

Probiotics for corals



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Abstract. Coral reefs are found in warm, oligotrophic, euphotic marine waters and occupy <0.1% of the sea floor, yet support ~25% of earth's marine species. They provide critical ecosystem services to human populations including coastal protection, food (e.g. fish) and personal income by way of fishing and tourism. However, recent pan-tropical coral 'bleaching' (the paling of corals due to the separation of corals and their algal endosymbionts following exposure to environmental stress) has led to coral mortality, thus jeopardising the persistence of reef ecosystems. Consequently, it has been recognised that direct interventions may be needed for coral survival, and 'manipulation of the community composition of microbial organisms associated with the coral holobiont' has been proposed as one solution. Such probiotic strategies would allow corals to adapt rapidly (days to weeks) to changing environmental conditions, relative to mutation and selection taking many years. This review describes corals, and research that has demonstrated the potential of probiotic approaches to protect them from environmental stressors.

Coral reefs provide critical ecosystem services including coastal protection, a source of food (e.g. fish) and a source of personal income by way of fishing and tourism. They also suffer from many challenges including climate change, shading from sediment runoff, pollution (e.g. oils), overfishing, and attacks from crown-of-thorn starfish. As a result of climate change, sea surface

temperatures are increasing and since 1901 by ~0.18°C per decade¹. The summers of 2014-2017 saw heat-induced pan-tropical coral 'bleaching'², which is the paling of corals due to separation of corals and their photosynthetic Symbiodiniaceae often leading to coral mortality and eventually to the collapse of reef ecosystems³.

Coral reefs are constructed by coral polyps as they secrete layers of calcium carbonate. These marine invertebrates (phylum Cnidaria, class Anthozoa) are typically found in warm, oligotrophic, euphotic marine waters, occupying <0.1% of the sea floor but supporting ~25% of Earth's marine species⁴. Each coral polyp is comprised of two cell layers (ectodermis and gastrodermis) separated by a largely cell-free mesoglea and include an external mucus layer⁵, as shown in Figure 1. They have a tentacle-ringed mouth leading to the gastrovascular cavity. A coral polyp is connected to the next genetically identical polyp by the coenosarc. Corals engage in endosymbioses with single-celled algae from the family Symbiodiniaceae, which reside *in hospite* (in gastrodermal cells) surrounded by a membrane complex of host and algal origin, called the symbiosome⁶. The symbiosis is mediated by exchange of organic and inorganic compounds from which both partners benefit; critically, corals gain the majority of their fixed carbon from Symbiodiniaceae. Corals engage in sexual reproduction via either broadcast spawning (release of eggs and sperm to the water → larvae form in water) or brooding (larvae formed inside polyps → released to the water).

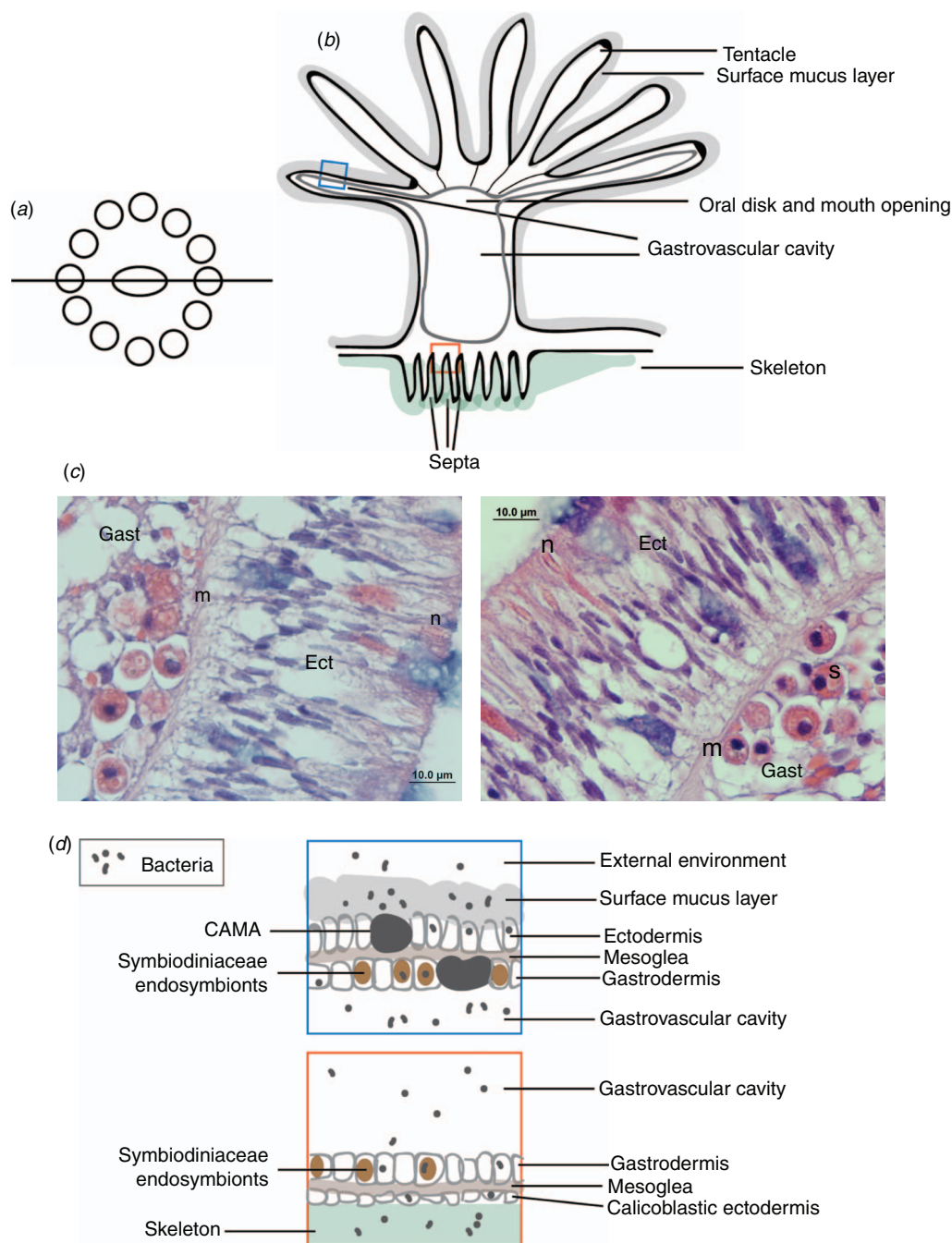


Figure 1. The body plan of a coral polyp, the location of bacteria within a polyp, and coral colony morphologies. (a) Plan view of a coral polyp with the horizontal line indicating the internal elevation view shown in (b); circles represent the tentacles and the oval represents the oral disk. (b) Internal elevation plan of a coral polyp showing the various microhabitats. Note that the gastrovascular cavity extends into the tentacles. (c) Brightfield microscopy image of a haematoxylin and eosin stained section through a coral larva (*Pocillopora acuta*) clearly showing the ectodermis (Ect), mesoglea (m) and gastrodermis (Gast) as well as Symbiodiniaceae (s), and cnidocyte showing coiled nematocyst (n). Photo credit: Katarina Damjanovic. (d) Diagrams of cross-sections through the tissue layers of a tentacle (top: blue boxed section from b) and the aboral part of a polyp (bottom: red boxed section from b) showing the various tissue layers and the location of bacteria. CAMA, coral-associated microbial aggregate.

Corals associate closely with prokaryotes (bacteria and archaea), viruses, microscopic eukaryotes, and, combined with Symbiodiniaceae, they are all collectively called the holobiont (for a review see ⁷). However, there is scant knowledge on what controls the community structure and function of most of the microbes in the coral holobiont. Hypotheses for structuring include coral-produced chemicals in the mucus and natural coral-associated microbe-produced chemicals (see ⁸ for more information). Many

functions of coral-associated bacteria are based on correlations between microbe identity and the phenotype of their closest relatives. However, proof for some phenotypic roles of bacteria have been provided, such as for nitrogen where nanoscale secondary ion mass spectrometry was used to show the incorporation and translocation of nitrogen from prelabelled bacteria into larvae of the coral *Pocillopora damicornis* and particularly into Symbiodiniaceae.

Probiotics for corals

'Manipulation of the community composition of microbial organisms associated with the coral holobiont' has been recognised as a direct measure needed to be a part of strategies to facilitate coral survival⁹. In line with this idea is the concept of probiotics for corals. Probiotics can be defined as, 'live microorganisms that are intended to have health benefits when consumed or applied to the body'¹⁰. In corals, probiotics are suggested as a rapid (day to weeks) natural strategy for corals to adapt to changing environmental conditions, relative to the alternative of mutation and selection taking many years¹¹. They could also be applied to aquacultured corals. This initial report¹¹ specifically discussed the development of coral disease resistance by beneficial microbes in the naturally occurring holobiont. Teplitski and Ritchie¹² described the development and application of probiotics for several marine species including trout, shrimp and eels, as paradigms for coral probiotics. Other terms also encompass coral 'probiotics' including 'beneficial microorganisms for corals'¹³ and 'microbiome engineering'¹⁴.

Since probiotics are live microorganisms that should colonise the inoculated host, information about how corals normally acquire their microbiome is relevant. It has been shown that bacterial communities in corals are distinct from those in the contiguous seawater¹⁵. However, there are conflicting reports about whether specific corals associate with particular microbes¹⁶, or whether the microbiome is shaped by the environment, location or weather. An experiment exploring whether adult corals are the source of bacteria for juveniles was carried out. Damjanovic *et al.*¹⁷ exposed 'recruits' of the brooding coral *Poc. acuta* to adult *Poc. acuta* and adult *Platygyra daedalea*. The findings showed that *Poc. acuta* recruits harbour dynamic and diverse bacterial assemblages, which were not influenced by nearby adult corals. Another investigation showed that *Poc. acuta* maternally transmits members of the *Rhodobacteraceae* and *Endozoicomonas* spp.¹⁸. The feasibility of coral early life stage microbiome manipulation (probiotics) was investigated by repeatedly inoculating coral recruits (*Acropora tenuis* and *Platy. daedalea*) with a mixture of seven marine bacterial isolates, which had no specific targeted phenotypes¹⁹. The cumulative inoculations had a strong effect on the bacterial community composition and diversity in recruits of both coral species, compared to control recruits, despite being reared in the same environment. The conclusion from this set of experiments was that host factors, as well as the environmental bacterial pool influence the microbiome of early life stages of corals. Host factors may include microbe transmission mode (horizontal versus maternal) and host specificity. While the long-term

stability of bacterial taxa as members of the host-associated microbiome remains to be evaluated²⁰, the findings provided support for the feasibility of coral microbiome manipulation, at least in a laboratory setting.

Use of coral bacterial inoculation

Two examples of practical applications of bacterial inoculation (probiotics) to corals are given below.

- (1) *Disease mitigation*. Some strains of the necrotizing coral pathogen, *Serratia marcescens* form a biofilm and disrupt the normal mucus layer on corals leading to the disease condition known as 'white pox'. Pure cultures of bacteria from the coral *Acropora palmata* were found to produce anti-bacterial chemicals against a broad spectrum of pathogens, including *S. marcescens*²¹. This work was extended to clarify that the mucus layer of healthy corals contain chemicals that inhibit biofilm formation (a noted virulence phenotype) in white pox pathogenic strains of *S. marcescens*⁸. Several marine bacteria from corals or Symbiodiniaceae were capable of affecting biofilm formation and swarming (also a prominent virulence phenotype) in the white pox *S. marcescens*⁸. These so-called 'antagonistic' strains were inoculated along with the white pox *S. marcescens* to the sea anemone *Exaiptasia diaphana* (formerly *Aiptasia pallida*), a coral model. The progression of white pox disease was minimised by the antagonists potentially due to antimicrobial properties of the inoculated bacteria. Although it was tested on anemones, this method was deemed to hold promise for other cnidarians, like coral⁸.
- (2) *Bioremediation of oil*. A good example of how microbes can facilitate coral survival in the face of environmental impact is research by dos Santos *et al.*²², where several bacterial species with the capacity to degrade water-soluble oil fractions were isolated from the coral *Mussismilia barti*. The health of *M. barti* subjected to petroleum hydrocarbons was negatively impacted according to photosynthetic efficiency; however, strictly this is a feature of Symbiodiniaceae, not corals *per se*. A single inoculum of an oil-degrading consortium composed of 10 bacteria (three *Bacillus* spp., *Acinetobacter calcoaceticus*, three *Paracoccus* spp., a *Psychrobacter* sp., *Vibrio alginolyticus* and *Pseudomonas stutzeri*):
 - improved the health *M. barti* when it was exposed to petroleum hydrocarbons, and
 - the bacterial mixture accelerated the degradation of petroleum hydrocarbons.

Mitigating coral bleaching with probiotics

Oakley and Davy²³ provided a recent summary of the cell biology of coral bleaching. Although there are several hypotheses for coral bleaching^{24–26}, one common theme revolves around damage to the Symbiodiniaceae photosystem II leading to the formation of reactive oxygen-centered radicals^{27,28} like singlet oxygen and superoxide²⁹. This partially occurs because more oxygen is produced by the Symbiodiniaceae than is used in the milieu leading to toxic accumulation of reactive oxygen species (ROS). ROS have several cellular damaging mechanisms including to photosystem II reaction centres in the Symbiodiniaceae, which can result in

Symbiodiniaceae being lost from host tissue. Corals and Symbiodiniaceae have ROS managing mechanisms like catalase and superoxide dismutase, which degrade ROS to oxygen and water²⁹.

To test the ability of probiotic inoculation to mitigate ROS and disease-induced coral bleaching, Rosado *et al.*³⁰ isolated bacteria including five *Pseudoalteromonas* spp., one *Halomonas taenensis* and a relative of *Colbetia marina* from the coral *Poc. damicornis* (grown in an aquarium) and its surrounding aquarium waters. Bacteria were screened for catalase activity, nitrogen metabolism (*nifH* and *nirK* genes via PCR), dimethylsulfoxide propionate demethylation (*dmdA* gene by PCR) and antagonistic activity against the coral pathogen *Vibrio coralliilyticus*; traits deemed relevant to protect corals against heat and disease stress. In controlled aquarium experiments, after a 10-day acclimatisation period, two stressors were evaluated. These were *Poc. damicornis* maintained in two temperature regimes, 26°C and 30°C (raised from 26°C over 9 days) and inoculation of *Poc. damicornis* with *V. coralliilyticus*. *Poc. damicornis* in both scenarios were inoculated with the seven-bacterial consortium on two occasions (days 10 and 15) and were maintained for 26 days. The method to determine bleaching was comparison of the coral tissue colour to a colour chart, and photosynthetic efficiency was also measured. It was concluded that the inoculated seven-bacterial consortium partially mitigated bleaching from temperature; although the reason was unclear as the inoculated bacterial consortium had diverse traits. In corals exposed to *V. coralliilyticus* and inoculated with the seven-bacterial consortium, no *V. coralliilyticus* were found after 26 days, demonstrating mitigation of this noted coral pathogen³¹.

Future directions

The field of coral probiotics is at a very early stage and is currently limited by a lack of definitive information about the functional roles of coral microbiome members, apart from Symbiodiniaceae. Information that would aid development includes determination of bacterial phenotypes that are beneficial to the host. These might include ROS metabolism, although other phenotypes are likely valuable. Testing the maintenance of introduced bacteria in the host is also required. Given the perilous situation facing coral reefs, including the broad GBR bleaching over the recent 2019–2020 summer, addressing these knowledge gaps to advance probiotic strategies is critical.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

Professor Linda I. 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. Her research has covered mammalian microbiomes of marsupials, humans, ruminants and horses; and the microbiota of non-mammals including corals and sponges. Environmental microbiomes explored in Linda's research span wastewater treatment (aerobic and anaerobic), solid waste digestion (landfill and composting), bioelectric systems and microbiologically influenced corrosion. The numerous methods she develops and employs in her research allow elucidation of microbial complexity and function in these diverse biomes.

Ms Ashley M Dungan is originally from New York. Ashley completed her Bachelor of Science degree in Biology in 2011, conducting a senior research project in the field of environmental microbiology. She completed her Master's degree at Nova Southeastern University in Florida under Dr Nicole Fogarty, where she studied the impact of ocean acidification on the calcification of Caribbean adult and juvenile corals. After graduating in 2015, she worked for Mote Marine Laboratory in the Florida Keys as a Staff Chemist in the Ocean Acidification program; there she continued her work with corals and began working with *Diadema antillarum*, the long-spined sea urchin. Beginning early 2017, Ashley joined Dr Madeleine van Oppen and Linda Blackall at the University of Melbourne as a PhD student. Ashley's current research focuses on characterising the microbiome of the model organism for corals, *Exaiptasia pallida*, for future use in assisted evolution research. Her research interests are in the field of climate change, coral reef ecosystems, and assisted evolution.

Mr Leon M Hartman recently completed a PhD through Swinburne University as part of the Blackall and van Oppen research group at the University of Melbourne using the sea anemone *Exaiptasia diaphana* as a coral model organism. His research has employed molecular and bioinformatic methods to study bacterial microbiomes, their relationship with their hosts, and response to environmental perturbation.

Professor Madeleine JH van Oppen is an ecological geneticist with an interest in microbial symbioses and climate change adaptation of reef corals. Her work has been published in >200 peer-reviewed papers and book chapters. Her early career focused on evolutionary and population genetics of algae and fish, and subsequently corals. She obtained a PhD in the molecular ecology of macroalgae in 1995 (U Groningen, The Netherlands) and is currently an Australian Research Council Laureate Fellow with part positions at the University of Melbourne and the Australian Institute of Marine Science. 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.



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