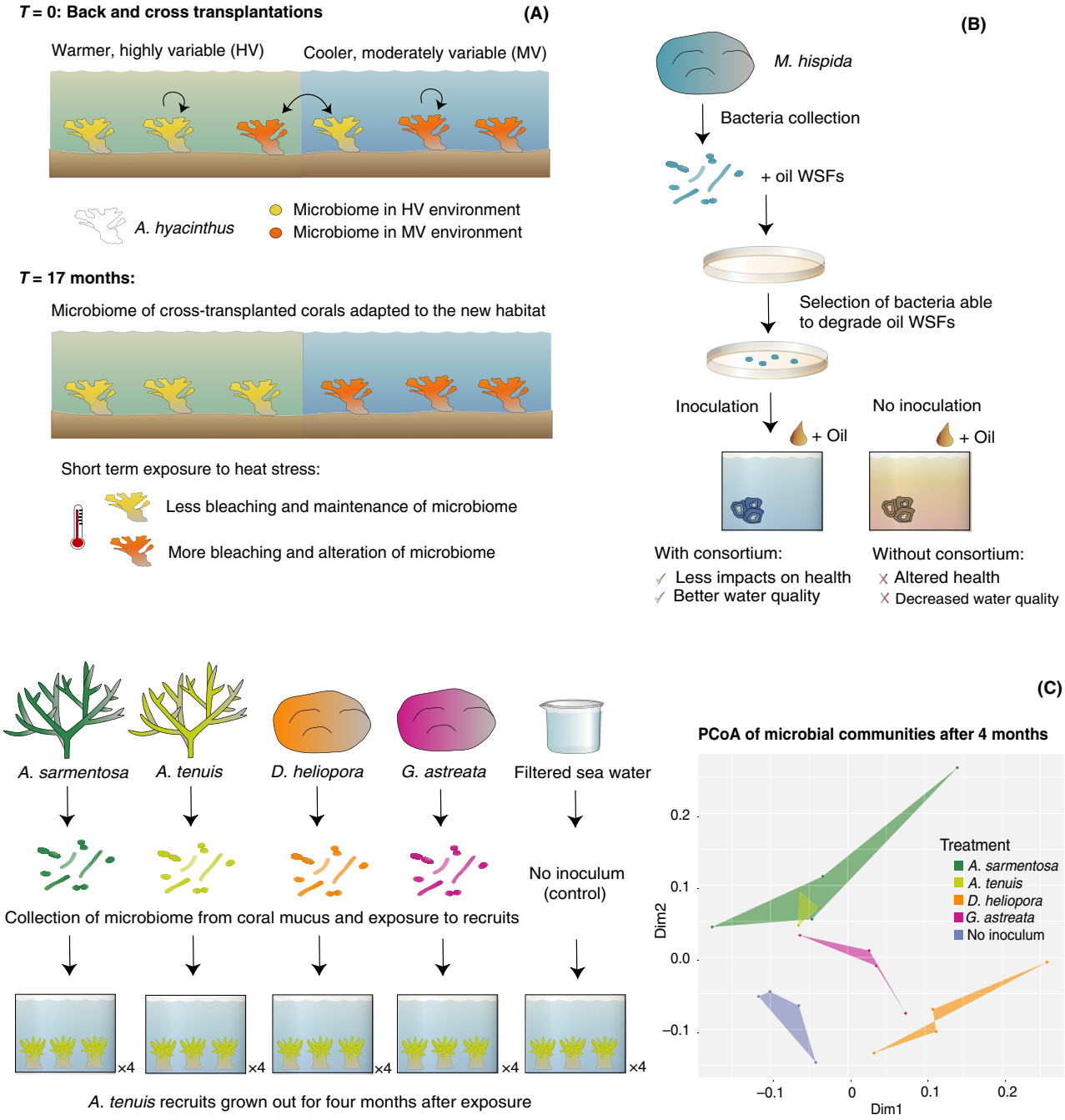

Fig. 1. Examples of studies involving coral microbiome manipulation.
A. Transplantation of *A. hyacinthus* fragments between regions of different thermal regimes induced a change in their microbiome (Ziegler *et al.*, 2017). After 17 months, corals that inhabited the highly variable, warmer environment (HV) harboured a microbiome that was distinct from corals located in the cooler and more stable environment (MV). When exposed to short-term heat stress, fragments from the HV environment bleached less, which could reflect a protective effect of their microbiome.
B. Bacteria isolated from *M. hartii* were selected for their ability to degrade oil WSFs (dos Santos *et al.*, 2015). Replicate coral colonies were inoculated with the selected bacterial consortium, while others were not exposed to these bacteria (controls). After subjecting the colonies to a treatment simulating an oil spill, the presence of bacteria able to degrade oil WSFs helped to preserve better water quality in the oil treatment and reduced negative effects on coral health.
C. *A. tenuis* larvae from a common pool were distributed across 20 experimental tanks. Filtered sea water or 5-μm filtered mucus collected from four different coral species were then introduced into four replicate tanks per treatment. Water flow was turned off overnight, and recruits were subsequently reared in flow-through filtered sea water. After four months, recruits were sampled for 16S rRNA gene amplicon sequencing. PERMANOVA of the Bray–Curtis dissimilarities at the OTU level based on 97% sequence identity detected significant differences in the prokaryotic communities associated with recruits that were exposed to distinct inocula (pseudo $F_{4,14} = 1.7015$, $P < 0.01$).

broadscale application in corals need to be addressed and overcome. First, the functions of the vast majority of coral-associated prokaryotes are yet to be deciphered. We are still at a stage of correlating the presence of specific microbes with coral features as exemplified by *Endozoicomonas* (frequently reported to be present on healthy corals) (Kvennefors *et al*., 2010; Morrow *et al*., 2012; Meyer *et al*., 2014; Glasl *et al*., 2016; Neave *et al*., 2017), *Roseobacter* (particularly common in juvenile corals) (Apprill *et al*., 2012; Ceh *et al*., 2012, 2013; Sharp *et al*., 2012; Lema *et al*., 2014) and diazotrophs (nitrogen provisioning to corals) (Lema *et al*., 2012, 2015; dos Santos *et al*., 2014). A deeper knowledge of functions exerted by particular taxa will help designing optimal microbial inocula. Recently, ~200 distinct bacterial OTUs were able to be obtained in pure culture from the coral model *Exaiptasia pallida* using conventional methods (Röthig *et al*., 2016). These pure cultures represented *E. pallida*'s 'key microbial associates', and given the functional redundancy among members of the coral microbiome (Bell *et al*., 2005; Blackall *et al*., 2015; Sunagawa, 2015), these cultures provide opportunities for formulating a bacterial cocktail to evaluate their benefit to the host.

Genetic engineering should also be considered as an avenue to generate coral microbial inocula that possess desired characteristics. Such technologies have been applied to a wide range of organisms including bacteria, plants and mammals to study gene function or enhance phenotypic traits. In marine microbes, genetic engineering has been successfully employed to express high-value bioactive compounds in the eukaryotic microalga, *Chlorella* (Yang *et al*., 2016), and for aquatic bioremediation and source of fuel in cyanobacteria (Lau *et al*., 2015). A framework has recently been proposed for creating transgenic *Symbiodinium*, which could ultimately lead to more stress-tolerant variants (Levin *et al*., 2017). Likewise, coral-associated bacteria could be transformed with genes of interest to produce strains that enhance the performance of the host under climate change. Genomes could for instance be edited at specific sites with the CRISPR-Cas9 system (Hsu *et al*., 2014) or with mini-Tn7 transposons (Lambertsen *et al*., 2004). The latter approach has already been used to label *Vibrio coralliilyticus* in corals and visualize host–pathogen interactions (Pollock *et al*., 2015). Developing genetically engineered symbionts could thus allow seeding vulnerable corals with organisms possessing proven beneficial properties.

Another challenge is the potential difficulty of manipulating microorganisms in open marine systems. While targeted microbiome transplants are performed in relatively closed systems by inoculating animal gut or soil, respectively, such precise interventions may prove less effective in the marine environment where the inoculum would dilute in sea water. Aquarium rearing would overcome this limitation by ensuring that a sufficiently high density of microorganisms reach corals. Propagation techniques of coral fragments and sexually derived propagules have considerably progressed over the last two decades for the purpose of coral reef restoration (Barton *et al*., 2015) and the *ex situ* rearing phase could theoretically be combined with microbiome inoculations.

A key uncertainty about the feasibility of manipulating microbes to enhance coral tolerance is whether the taxa in the inoculum will remain associated with the coral over time. The inherent variability of the microbiome (Escalante *et al*., 2015) may limit the utility of microbiome manipulation for sustainable development. Engineered microbiomes that are acquired environmentally, such as via the rhizosphere in the case of plants, are likely to require continuous selection for retention of the beneficial properties that are conferred to the host. However, members of the core microbiome may be more stably associated with their coral host, and this is a knowledge gap that needs to be urgently addressed. The coral core microbiome comprises bacterial taxa consistently associated with given coral species at a global scale despite contrasting environments (Ainsworth *et al*., 2015; Chu and Vollmer, 2016; Hernandez-Agreda *et al*., 2016b). Such ubiquitous interactions across spatiotemporal boundaries suggest that these bacteria might perform critical functions for the host (reviewed in Hernandez-Agreda *et al.* (2016a,b)). It has been reported that some members of the core microbiome are endosymbionts, residing both within host tissue (Ainsworth *et al*., 2006, 2015; Ainsworth and Hoegh-Guldberg, 2009) and *Symbiodinium* cells (Ainsworth *et al*., 2015). We postulate that vertically inherited prokaryotes are more stably associated with the coral host compared to those that are horizontally acquired, as observed in other species such as the honeybee gut microbiome (Powell *et al*., 2014; Mueller and Sachs, 2015). The existence of conserved and intimate relationships between corals and microbes could open new avenues of research, where these stable communities would be targeted for manipulation.

Despite the recognition that coral reefs are threatened by human activities, measures to reduce negative impacts are insufficient. The United Nations have put forward targets that ought to be reached in the coming decade to protect marine ecosystems and avoid further adverse impacts (United Nations 2017). These goals include the sustainable management of marine zones by reducing pollution and destructive fishing, as well as the transfer of scientific knowledge to improve ocean health and support developing countries that rely on

coral reefs. Intervention methods such as manipulation of the microbiome and genetic engineering have been successfully applied to terrestrial organisms to increase their tolerance to stress. Numerous lines of evidence suggest that a translation of these technologies to corals and their symbionts might effectively enhance coral resilience and contribute to the success of coral reef restoration efforts.

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Conflict of interest

None declared.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Relative abundance of phyla present in water (left) and coral (right) samples.
Fig. S2. PCoA plot of the Bray-Curtis dissimilarities calculated at the phylum level depicts a striking difference between microbial communities present in coral and water samples.
Fig. S3. Box plots indicating beta diversity values in coral and water samples within each of the five inoculation treatments.
Data S1. Methods and results.
Data S2. Read counts for each sample at the genus level.
Data S3. Read counts for all samples at the phylum level.

