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https://doi.org/10.1071/MA23009
Microbiology Australia

Advancing coral microbiome manipulation to build long-term climate resilience

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Received: 15 January 2023
Accepted: 1 March 2023
Published: 9 March 2023

Cite this:
Doering T *et al.* (2023)
Microbiology Australia
44(1), 36–40. doi:10.1071/MA23009

ABSTRACT

Coral reefs house one-third of all marine species and are of high cultural and socioeconomic importance. However, coral reefs are under dire threat from climate change and other anthropogenic stressors. Climate change is causing coral bleaching, the breakdown of the symbiosis between the coral host and its algal symbionts, often resulting in coral mortality and the deterioration of these valuable ecosystems. While it is essential to counteract the root causes of climate change, it remains urgent to develop coral restoration and conservation methods that will buy time for coral reefs. The manipulation of the bacterial microbiome that is associated with corals has been suggested as one intervention to improve coral climate resilience. Early coral microbiome-manipulation studies, which are aimed at enhancing bleaching tolerance, have shown promising results, but the inoculated bacteria did generally not persist within the coral microbiome. Here, we highlight the importance of long-term incorporation of bacterial inocula into the microbiome of target corals, as repeated inoculations will be too costly and not feasible on large reef systems like the Great Barrier Reef. Therefore, coral microbiome-manipulation studies need to prioritise approaches that can provide sustained coral climate resilience.

Keywords: assisted evolution, coral bleaching, coral microbiome, microbiome manipulation, probiotics.

The threat of climate change to coral reefs

Tropical coral reefs are biodiversity hotspots, protect our coastlines from floods and storms, are socioeconomically important because they provide employment and support several industries, and have high cultural and spiritual value. However, tropical coral reefs are disappearing due to the impacts of climate change, which is causing a gradual increase in sea surface temperatures (SSTs), and also an increase in the frequency, intensity and duration of summer heatwaves. Higher-than-usual SSTs in combination with high irradiance levels, which often occur during these extreme summer events, are the main cause of mass coral bleaching.[1] Coral bleaching is the loss of dinoflagellate photosymbionts (Symbiodiniaceae family) from coral tissues. Since Symbiodiniaceae provide corals with most of their energy, bleaching is often followed by coral starvation and death, and reef degradation.[2] On the Great Barrier Reef (GBR), seven large-scale bleaching events have occurred since 1998, and <2% of the GBR has never bleached.[3] Effective bleaching mitigation and restoration approaches that will buy time for coral reefs until global warming is curbed are therefore urgently required. Accelerating evolutionary processes to enhance coral bleaching resilience through assisted evolution[4] is being explored as one option, which includes the manipulation of the coral bacterial microbiome.[5]

The coral host together with its associated microorganisms, including bacteria, archaea, Symbiodiniaceae and other protists, viruses and fungi,[6] are referred to as the coral holobiont. Coral-associated bacteria are diverse and believed to play important roles for the holobiont such as cycling nutrients,[7] producing essential vitamins and amino acids, regulating the bacterial community and warding off pathogens.[8] The composition of coral-associated bacterial communities is sometimes correlated with heat tolerance of the coral host,[9] suggesting a bacterial role in the coral heat stress response.

There are currently three hypotheses that explain the cellular mechanisms underpinning coral bleaching. The oxidative stress theory posits that high SSTs and irradiance impair the Symbiodiniaceae photosystem, triggering an overproduction of toxic reactive oxygen species (ROS) that leak into coral cells where they cause a cellular cascade that results in the separation of Symbiodiniaceae from the host.[10] The second hypothesis suggests bleaching is triggered by an accumulation of ROS and damage to the Calvin–Benson–Bassham cycle, due to the host not meeting the CO_2 demands for the faster-growing algal endosymbionts under elevated temperatures.[11] The third hypothesis poses that heat stress heightens host catabolism, which increases available ammonium for Symbiodiniaceae, fuelling algal growth and carbon usage.[12] This results in carbon limitation of the host and phosphorus limitation of Symbiodiniaceae, causing damage to the algal photosystem and its membranes[13] and, again, leading to the overproduction of ROS, which triggers bleaching. Thus, potentially relevant bacterial traits for microbiome manipulation to boost thermal bleaching resilience include the neutralisation of ROS by antioxidants or the supply of carbon to the host to minimise its starvation.

Microbiome manipulation as a tool to enhance coral climate resilience

Here, we define microbiome manipulation as the directed alteration of the microbiome by humans, with the overall goal to provide host health benefits. This can be achieved by approaches such as probiotics or the transplantation of microbial communities. Microbiome manipulation is a common approach in medicine, including, for instance, the use of fecal microbiome transplantation to treat *Clostridium* infections.[14] However, microbiome manipulation in corals is still at an early stage. Generally, its feasibility has been demonstrated by showing that the composition of coral-associated bacterial communities can be modified through bacterial inoculation.[15] Initial coral microbiome-manipulation studies have focussed on disease treatment, such as white pox disease,[16] or on the bioremediation of oil pollution.[17] Recent microbiome-manipulation studies to enhance coral bleaching resilience are promising, although clear correlations between the presence or abundance of inoculated bacteria and coral bleaching tolerance have not been proven yet. One study determined that bacteria isolated from corals and surrounding seawater had putative beneficial functions such as antagonistic activity against a common coral pathogen, activity of the ROS-scavenging enzyme catalase, and potentially contributed to sulfur and nitrogen cycling (by determining the presence of genes involved in the pathways).[18] Inoculating the coral *Pocillopora damicornis* with this bacterial cocktail partially inhibited thermal coral bleaching and pathogen infection.[18] A similar study selected candidate probiotic bacteria from *Mussismilia hispida* by screening for the same attributes as above, and showed that administering the probiotic mix to *M. hispida* reduced bleaching and improved recovery after thermal stress.[19] An additional study showed short-term bleaching mitigation of previously heat-sensitive corals after they were inoculated with a microbiome obtained from heat-tolerant corals from the same species.[20] Even though all three studies applied no-inoculum controls, increased bleaching tolerance might stem from the bacterial cocktail acting as a source of nutrition for the coral host growing heterotrophically. Integrating non-beneficial or dead bacteria as an additional negative control that might act as a food source without providing any other benefits may help decipher the impact of heterotrophic feeding *v.* microbiome manipulation on thermal tolerance. A recent study inoculated the coral model sea anemone *Exaiptasia diaphana* with either a consortium of bacterial strains with high ROS-scavenging abilities, a negative control that contained closely related bacterial strains with no ROS-scavenging abilities (to control for the effect of heterotrophic feeding), or a no-inoculum control.[21] The inoculated bacteria were lost from the *E. diaphana* host prior to heat stress application precluding any conclusions on their impact on bleaching resilience to be drawn.

Overall, these studies showed limited and short-term uptake of some of the inoculated bacteria,[20,21] or restructuring of the bacterial community composition following inoculation.[18,19] However, no study has demonstrated long-term uptake and temporal stability of the inoculated bacteria, although divergent microbiome communities were observed in coral juveniles 4 months after a single microbiome transplant from each of four different species of adult corals, including one adult coral that was conspecific to the larval recipients.[15] To provide long-term benefits and to create a sustainable solution to build coral bleaching resilience, putative beneficial coral bacteria need to form a temporally stable association with the coral host, thereby limiting the need for repeated inoculations across vast geographical scales.

Using stably associated bacteria for long-term benefits

Several aspects need to be considered to ensure uptake and persistence of inoculated bacteria by the host to guarantee long-term beneficial effects on holobiont performance (Fig. 1). First, we recommend focussing on bacteria that are stably associated with the host. Although corals associate with a high portion of ephemeral bacteria, there is a smaller portion that forms a more temporally stable symbiosis with the coral host.[22] Stable members of the coral microbiome are more likely to be found in the coral tissues,[23] where some are described to form bacterial aggregates,[24] and sometimes they are vertically transmitted to coral offspring. A successful example from another biological system is the use of the vertically transmitted, intracellular bacterium *Wolbachia* to reduce the spread of the viral disease, dengue. When introduced into *Aedes aegypti* mosquitoes, *Wolbachia* provides arboviral protection to mosquito hosts and spreads quickly and efficiently through wild populations following the release of infected mosquitoes, without the need for further intervention.[25] This is despite *A. aegypti* not being a natural host for *Wolbachia*. Therefore, temporal stability of bacteria within the host microbiome should be studied over longer timescales (e.g. months and longer), as most previous experiments have tested a duration of 24 h,[20] 5,[19] 11[18] and 35 days,[21] except for one study testing over 4 months,[5] and over multiple generations.

Second, we propose to source bacteria from coral microhabitats where beneficial functions are required. One of the key mechanisms in bleaching involves the overproduction of ROS by Symbiodiniaceace in host gastrodermal cells. Therefore, ROS-scavenging bacteria that closely associate with

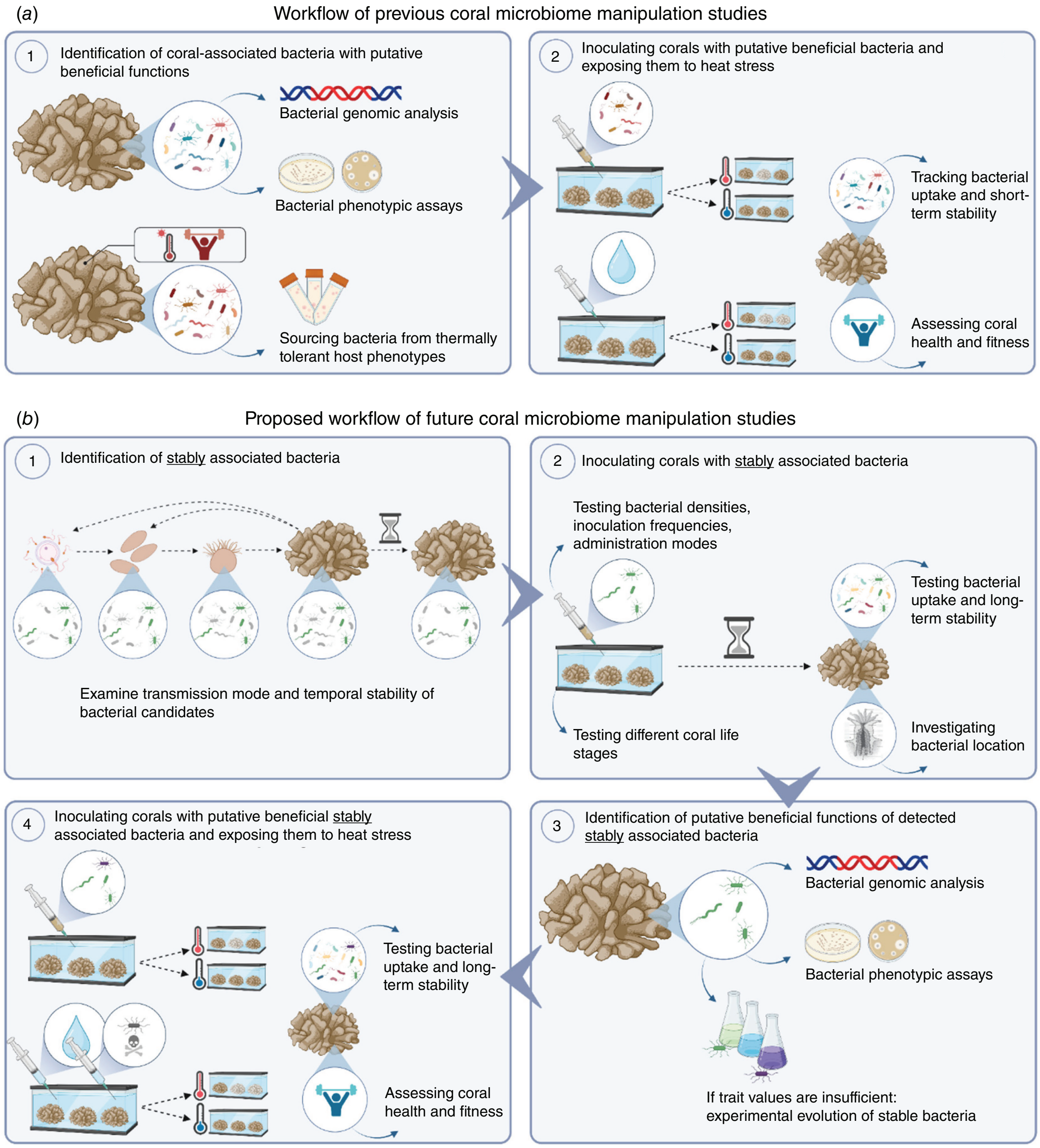

Fig. 1. Conceptual figure describing (*a*) the workflow of previous coral microbiome-manipulation studies that aimed to enhance coral bleaching resilience and (*b*) our proposed workflow for future studies. Previous coral microbiome-manipulation studies (*a*) started with (1) the identification of coral-associated bacteria with putative beneficial functions by performing bacterial genomic and phenotypic analyses, or by sourcing putative beneficial bacteria from thermally tolerant coral host phenotypes. This was followed by (2) inoculating corals with identified putative beneficial coral-associated bacteria or filtered seawater (negative control), exposing treated corals to heat stress or ambient temperatures and examining bacterial uptake and short-term stability, as well as determining coral holobiont health and fitness. We propose (*b*) for future coral microbiome-manipulation studies aiming for long-term coral bleaching resilience to start with (1) identifying stably associated bacteria by examining the transmission mode of bacterial candidates throughout different coral life stages and their temporal stability within adult corals. Subsequently, we propose to (2) inoculate corals with stably associated bacteria to test bacterial uptake and long-term stability, as well as investigating the location of the bacterial candidates within the coral holobiont. Here, we also propose to test the effect of different bacterial densities, inoculation frequencies, administration modes and coral life stages on the bacterial uptake and stability. Afterwards, we propose to (3) identify putative beneficial functions of stable coral-associated bacteria by bacterial genomic and phenotypic analyses. If functions of interest of stable bacteria are insufficient, we recommend experimental evolution to enhance their functional potential. If multiple strains are chosen, we also propose to test for interspecific interactions including effects on growth rates and the efficiency of the putative beneficial functions of interest. Finally, we advise to (4) inoculate corals with putative beneficial stable coral-associated bacteria, filtered seawater (first negative control), and heat-killed bacteria (second negative control), expose them to heat or ambient temperatures, assess coral health and fitness and track the uptake and temporal stability of inoculated bacteria within the coral holobiont. Created with BioRender.com.

Symbiodiniaceae or are present in the gastrodermis would be relevant. Some bacteria co-localise with Symbiodiniaceae in culture and *in hospite*[26,27] and may play a role in Symbiodiniaceae and coral health.[28]

Once stable members from coral microhabitats are identified, the next critical step for candidate selection will be to understand their functions, especially with regards to coral bleaching and climate resilience. This can be achieved through genomic analyses and phenotypic assays. If candidates of interest exhibit limited functional ability, such as low ROS-scavenging abilities, laboratory evolution experiments may be used to enhance their abilities, for example through long-term exposure to oxidative stress conditions.[29]

In summary, the selection of bacterial candidates based on their temporal stability and location within the coral holobiont, followed by their bleaching mitigation functions are critical steps in developing microbiome-manipulation techniques that aim to build long-term bleaching resilience. Since recent models predict that environmental conditions will become unsuitable for coral reefs by 2035,[30] the next decade will be crucial to curb greenhouse gas emissions and develop effective and sustainable conservation methods to buy time for corals.

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Data availability. Data sharing is not applicable as no new data were generated or analysed during this study.

Conflicts of interest. The authors declare that they have no conflicts of interest.

Declaration of funding. This research was funded by the by the Australian Research Council Laureate Fellowship FL180100036 to M. J. H. van Oppen. T. Doering was supported by a University of Melbourne Scholarship by the Environmental Microbiology Research Initiative.

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Biographies

Talisa Doering is currently a PhD student at the University of Melbourne, supervised by Prof. Madeleine J. H. van Oppen and Prof. Linda L. Blackall. She completed her Bachelor of Science degree in Biology at the Free University of Berlin, studying evolution of marine fishes to climate change regimes. She obtained her Master of Science degree in Biological Oceanography at Kiel University and GEOMAR Helmholtz Centre for Ocean Research Kiel, where she conducted a coral microbiome transplant experiment from heat-sensitive to heat-tolerant coral conspecifics in order to build coral climate resilience. Her current PhD research focuses on understanding coral bleaching mechanisms, identifying beneficial coral-associated bacteria and developing successful microbiome-manipulation approaches that can enhance coral bleaching resilience.

Dr Justin Maire is currently a postdoctoral fellow at the University of Melbourne. He obtained his PhD in 2018 from Institut National des Sciences Appliquées de Lyon, France, on symbiotic interactions between insects and bacteria and their impact on host immunity and development. In 2019, he moved to the University of Melbourne, where his research is focused on coral–bacteria associations. He specifically focuses on closely associated symbionts, e.g. intracellular, vertically transmitted symbionts, and their potential use as bacterial probiotics and microbiome engineering as an approach for the mitigation of coral bleaching and coral reef conservation. This includes characterising the taxonomy, localisation and functions of symbionts of interests.

Prof. Madeleine J. H. van Oppen is an ecological geneticist with an interest in microbial symbioses and climate change adaptation of reef corals. Her early career focused on evolutionary and population genetics of algae and fish, and subsequently corals. Currently, her team is using bioengineering approaches aimed at increasing coral climate resilience and the likelihood that coral reefs will survive this century. These interventions include coral host hybridisation and conditioning, directed evolution of microalgal symbionts and bacterial probiotics.

Prof. Linda L. Blackall is an environmental microbial ecologist, who has studied many different complex microbial communities ranging from host associated through to free living in numerous environments. One of her research fields is the microbiota of corals and sponges. The numerous methods she develops and employs in her research allow elucidation of microbial complexity and function in these diverse biomes.