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Coral holobionts and biotechnology: from Blue Economy to coral reef conservation

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Corals are of ecological and economic importance, providing habitat for species and contributing to coastal protection, fisheries, and tourism. Their biotechnological potential is also increasingly recognized. Particularly, the production of pharmaceutically interesting compounds by corals and their microbial associates stimulated natural product-based drug discovery. The efficient light distribution by coral skeletons for optimal photosynthesis by algal symbionts has led to 3D-printed bionic corals that may be used to upscale micro-algal cultivation for bioenergy generation. However, corals are under threat from climate change and pollution, and biotechnological approaches to increase their resilience, like 'probiotics' and 'assisted evolution', are being evaluated. In this review, we summarize the recent biotechnological developments related to corals with an emphasis on coral conservation, drug discovery and bioenergy.

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Introduction

Corals have generally been considered 'simple animals' because of their simple body plan and lack of organs, but it turns out that they are in fact highly complex 'meta-organisms' when considering their microbiota. The coral host and its microbial symbionts (e.g. archaea, bacteria, micro-eukaryotes) form an entity called the coral 'holobiont' [1]. The bacterial fraction of the microbiota is immensely diverse with up to 100 000 different species/phylotypes and a bacterial density ranging between $10^5 - 6 \times 10^7$ per cm^2 of colony surface and $\sim 10^7$ bacteria per mL of mucus. Among the other coral-associated microbes, the algae of the Symbiodiniaceae family are the most characteristic symbionts of tropical shallow corals: they provide the coral with large amounts of food (amino acids and carbohydrates) produced during photosynthesis. And the corals have optimized the (micro)structure of their skeletons to ensure a light environment optimal for photosynthesis by the algae [2]. However, this symbiosis may breakdown under stressful conditions leading to 'coral bleaching' and ultimately starvation of the host. This may provide an opportunity for pathogenic microbes to proliferate and cause disease. Under normal conditions, the composition of the coral holobiont is quite stable and an evolutionary history between coral species and their microbiota exists [3,4]. However, coral holobionts do have the capacity to modulate their composition and select microbes with beneficial characteristics to acclimate to the prevailing environmental conditions and protect themselves from pathogens, parasites, and predators. There are thus many inter-species, intra-species and host-microbiota interactions within the holobiont aimed at nourishing it and protecting its integrity.

Corals have evolved over millions of years in the true microbial world that the oceans are. The stable symbiotic associations and optimized structures of the coral skeleton that developed contribute to the success of corals. As a result, corals have also drawn the attention of the field of biotechnology. Microbiome regulation and chemical warfare by corals has raised interest into isolating compounds with antibiotic or antifouling properties from coral holobionts. And studies on the coral skeletons have led to a model to improve algal cultures for bioenergy and biocompound production. Biotechnological approaches are now also being explored to generate coral holobionts that are more tolerant to stressors that severely impact coral populations, such as climate change and pollution, and to preserve coral ecosystems. These ecosystems provide many important services with an estimated worth of $375 billion annually, including the use of resources (e.g. fisheries), tourism and coastal protection. All these directions are thus covered under the 'Blue Economy' concept that encompasses all economic activities linked to the oceans. In this review, we discuss the advances that have been made in these fields, demonstrating the biotechnological potential of coral holobionts, and identify

priority research areas for biotechnological applications for the benefit of human and ocean health.

Bioprospection for natural products

Coral holobionts produce a vast array of compounds, such as chemicals to deter predators, cytotoxic substances to compete for space with other benthic organisms and microbiome regulatory compounds to interact with symbionts and eliminate pathogens [5]. Over the past five years, over 200 bioactive substances were isolated from coral holobionts, making them one of the most important sources of marine natural products together with sponges, and free-living bacteria and fungi. Research into these compounds is both of biotechnological and economic interest, as it can lead to the development of novel medications as well as products to prevent biofilm formation in medical equipment, industrial facilities and biofouling on ships.

Initial natural product (NP) discovery efforts extracted products from the whole coral holobiont, thus not knowing which member produced which compound: the coral host or coral-associated microbes (CAM)? But as the importance of CAMs is increasingly recognized, the focus has now also shifted specifically to bioactive compounds produced by CAMs (reviewed in Refs. [6,7]). Of all CAM-derived NPs, fungi and bacteria are responsible for respectively ~90% and ~10%. Advances in the discovery and characterization of coral holobiont-derived bioproducts as well as their synthesis are regularly reviewed (overview in Ref. [6]; yearly updates by Carroll *et al.*, for example, Ref. [8•] and earlier), and generally found to exhibit cytotoxic, anti-cancer, anti-inflammatory, antibacterial, anti-fungal, anti-viral or anti-fouling activities.

Particularly CAMs living in the surface mucus layer (SML) of corals may be of interest as they produce anti-fouling and anti-microbial compounds aiding the holobiont to prevent overgrowth by biofilms, regulate the microbiota, and eliminate pathogens [9,10]. Interestingly, the anti-fouling substances from CAMs are more potent than those derived from free-living bacteria [11]. And CAM-derived anti-microbial NPs have proven effectiveness against clinically important pathogens [10,12,13]. A focus on microbes from the SML to find new antibiotics may thus be warranted. However, when screening for anti-microbial effects, the doses assessed should be carefully taken into consideration as some anti-microbials from soft coral holobionts had quorum quenching activity at high doses but stimulated microbial growth at low concentrations [14•]. Other interesting CAM-derived NPs are biosurfactant substances that could replace chemical oil dispersants that are harmful to marine life [15], chitin-modifying enzymes with possibly large-scale industrial applications [16], and carotenoids that can potentially be used in food, cosmetic and pharmaceutical industries [17].

The coral but primarily its algal symbionts of the Symbioniaceae also produce compounds of cosmetic interest: mycosporine-like amino acids (MAAs). These natural filters of ultraviolet light are promising, ecologically friendly alternatives for use in sun care products [18], and also possess anti-oxidant, anti-inflammatory and anti-ageing properties [19]. Nonetheless, MAAs are not widely utilized in cosmetic or sunscreen products yet. But the increased stability and UV-protective properties of red alga-derived MAAs through conjugation to chitosan hold promise for a wider use of these NPs to replace environmentally harmful chemical-based sunscreens [20].

NP discovery efforts from coral holobionts have focused on only a few coral species and only a few thousand CAMs have so far been isolated. Considering that there are hundreds of coral species with each thousands of different microbial symbionts, it is clear that the full potential of coral holobionts in NP discovery is still largely untapped. Particularly, coral species from mesophotic (>50 m depth), deep-sea and cold-water (>200 m depth) environments remain largely unexplored due to the technical challenges to reach these depths. As a result, less than 2% of the >34 000 marine NPs described come from organisms from deep-water environments [21]. However, significant challenges also exist in isolating relevant CAMs as the vast majority of microbes are 'uncultivable' — or better said their culturing requirements are as yet unknown. These challenges are discussed in more detail in the following chapter.

Challenges in natural product discovery and production

Most microbes have not been cultured yet as their culture requirements are still unknown. And in symbioses and its natural environment, hosts will likely produce most of their chemical defense repertoire with all intra-holobiont and competitive interactions in place. As such, more advanced methods will be required to tap into the full potential of coral holobionts for NP discovery. Besides, it is necessary to produce the compounds in sufficiently large amounts for use in pharmaceutical, cosmetic and food products. As harvesting coral holobionts from the wild is not sustainable, microbes can be hard to grow and chemically synthesizing the compounds is often challenging, alternative production methods are also needed. In the following sections, we briefly describe the most recent advances in the cultivation and genome analyses of CAMs for NP discovery, as well as the establishment of coral cell cultures and the use of coral skeleton-inspired microalgal cultivation systems for NP production.

Microbial cultivation and NP discovery

Recently, an assessment of the cultivation success of CAMs and comparative genomics analyses of isolated

bacteria were made by Sweet *et al.* [22]. They found that among the 1045 isolated CAMs with a full-length 16S rRNA gene sequence available at least 150 different genera are represented, including ~60% of genera considered beneficial symbionts of corals. This shows that a large and diverse range of CAMs are likely amenable to cultivation using different culture media. However, the diversity of bacteria grown differed significantly between the various media used, with nutrient agar and R2A supporting some of the highest diversity [22]. Overall, this is very promising. However, we have not been able to cultivate many other CAMs using these traditional culture methods also, and these difficulties show the need for new approaches.

Modifications to traditional plate-based methods have successfully increased the diversity of CAMs retrieved. For example, ~62% of bacterial species detected with metabarcoding on the temperate gorgonian *Eunicella labiata* were isolated by cultivation on low-nutrient media at lower-than-usual temperatures for prolonged periods of time [23]. And while adding coral-derived NPs with quorum sensing interference activity to culture media appears to favor growth of CAMs [14•], media enriched with dimethylsulfoniopropionate (DMSP) shows very high diversity among the bacteria isolated [22]. More advanced technologies for cultivating microbes are presented in Box 1. To better understand the culture conditions, 'reverse genomics' approaches are also useful tools. Reverse genomics-enabled cultivation [24] uses metagenomics or single-cell genomics techniques to identify specific antibody targets on the microbe of interest allowing antibody-mediated isolation. However, it may also be used to study the metabolic capacities encoded in the genomes of microbial species, which can in turn inform which nutrient supplements and conditions are required to culture them, and to identify the genes involved in the biosynthesis of secondary metabolites. The bioinformatics tool *antiSMASH* [25] has been used for over a decade to predict such biosynthetic gene clusters (BGC). Recent metagenomics studies on Mediterranean gorgonian octocorals [26••] identified a large number of BGCs (particularly anti-microbial peptides) in the microbiota of *Leptogorgia* sp., whereas very few were detected in *Eunicella* spp. The most prolific source of BGCs, however, were the microbial communities of diseased *Eunicella* spp. Interestingly, ~87% of all BGCs detected in that study did not share homology with known BGCs, showing the hidden potential of coral pathogens for new NP discovery.

Data mining of all 74 isolated CAMs whose genomes have been sequenced also revealed a large repertoire of BGCs present in CAMs [22]. Particularly Rhodobacteraceae (Alphaproteobacteria) possess many BGCs (on average ~7.5 per strain), which is significantly more than in the Gammaproteobacterial coral symbionts *Endozoicomonas* and *Pseudoalteromonas* (~3.8 and ~3.6 BGCs per strain, respectively) but similar to pathogens (~7.2 BGCs per strain). This confirms that coral pathogens but also Rhodobacteraceae are of interest for NP discovery. However, pathogenicity or relatively slow growth may make CAMs unsuitable for biotechnological applications, whereas the cultivation conditions of other CAMs may be unknown. A solution may thus be to clone the BGCs for heterologous expression in resistant, easy-to-grow microbes that can be employed for mass production, thereby bypassing the need to cultivate CAMs.

Coral cell culture and engineering

Coral animals also produce compounds, which may be of biotechnological interest, to regulate their microbiota and prevent biofouling. However, these compounds may be difficult to synthesize by chemistry as the biosynthesis pathways and post-translational modifications are unknown, and identifying the genes and processes involved is therefore crucial. Genetic engineering of established coral cell cultures to overexpress genes involved in the production of these compounds may be a viable solution. Over the past few decades, scientists have made several attempts to grow coral cells *in vitro* but with varying success [33,34]. Although some advances in cell culture preparation methods were made recently [35,36], microbial contaminations still posed problems for long-term maintenance of cultures. However, a significant breakthrough was made by Kawamura *et al.*, reporting the establishment of *in vitro* cultures of eight morphologically distinct stable cell lines of *Acropora tenuis* that had been maintained for 10 months [37••]. These cell lines exhibit distinct gene expression profiles but with characteristics of different cell layers, indicating that each cell line consists of a multiple cell types. Fluorescence-activated cell sorting (FACS) may be used to obtain pure cell lines and minimize microbial contamination, and has already been successfully applied to obtain live cell populations for short-term culture [36,38].

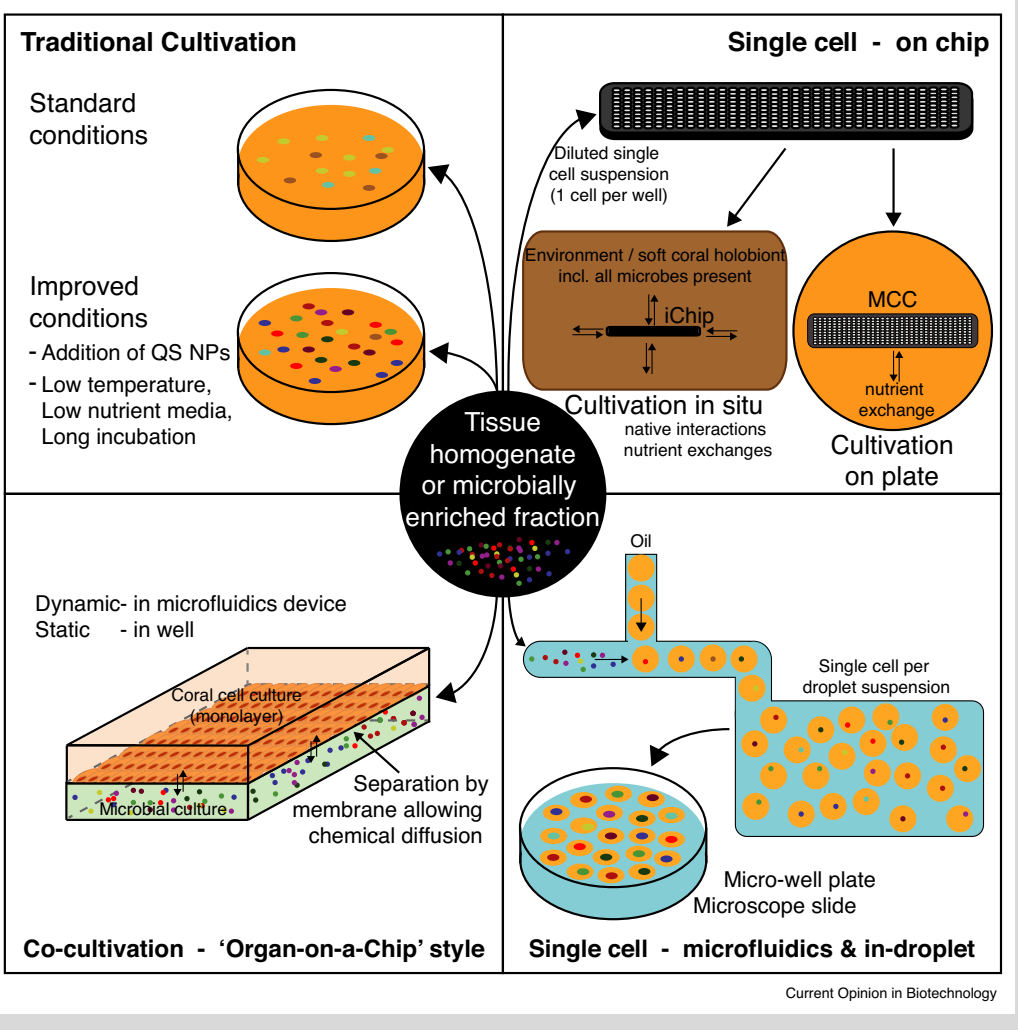
Although coral cells have not yet been genetically engineered in culture, two recent studies succeeded in using CRISPR/Cas9 technology to knock-down gene expression in coral larvae [39•,40••]. As such, it is likely that genome modification approaches can be applied to established coral cell cultures. Using genomics to identify the bioproduct synthesis pathways, single cell transcriptomics [41,42] combined with FACS or microfluidics technologies to characterize and select the best coral cell types, and engineering coral cells with CRISPR activator (CRISPRa) [43] or knock-in systems [44] to overexpress genes involved in biosynthesis pathways will be the next steps in improving the production of coral-derived bioproducts.

Box 1 Advanced technologies for cultivating the 'uncultivated'

In the quest for cultivating the microbes that have not yet been cultured, several advanced methods have been developed (Figure B1). Single-cell technologies have tremendous potential. For instance, droplet microfluidics can be used as a high-throughput method to isolate individual cells in tiny droplets followed by in-droplet culturing and selection [27••]. Diffusion-based methods using multi-(micro)well chip devices (e.g. miniaturized culture chip [28]) are another approach, where a complex community is plated on a chip ensuring a maximum of one microbe per microwell and the chip is placed on cultivation media allowing diffusion of nutrients. The isolation chip (iChip) [29] follows a similar approach, but as the chip is sealed with a membrane that allows chemical diffusion, it can be employed *in situ* to cultivate soil and aquatic microbes by allowing native conditions and metabolic interactions for microbial growth. Cultivating CAMs within such chips within a coral is unlikely though due to the thin tissues of skeleton-building corals. However, it may be possible to implant iChips in fleshy soft corals, which are considered the most versatile NP producers among corals, similar as recently done in a sponge [30•]. Co-culturing of microbes and coral cells (see section 'Coral cell culture and engineering') may allow 'host regulation' of microbial growth and therefore open new avenues for CAM isolation. While standard cell culture inserts with 0.22 μm pore sizes may be useful, microfluidics and diffusion-based methods have been combined to mimic specialized environments or organs and simulate flow and mechanical movements, for example to study the gut microbiota ('Gut-on-a-Chip' [31]). Such approaches may help simulating the coral environment to cultivate CAMs under controlled conditions. While these methods may allow the cultivation of the 'yet-to-be cultured' fraction of the microbiota, the culture conditions for large scale cultivation will still need to be optimized.

Microbes may also produce some secondary metabolites at high amounts, masking the presence of other compounds. Recently, it was found that CRISPR/Cas9-mediated knockdown of known BGCs in fungi reveals novel variants of antibiotics [32•]. Screening for secondary metabolites after knockdown of known BGCs may thus be another promising lead to discover the full NP biosynthetic potential provided by coral-associated microeukaryotes.

Figure B1



Methods to cultivate the 'yet-to-be-cultured' microbes. Four approaches are presented: 1) modifications to the conditions and media used in traditional cultivation; 2) cultivation of single microbial cells in well under *in situ* conditions or in the laboratory; 3) isolation of single cells using microfluidics technologies; and 4) co-cultivation of microbes and cells of the host organism separated by a diffusion membrane allowing native chemical interactions to select for microbial symbionts.

3D-printed bionic corals

Micro-algal cultivation is of interest for the production of bioenergy and bioproducts for pharmaceutical, nutraceutical and cosmetic use, but the self-shading of algal cells in culture facilities limits the scalability as large surface areas are required. Interestingly, corals have evolved microstructurally complex skeletons to enhance light availability for photosynthesis by their symbiotic Symbiodiniaceae algae. By optimizing light scattering within the coral tissues, even algae that would otherwise be shaded by other algal cells or coral tissues receive sufficient light levels [2]. These adaptations have turned corals into highly efficient 'photon augmentation systems', which is particularly true for mesophotic corals that thrive at low-light conditions [45,46]. Inspired by the optical properties of corals, a '3D-printed bionic coral' model made from gelatin-based hydrogels and biopolymers was recently developed to address the problem of self-shading in micro-algal cultivation systems [47••]. The authors showed that by growing micro-algae in the hydrogel 'tissues' of the bionic coral, 10-fold higher cell densities were reached in comparison with standard media-based cultivation, and that in contrast to biofilm-based photoreactors light attenuation was limited. The productivity of the algal cells grown in such hydrogels can even be boosted via co-cultures with *Erythrobacter* bacteria [48], and possibly genetic engineering using protoplast technology [49•]. These studies show significant promise for the use of 3D-printed bionic corals for micro-algal cultivation. However, upscaling the capacity is needed to turn this into an economically viable application at industrial scales to produce oils for bioenergy and commercially relevant bioproducts [50].

Coral holobionts have a tremendous biotechnological potential — from a resource of compounds with potentially medical and industrial applications, to coral-inspired production systems. This biotechnology-based ecosystem service of corals may become highly profitable and thus contribute substantially to a 'Blue Economy'. However, the survival of corals is under threat due to urbanization and the increasing seawater temperatures due to climate change. Protecting the diversity of corals is thus of the utmost importance, or we may risk losing the still hidden treasures harbored within coral holobionts.

Biotechnology for coral reef conservation

Anthropogenic impacts such as pollution and global climate change have significantly impacted coral ecosystems. To preserve the biodiversity and ecosystem services they provide, many coral reef restoration initiatives are underway, transplanting asexually produced coral fragments grown in coral nurseries onto denuded reefs. As the native corals used for restoration efforts are unlikely to have increased tolerance to climate change or pollution, some scientists have advocated more drastic interventions using biotechnological approaches, like 'assisted evolution' and 'coral probiotics'.

Biotechnological asexual reproduction of corals

Most coral restoration efforts use asexual reproduction by generating many small coral fragments from large colonies to increase colony numbers. These micro-fragments are then maintained in 'coral nurseries' until they are ready for outplanting onto degraded reefs. This relatively low-tech but labor-intensive practice is known as 'Coral Gardening'. Recently, a new direction for 'Coral Gardening' was proposed, advocating to embed coral gardens into architectural landscapes of coastal cities on a large scale to stimulate (eco-)tourism, raise awareness and educate people about the marine environment [51]. As this may generate additional revenue for participating businesses, it will likely encourage further investments in coral restoration efforts. In contrast to traditional coral gardening in ocean-based coral nurseries, culturing conditions can be modulated in land-based coral gardens, which will allow the selection for and the cultivation of corals with increased stress resilience required to survive under expected future climate conditions thereby likely increasing the long-term success of such reef restoration efforts.

The rate limiting step in coral colony growth and thus reef restoration, however, is the calcification process in corals to build a skeleton. On the contrary, coral tissue growth is remarkably rapid. Therefore, a new approach has been developed: the 3D-printing of coral skeletons with calcium carbonate photoinitiated (CCP) ink using 3D scans of coral skeletons as a blueprint, followed by the addition of a small colony whose tissues will rapidly overgrow the 3D-printed skeleton [52]. The advantage of this method is that it is possible to generate large coral colonies within a relatively short timeframe, and using these will likely restore the ecological function and ecosystem services of a reef faster. Besides, as colony size matters for the reproduction of corals, these colonies may be reproductively active much sooner than those raised through traditional 'Coral Gardening', thereby enhancing natural processes of reef restoration. This method may in particular be a solution to rapidly expand the number of slow-growing coral species. To fully restore a reef, however, thousands if not millions of colonies will be needed, and 3D-printing such numbers and maintaining them in nurseries until outplanting will require a significant investment in logistics and infrastructure.

The use of coral cell and tissue cultures (discussed earlier) may be other alternative methods for asexual reproduction. Particularly the feasibility of long-term culture [37••] and the observation that cell cultures from adult tissues resemble larvae [53], suggest that if these cultures can be stimulated to develop into corals, this may have potential for rapidly expanding juvenile-like corals. This can then

be combined with the 3D-printing of coral skeletons to boost colony development.

Native corals that may be used in the above-described methods for reef restoration are, however, unlikely to have resilience against pollution or climate change-related environmental stressors. Increasing the stress-tolerance of corals is thus of high importance. The various biotechnological avenues that are currently being explored are discussed in the next sections, and may be combined with asexual reproduction methods to boost the numbers of resilient corals for outplanting.

Assisted evolution

High seawater temperatures can lead to a breakdown of the coral-Symbiodiniaceae symbiosis, causing 'bleaching' events and subsequent coral loss on massive scales. Hence, van Oppen *et al.* [54] coined the idea to use 'assisted evolution' to create corals resilient to the ever-increasing stressors related to climate change by accelerating natural processes of selection, and later provided guidelines for implementation into coral reef conservation [55]. However, ethical concerns about assisted evolution remain [56•] as it is focused on 'designing a 'natural' system' to preserve its function rather than restoring reef coral communities to their original state, and risks of such interventions on ecosystems are unknown.

Research into assisted evolution has taken several different avenues. As coral bleaching is primarily the result of dysfunctioning algal symbionts, one of the goals is to increase the heat tolerance of Symbiodiniaceae by culturing them *in vitro* at elevated temperatures and use them to inoculate coral recruits (Figure 1). Overall, it was found that four years (~120 generations) of heat evolution were needed in order for some strains of Symbiodiniaceae to provide sufficient protection against bleaching [57••], and that 2.5 years (~80 generations) was not enough [58]. The 'heat-evolved' algal strains that conveyed bleaching resilience to the coral holobiont had increased the expression levels of genes linked to heat tolerance in the coral host and to carbon fixation in the algal symbionts [57••].

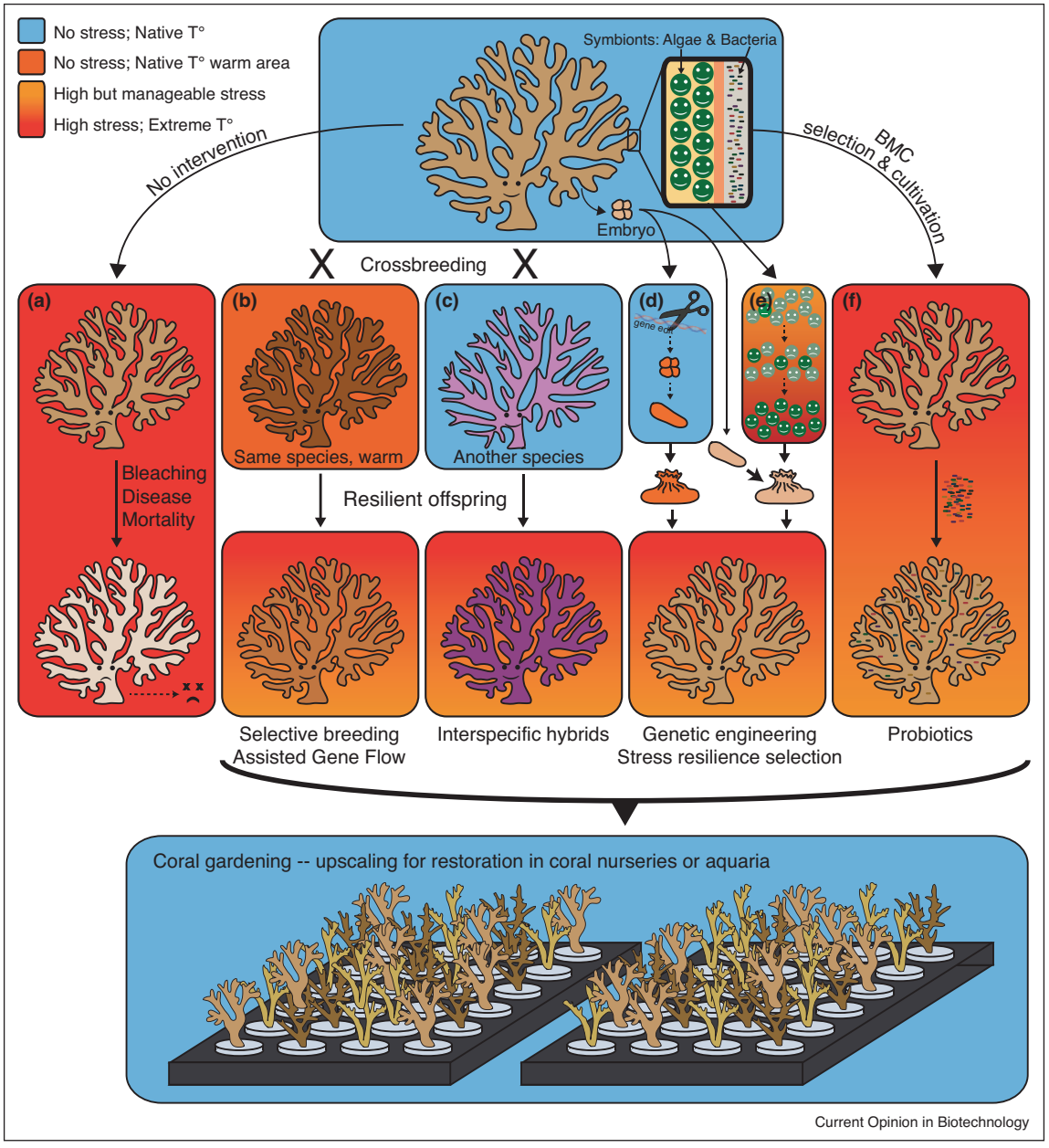
Other assisted evolution approaches also under investigation are coral host-centric, as many generations will be needed to establish adaptive gene variants in sensitive populations without interventions [59]. These approaches are: (1) 'assisted gene flow', where corals with high thermal history are transplanted to colder reefs with the aim to introduce alleles linked to thermotolerance; (2) 'selective breeding', where corals with heat tolerant properties are crossed *ex situ* with corals from colder areas to create more resilient crossbreds (Figure 1); and (3) 'inter-specific hybridization', where hybrids of two coral species are created *ex situ* (Figure 1). Inter-specific *Acropora* hybrids have indeed higher growth rates and tolerance to elevated temperatures and ocean acidification than purebred juvenile colonies [60•]. And selective breeding has successfully increased survival rates by >13-fold under thermal stress conditions in hybrids in comparison with purebreds from colder reefs [61•], however, bleaching sensitivity was still mostly driven by the associated Symbiodiniaceae. Although some promising advances have been reported, all studies so far focused on broadcast spawning *Acropora* species and to maintain diversity and structural complexity on reefs other taxa still need to be evaluated. As native species will be used, these methods are believed to carry relatively little risk when implemented *in situ*. However, as these improved corals do have a competitive advantage over native colonies, there is a risk for change in reef diversity and ecosystem functioning. Besides, whether it is feasible to transplant sufficiently large numbers of reproductive heat-tolerant corals or laboratory-grown hybrids onto reefs will be logistically incredibly challenging. Therefore, it will be necessary to study population genetics and reef connectivity to identify reefs with high connectivity and use those to develop a select number of 'designer reefs' that can seed connected reefs to rapidly spread the adaptive gene traits.

Developed to unravel the functional biology of corals and their algal symbionts at the molecular level, the successful genetic engineering of coral larvae with CRISPR/Cas9 technology [39•,40••] (Figure 1) and the development of protoplast technology for Symbiodiniaceae has also opened avenues to engineer genetically improved coral holobionts with higher thermal tolerances [49•]. Contrary to the other assisted evolution approaches, genetic modification of corals and introduction into the wild for reef restoration is highly controversial and whether it should be pursued at all is still being debated. The consensus is that this should only be considered a last resort for coral reef restoration crisis management and approached with extreme caution as the consequences of introducing engineered organisms for other animals and the ecosystems are still unknown. However, developing these techniques further to study coral biology and stress resilience should be encouraged, and will also guarantee that if genetic modification of reef corals will, for some reason, become necessary in the future, the toolkit is optimized and can be readily applied. Regardless of these biotechnological approaches, however, the priority remains to reduce pressures, such as chronic pollution and greenhouse gas emissions, on coral reefs to conserve coral reef ecosystems.

Coral probiotics

The Coral Probiotic Hypothesis was coined by Reshef *et al.* in 2006 [62] positing that dynamic changes in the microbial symbiont community in response to changing environmental conditions are based on the selection for a

Figure 1



Biotechnological approaches for coral conservation — Assisted Evolution and Coral Probiotics. Stress tolerance-increasing interventions are needed as most corals are not resilient to current summer temperature extremes and future climate scenarios. Under these conditions, corals may bleach and/or contract disease, both of which may ultimately lead to mortality **(a)**. Crossbreeding sensitive corals with those from areas with naturally higher temperatures **(b)** or closely related species **(c)** has been shown to increase stress resilience in their intra-specific or inter-specific hybrid offspring, respectively. Genetic engineering of coral embryos, which further develop into larvae and settle to grow out to adult colonies, to overexpress stress-tolerance genes **(d)** may be another approach, but ethical concerns still exists and the time to reach sexual maturity is long. Manipulation of the microbial symbionts of corals is another angle that is being explored. Cultivating algal symbionts over many generations at increasing/elevated temperatures can boost their thermal tolerance, subsequently improving holobiont resilience in inoculated corals **(e)**. Corals that are experiencing stress can also be treated using 'Beneficial Microbes for Corals' (BMC) to increase the coral's chance of survival **(f)**, an approach that not only impacts the microbiota but also stimulates coral physiology. Coral gardening can be employed to further increase the number of corals with increased stress tolerance for outplanting on degraded reefs for restoration.

well-adapted/acclimatized holobiont. Since then, a framework for the active manipulation of the coral microbiota with 'probiotics' was proposed [63] and has been recognized as a potentially powerful way to improve coral resilience to stressors and control coral diseases (Figure 1). The coral probiotics field has been expertly reviewed recently [64••] and in this special issue [65]. Therefore, we will only briefly discuss it here. The approach taken is to first isolate CAMs from the target coral species and select those that display putatively beneficial roles (e.g. stress mitigation; degradation of pollutants; provision of nutrients; protection against pathogens) [66]. Then, tailored consortia of those Beneficial Microorganisms for Corals (BMCs) [63] are developed and used for inoculation to increase their abundances in the holobiont with the aim to mitigate the threat [64••]. This approach can thus be considered as Customized Medicine for Corals [67]. Coral probiotics have been successfully applied to treat corals in experimental conditions. For example, the addition of a consortium of BMCs to *Pocillopora damicornis* mitigated coral bleaching and mortality under thermal stress conditions and in combination with pathogen exposure [68••]. Similarly, direct coral microbiome transplantation (CMT) from heat-tolerant corals to their heat-sensitive counterparts also reduced bleaching in the inoculated corals experiencing thermal bleaching conditions [69]. While inoculation of thermally stressed corals with Symbiodiniaceae probiotics reduced bleaching and mortality as well, this was likely related to predation on the algae [70]. In-depth studies on *Mussismilia hispida* treated with probiotics have linked the increased coral survival following heat stress to the BMC-induced restructuring of the microbiota and the subsequent changes in the coral's metabolism and stress response [71]. The BMC inoculations stimulated in fact the expression of genes involved in stress protection and lipid biosynthesis in the thermally stressed coral host, thereby mitigating stress as evidenced by reduced inflammation responses and apoptosis, and stimulated sulfur metabolism processes in the holobiont that are related to antioxidant production and disease mitigation. The transcriptome reprogramming has raised the question whether CAMs may modify a coral host's epigenome [72] — an exciting hypothesis, because it could mean that BMC treatments may potentially provide long-term benefits for coral stress resilience as epigenetic changes can be transferred to offspring. BMCs can also be used for bioremediation of oil pollution [73], and may be a better and safer alternative to the highly toxic chemical oil dispersants commonly used [74••].

Phage therapy is another probiotic technique which uses the natural properties of lytic phages to eliminate specific bacterial pathogens. Phages that infect *Vibrio coralliilyticus* have received particular attention as this pathogen is implicated in coral bleaching and coral diseases [75]. Although such phages have been shown to prevent and treat infections of Symbiodiniaceae algae in culture [75,76], whether phage therapy of diseased corals holds promise remains unclear. Pathogens can, however, develop resistance to phages and a phage-cocktail, such as recently employed to prevent *V. coralliilyticus* infection in oyster larvae, may be the best approach [77].

While preliminary studies implementing probiotic applications have shown promising outcomes in experimental settings, the next challenges are to isolate novel microbes (discussed earlier) and to deliver the inoculum of BMCs to the host in open marine systems [64••,78]. While direct delivery of the microbial consortium may be impractical and sacrificing heat-tolerant corals for CMT is unwanted, other proposed options for delivery involve encapsulating the microbes in carriers, biopolymers or food [64••]. For example, rotifers can be used as BMC delivery vehicles as they uptake BMCs efficiently and corals predate on them [79]; but whether a symbiosis between the coral and the BMCs subsequently develops remained unclear. Regardless, BMC delivery to the target host remains challenging as it is unclear how specific target inoculations can be reached. Another complication is the observation that the natural tendency to allow change in the microbiota — termed 'microbiome flexibility' — is coral host species specific [80•,81••]. Whereas some species restructure their microbiota in response to environmental change ('microbiome conformers'), other species may regulate it to maintain a consistent microbiome structure ('microbiome regulators') [80•,82]. Most holobionts can likely not be strictly assigned to one group, and a spectrum of microbiome flexibility with the 'conformers' and 'regulators' on either end of the scale should thus be considered. As such, we need to investigate where on the microbiome flexibility spectrum each coral species is to assess the likelihood of success of probiotics treatments, particularly when introducing new putatively beneficial microbial species.

Various biotechnological approaches are currently used or being investigated with the aim to restore and conserve coral ecosystems. Protecting coral ecosystems is not only of ecological importance, but will also safeguard their $375 billion annual worth to the 'Blue Economy' through the ecosystem services provided. Afterall, the lives of millions of people and local communities depend on coral ecosystems – from a food resource through fisheries and mariculture, and a source of income via tourism to the protection of coast lines. It is thus of the utmost importance to ensure that corals will be resilient and able to survive future climate conditions to safeguard the livelihoods of millions of peoples, while in the meantime finding solutions to boost the chances of survival of corals experiencing potentially lethal stress. A lot of work remains to be done, however, to scale up the efforts and increase the feasibility of these approaches.

Conclusion

The biotechnological potential of coral holobionts for natural product discovery and the biotechnological applications for coral ecosystem conservation fall within the 'Blue Economy' principle and are focused on 'Blue Growth'. This concept is aimed at 'fostering economic growth and development while ensuring that natural assets of aquatic ecosystems continue to provide the resources and environmental services on which our well-being relies' (Organization for Economic Co-operation and Development).

Natural products harbored within coral holobionts may be a high value resource. Their pharmaceutical, cosmetic and anti-fouling properties show promise for applications in medicine as well as industrial processes and shipping. Particularly given the threat of antibiotic resistance and viral pandemics it is important to search for novel antibiotic and antiviral compounds, sometimes in unusual places, like corals. Currently, the potential of NPs from coral holobionts is still largely untapped as mostly some shallow coral species have been investigated. Exploring the NP repertoire of corals from the deep sea and cold waters may be a good way forward as extreme conditions require extreme adaptations. But a tremendous potential is likely also hidden within the microbial symbionts of corals — not only NPs but likely also novel OMEGA gene-editing tools related to the CRISPR/Cas9-system [83]. Despite some successes, the cultivation of those microbes remains challenging as culture conditions are often unknown. Therefore, it is worth exploring new microfluidics-based technologies for single-cell isolation and cultivation of microbes under near-native conditions in specialized chips. At the same time, we need to invest in (meta)genomics and genome engineering, which have shown their usefulness in identifying gene clusters involved in NP biosynthesis, which can be used for heterologous expression in biotechnologically important microbes, and for improved NP production. But a 'reverse genomics' approach should also be considered as genome data can provide information on the metabolic requirements of microbes, allowing us to formulate custom media optimized for their isolation and growth.

Products from the eukaryotic partners within the coral holobiont may also be of high medical and industrial value. While the algal symbionts have been cultivated for decades, establishing coral cell cultures was only recently achieved and still needs to be further optimized. The development of novel coral cell culture media that allow expansion of cultures would be a first priority to further coral cell biology studies and the production of coral NPs *via* overexpression (e.g. CRISPRa systems) of biosynthesis pathways and upscaling. In contrast, algae-derived products, such as oils for bioenergy, may see a significant boost in yields thanks to recently developed coral-inspired 3D-printed bionic coral production systems, although investments will be required to scale up these systems for industrial applications to be economically viable.

As corals are threatened by climate change and pollution, so is the still untapped potential for NP discovery. However, the importance of coral reefs and animal forests for coastal protection, tourism and fisheries are equally under threat. But as long as greenhouse gas emissions and pollution have not been substantially and adequately reduced, new biotechnological intervention strategies are needed to preserve these coral ecosystem services. Research in 'Assisted Evolution' and 'Coral Probiotics' has made significant steps to develop future-proof coral holobionts, and treat the impacts of stress in the short term, respectively. However, we need to remain cautious when implementing biotechnologically-improved corals as many ethical concerns and uncertainties about potential ecological impacts remain.

Overall, substantial progress has been made on the biotechnological exploitation of and conservation applications for coral holobionts. Current research and the use of novel technologies will no doubt further reveal the biotechnological potential harbored within coral holobionts. One of the main challenges is, however, scalability — how can we stimulate investments in coral-based biotechnology for coral conservation and 'Blue Growth'. Last year, the World Coral Conservatory (WCC) [84] was launched: an initiative to collect all coral species and house them in several public aquaria around the world for preservation, reef restoration and education of the public. We can use this opportunity to ambitiously scale up the efforts under the WCC umbrella by perhaps not only using public aquaria but implement it also in the architectural landscape as suggested by Schmidt-Roach *et al.* [51] which contributes to public education and possibly generates additional income for participating businesses. This high coral diversity coral gardening could even be combined with assisted evolution approaches, once ethical concerns have been addressed, to boost feasibility to 'future-proof' coral ecosystems. As such a collection will likely also be a treasure trove for natural product discovery, investments from biotechnology and pharmaceutical companies may be solicited, possibly stimulated through tax benefits for sustainable development.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Jeroen AJM van de Water: Conceptualization, Writing – original draft, Writing – review & editing, Visualization. **Romie Tignat-Perrier:** Writing – original draft, Writing – review & editing. **Denis Allemand:** Writing – review & editing. **Christine Ferrier-Pagès:** Conceptualization, Writing – original draft, Writing – review & editing.

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