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Symbiodiniaceae-bacteria interactions: rethinking metabolite exchange in reef-building corals as multi-partner metabolic networks.

Running Title: Corals as multi-partner metabolic networks.

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Summary

The intimate relationship between scleractinian corals and their associated microorganisms is fundamental to healthy coral reef ecosystems. Coral-associated microbes (Symbiodiniaceae and other protists, bacteria, archaea, fungi, and viruses) support coral health and resilience through metabolite transfer, inter-partner signalling, and genetic exchange. However, much of our understanding of the coral holobiont relationship has come from studies that have investigated either coral-Symbiodiniaceae or coral-bacteria interactions in isolation, while relatively little research has focused on other ecological and metabolic interactions potentially occurring within the coral multi-partner symbiotic network. Recent evidences of intimate coupling between phytoplankton and bacteria have demonstrated that obligate resource exchange between partners fundamentally drives their ecological success. Here, we posit that similar associations with bacterial consortia regulate Symbiodiniaceae productivity and are in turn central to the health of corals. Indeed, we propose that this bacteria-Symbiodiniaceae-coral relationship underpins the coral holobiont's nutrition, stress tolerance, and potentially influences the future survival of coral reef ecosystems under changing environmental conditions. Resolving Symbiodiniaceae-bacteria associations is therefore a logical next step towards understanding the complex multi-partner interactions occurring in the coral holobiont.

Keywords: Symbiodiniaceae; microbiome; bacteria; coral; metabolism; symbiotic interactions

Originality-Significance Statement

Complex interspecific interactions between coral hosts and the multitude of symbiotic microorganisms they harbour underpins the health and survival of coral reefs. However, research to date has focused on coral host-unicellular algae (Symbiodiniaceae) or coral host–prokaryote interactions, while other relationships within the coral holobiont have been relatively overlooked. We propose to view the coral holobiont as a multi-partner metabolic network, providing a paradigm shift from a bipartite symbiosis to a community-based

integration of factors that determine the resilience of coral reef organisms. We review emerging evidence of the importance of the ecological relationships occurring between marine phytoplankton and bacteria, and review Symbiodiniaceae-bacteria interactions that could underpin Symbiodiniaceae ecological success and contribute to coral holobiont fitness. Understanding these interactions is of critical importance for predicting the resilience and adaptability of coral reefs to environmental change.

Introduction

Reef-building corals are holobionts, comprising the cnidarian host and its associated microbes: dinoflagellates [Symbiodiniaceae, (LaJeunesse et al., 2018)] and other protists, bacteria, archaea, fungi, and viruses (Rohwer et al., 2002; Kwong et al., 2019; Pernice et al., 2019). The cnidarian host relies on the resources from these numerous symbionts to support its energy budget, growth rate, fecundity and metabolism (Peixoto et al., 2017; Torda et al., 2017). Indeed, coral-microorganism interactions have influenced the genomic evolution of both the host and microorganism partners, shaped coral development, and defined the ecological success of the coral holobiont (Thompson et al., 2015). Yet, of the multiple potential interactions within the coral holobiont, research has generally focused on the coral-dinoflagellate and coral-bacteria interactions e.g. (Webster et al., 2016; Hillyer et al., 2018), and relatively few studies have investigated other potential inter-partner interactions (Fig. 1; but see Table 1).

Numerous studies have demonstrated that both endosymbiotic Symbiodiniaceae and associated bacteria support the persistence of corals through the exchange of metabolites and bioactive compounds (Rohwer et al., 2002; Cantin et al., 2009; Ainsworth et al., 2010; Bourne et al., 2013; Glasl et al., 2016; Peixoto et al., 2017; Hillyer et al., 2018; Matthews et al., 2018). Yet, remarkably, the role of bacteria in regulating Symbiodiniaceae resource acquisition, competitive performance, and functional diversity (as both free-living and endosymbionts) is relatively unexplored (Ritchie, 2012; Bourne et al., 2013; Ainsworth et al., 2015; Peixoto et al., 2017; Silveira et al., 2017; Bernasconi et al., 2018); Table 1). This fundamental gap in knowledge wholly constrains our understanding of how microbes act in concert to regulate the health of coral holobionts, especially given the importance of bacterial-algal interactions for nutrient cycling, signal transduction and gene transfer as demonstrated for other microalgal taxa (Seymour et al., 2017). Emerging evidence suggests that interactions with bacterial associates may be important for Symbiodiniaceae nutrition and survival in their free-living state (Jeong et al., 2012; Frommlet et al., 2015; Lawson et al., 2018). Furthermore, global co-occurrence of specific bacterial taxa and Symbiodiniaceae in corals (Bernasconi et al., 2018) and co-localisation of distinct bacterial taxa with Symbiodiniaceae in cnidarian host tissues (Ainsworth et al., 2015) suggest specific Symbiodiniaceae-bacterial interactions may be crucial to support holobiont metabolic functioning. Exploring Symbiodiniaceae-bacteria associations is therefore a logical next step towards fully understanding the complex symbiotic interactions occurring in the coral holobiont, and assists development of conservation strategies for reefs under global climate change (van Oppen and Blackall, 2019).

Unlocking the significance of these interactions in Symbiodiniaceae ecological success could help answer fundamental questions surrounding coral reef function and resilience, including: 1) do obligate associations with bacterial symbionts enable Symbiodiniaceae to thrive as both free-living cells and endosymbionts?; 2) do interactions with bacteria promote the immense functional diversity observed for Symbiodiniaceae?; and 3) what role, if any, could Symbiodiniaceae-bacterial interactions play in the ecological success of the coral holobiont? Here, we consider that Symbiodiniaceae may hold an innate dependency on specific bacteria for resource exchange, thus forming an intimate association that may support optimum metabolic fitness of the coral holobiont. We present the current data supporting these relationships in the coral symbiotic network and explore how evidence from other algal-bacterial systems suggest Symbiodiniaceae-bacteria interactions may be a hidden key in determining the overall health and resilience of the coral holobiont.

Are bacteria obligate symbionts and “resource surrogates” for free-living Symbiodiniaceae?

Metabolic exchange between autotrophic organisms and bacteria underpin the ecological success of many plant and algal species across marine and terrestrial systems (Ramanan et al., 2016). Bacterial and archaeal associates protect many eukaryotic hosts against pathogens, supply and recycle essential nutrients (Ramanan et al., 2016), and even contribute to the acclimatisation and adaptation of their host to environmental change (Schönknecht et al., 2013). Phytoplankton-bacteria interactions drive the major oceanic biogeochemical cycles, contribute to energy transfers to higher trophic levels, and to the emission of climate-regulating gasses (Seymour et al., 2017). More specifically, bacteria can enhance the growth of dinoflagellates through specific nutrient exchange (Croft et al., 2005), while dinoflagellate-bacteria interactions may play a role in algal toxin production, e.g. neurotoxin production by *Alexandrium tamarense* (Zhang et al., 2015). Furthermore, specific bacterial taxa are consistently present in dinoflagellate cultures, including Symbiodiniaceae (Frommlet et al., 2015; Lawson et al., 2018), which strongly suggests that associated bacteria carry out important functions for these microalgae (Zhang et al., 2015).

The functional diversity of Symbiodiniaceae in terms of stress tolerance provides corals with the capacity to respond to environmental change, and thus acquiring new Symbiodiniaceae species from the environment or undergoing changes in the relative abundance of species already present in the coral tissues can enable corals to acclimatise to increasing environmental threats (Baker, 2003; Berkelmans and Van Oppen, 2006; Loram et al., 2007; Stat and Gates, 2011; Boulotte et al., 2016; Hume et al., 2016). Recent evidence has demonstrated the uptake of exogenous, thermally tolerant Symbiodiniaceae species by corals in response to repeated temperature stress (Boulotte et al., 2016). Furthermore, molecular studies have now confirmed that most Symbiodiniaceae genetic variants forming symbioses spend part of their life-cycle as free living cells before colonising new coral recruits (Cunning et al., 2015). Yet despite over 50 years of research examining Symbiodiniaceae biology and ecology, relatively little is known about the free-living life stage of Symbiodiniaceae (Stat et al., 2006; Takabayashi et al., 2012; Cunning et al., 2015; Suggett et al., 2017; LaJeunesse et al., 2018). For instance, it is still unclear how Symbiodiniaceae thrive outside of the coral tissues in oligotrophic waters and sediments without the concomitant provision of essential nutrients.

Cultured Symbiodiniaceae predate on bacteria under both nutrient-replete and -depleted conditions, demonstrating that heterotrophic feeding may be a survival strategy for Symbiodiniaceae cells when free-living (Jeong et al., 2012). However, dependency upon bacteria for resource acquisition by free-living Symbiodiniaceae likely extends far beyond predation, and may also involve exchange of key metabolites (Seymour et al., 2017). For example, *Sulfitobacter* species promote diatom growth through the secretion of indole-3-acetic acid synthesised from diatom-derived tryptophan, demonstrating how mutual exchanges of metabolites can influence the success of bacterial and phytoplankton partners (Amin et al., 2015). Evidence for algal acquisition of vitamins (e.g. vitamin B12; (Croft et al., 2005)), and enhanced micronutrient bioavailability (e.g. Fe (Amin et al., 2015), Zn (Ferrier-Pagès et al., 2005)) through symbiotic relationships with specific bacteria further highlights the important nutritional links between these groups of aquatic microorganisms.

Most dinoflagellates are difficult to grow axenically, which suggests that associated bacteria provide necessary metabolic resources for successful dinoflagellate growth outside of the host, and that these bacterial partners might operate as “resource surrogates” (Ritchie, 2012; Suggett et al., 2017). Indeed, changes in bacterial community composition modify the growth of the dinoflagellate *Gymnodinium catenatum* by approximately four-fold, which is equivalent to growth enhancements triggered by a 5°C difference in temperature or a six-fold difference in light intensity under laboratory conditions (Bolch et al., 2017). A recent study confirmed that bacteria are abundant and diverse within most Symbiodiniaceae laboratory cultures, and that some bacterial taxa are conserved across Symbiodiniaceae genera (Lawson et al., 2018). Notably, members of the Rhodobacterales, a family of α-proteobacteria commonly associated with many phytoplankton taxa, have been found in Symbiodiniaceae cultures all over the world, suggesting these bacteria are conserved mutualists of Symbiodiniaceae (Ritchie, 2012; Lawson et al., 2018). In culture, Symbiodiniaceae spp. and associated bacteria form calcifying structures known as symbiolites (i.e. aragonitic microbialites in which Symbiodiniaceae are encased) (Frommlet et al., 2015). Evidence suggests symbiolite formation is a photosynthesis-induced bacterial-Symbiodiniaceae calcification, which likely occurs in reef sediments as well (Frommlet et al., 2018). This is the first evidence of metabolic coupling between Symbiodiniaceae and associated bacteria, and identify an entirely new endolithic niche for free-living (benthic) Symbiodiniaceae. The formation of symbiolites is likely ecologically important, as it can protect Symbiodiniaceae from grazers and UV radiation, while still permitting photosynthesis (Friedmann, 1982; Shashar et al., 1997; Jeong et al., 2012; Frommlet et al., 2018). These novel insights into bacterial-Symbiodiniaceae associations reinforce how this coupling may be essential to the fitness and life-cycle viability of the dinoflagellates, and may ultimately influence their availability to new coral recruits. However there is as yet very little understanding of the metabolic exchange between Symbiodiniaceae and their bacterial partners in the water column, sediments and in endosymbiosis (Fig. 3).

Genome reduction and the exchange of metabolic intermediates between hosts and symbionts are common phenomena of obligate symbioses, and the absence of important metabolic pathways within an organism can point towards dependencies of symbiotic partners. For example, the abundant coral genus *Acropora* lacks the enzyme responsible for cysteine biosynthesis, implying this coral is dependent on its microbial symbionts for provision of this amino acid (Shinzato et al., 2011). While still large compared to other microalgae, as a result of idiosyncratic features such as multiple gene copies, Symbiodiniaceae genomes are small relative to other dinoflagellates (Fig. S1), suggesting this family could have also lost necessary components, such as key enzymes, due to obligate interactions with bacterial symbionts, coral hosts, or other symbionts. Symbiodiniaceae-associated bacteria may provide metabolic capabilities that are missing in the Symbiodiniaceae genome. Examples of potential obligate bacterial contributions observed in other microalgae-bacteria interactions include the ability to degrade complex polysaccharides, to reduce competition (Ramanan et al., 2016) or to acquire bioavailable iron (Hopkinson and Morel, 2009)). Iron is essential for a range of physiological processes in microalgae, including photosynthesis, respiration and nitrogen fixation (Behrenfeld et al., 2009). Although, Symbiodiniaceae in a free-living state can acquire bioavailable trace metals from ambient seawater using specific transporters (Morel and Price, 2003), the concentrations of bioavailable iron in much of the global ocean are too low to support microalgal growth (Hopkinson and Morel, 2009). Recent culturing work examining trace metal availability demonstrated that the free-living *Fugacium kawagutii* (previously *Symbiodinium kawagutii*) requires high concentrations of bioavailable iron to achieve optimum growth (Rodriguez and Ho, 2018). Marine bacteria produce siderophores, which bind and concentrate iron into bioavailable forms, enabling the uptake of this limiting micronutrient by phytoplankton; for example, the production of siderophores by the γ-proteobacterium *Marinobacter* promotes the growth of its dinoflagellate partner, *Scrippsiella trochoidea* (Amin et al. 2009). Intriguingly, *Marinobacter* was also part of the "core" bacterial-associates shared between diverse Symbiodiniaceae cultures (Lawson et al., 2018). It is therefore likely that Symbiodiniaceae depend on bacterial associates to fulfil their needs for bioavailable iron (Fig. 2).

Another important micronutrient is Vitamin B_{12} (cobalamin), a cofactor involved in the production of the amino acid methionine, which is needed to synthesize every protein as well as in diverse metabolic pathways including the generation of antioxidants glutathione and DMSP (Croft et al., 2005). Cobalamin is synthesised by many heterotrophic bacteria and nearly all oxygenic photosynthetic cyanobacteria but as with many algal protists, Symbiodiniaceae require exogenous cobalamin for growth as they do not possess the genetic machinery to generate the active form of this vitamin (Supplemental Information S1). Genomic evidence suggests Symbiodiniaceae possess cobalamin-dependent methionine synthase and not cobalamin-independent methionine synthase (Supplemental Information S1), implying that free-living Symbiodiniaceae depend on bacterial symbionts to gain access to this important cofactor.

Do bacteria drive Symbiodiniaceae functional diversity?

Substantial genetic and functional diversity of Symbiodiniaceae provides capacity for the coral holobiont to respond to a broad range of environmental conditions (Sampayo et al., 2009; Pettay et al., 2015; Boulotte et al., 2016; Hume et al., 2016; Silverstein et al., 2017; Suggett et al., 2017; LaJeunesse et al., 2018), which is of immense ecological importance to the survival and growth of coral reefs. There is high genetic divergence between Symbiodiniaceae species (even those in the same genus) (Liu et al., 2018; González-Pech et al., 2019); however, many of the drivers of the large functional diversity of Symbiodiniaceae remain largely unresolved. Gene duplication and horizontal gene transfer are two drivers of genome evolution, which promote the emergence of novel traits. Long-term symbiosis can lead to the sharing of genetic traits between algae and their associated microbiome, which has been proposed to have had a major impact on the gene content of algae (Keeling, 2009; Schönknecht et al., 2014; Husnik and McCutcheon, 2018). For example, emerging genomic evidence has shown that horizontal gene transfer from bacteria and archaea facilitates the survival of the extremophilic, unicellular red alga, *Galdieria sulphuraria*, which lives in hot, metal-rich, toxic and acidic environments (Schönknecht et al., 2013). A growing body of evidence indicates that horizontal gene transfer is an important source of gene innovation in dinoflagellates (Wisecaver et al., 2013). Gene transfer from associated bacteria may therefore provide a similar adaptive mechanism for Symbiodiniaceae and may explain the large functional diversity evident across the Symbiodiniaceae family. For example, members of the Rhodobacterales, which have been found in close association with corals (Apprill, 2017) and are an important component of the Symbiodiniaceae bacterial-associates (Lawson et al., 2018), have a large propensity for transferring genes to other microbes in the marine environment (McDaniel et al., 2010; Webster and Reusch, 2017). It is therefore plausible that Symbiodiniaceae could acquire foreign genes from associated bacteria via bacteriophages or similar genetic elements such as gene transfer agents (Solioz and Marrs, 1977; Shumaker et al., 2019). Furthermore, microbes in coral reef environments show a much higher rate of novel-trait acquisition by horizontal gene transfer than by spontaneous mutation, suggesting an alternate mode of adaptation via swapping of potentially beneficial genes between marine microbes (McDaniel et al., 2010), and possibly Symbiodiniaceae.

Horizontal symbiont acquisition, where offspring acquire symbionts from the environment, could provide an opportunity for free-living Symbiodiniaceae and/or bacterial communities to establish novel, potentially advantageous, associations with a cnidarian host. This mode of Symbiodiniaceae transmission is observed in the majority of coral species and could allow offspring to select a symbiont community that confers advantages locally. The role of bacteria in facilitating functional diversity in Symbiodiniaceae is entirely unexplored, but raises intriguing questions about whether the variability of Symbiodiniaceae fitness (and stress resistance) is determined purely by genetic differences between Symbiodiniaceae species (Sampayo et al., 2009; Pettay et al., 2015; Boulotte et al., 2016; Hume et al., 2016; Silverstein et al., 2017; Suggett et al., 2017; LaJeunesse et al., 2018; Liu et al., 2018; González-Pech et al., 2019) or whether other mechanisms play a role.

Dynamic resource exchange supports coral holobiont health and survival

Obligate associations between the cnidarian host and Symbiodiniaceae are essential for coral reef growth and survival. The host coral provides the endosymbionts with substrates for photosynthesis, which in turn fuels the autotrophic production of metabolites that the host cannot synthesise independently (e.g. palmitoleic acid in *Exaiptasia pallida*, (Matthews et al., 2018)). This resource exchange is one of the defining features allowing reef-building corals to flourish in otherwise nutrient-poor environments. Bacteria are also key ecological partners of cnidarians and are increasingly recognised as crucially important for the health of the holobiont (Bourne et al., 2016; Raina et al., 2016; Hernandez-Agreda et al., 2017; Peixoto et al., 2017; Brener-Raffalli et al., 2018) (Fig. 1). Gammaproteobacteria in the genus *Endozoicomonas* are a ubiquitous endosymbiont for corals (Bayer et al., 2013), and comparative genomic analysis of different strains of *Endozoicomonas* from different hosts suggests a common role in carbohydrate cycling and potential specificities in amino acid synthesis (Neave et al., 2017). Recent advances have allowed a better understanding of the functional significance of coral-associated bacterial communities, their roles in resource provision and nutrient cycling (Rädecker et al., 2015; Bourne et al., 2016), their responses to shifts in environmental conditions (Brener-Raffalli et al., 2018), and their potential "probiotic" role in mediating holobiont health and survival (Damjanovic et al., 2017; Peixoto et al., 2017; Webster and Reusch, 2017; Rosado et al., 2019; van Oppen and Blackall, 2019).

Only a few studies have specifically considered Symbiodiniaceae-bacterial interactions in the coral holobiont, with their results pointing to the potentially critical role that these partnerships might play in regulating holobiont nutrient cycling and competitive fitness (Ritchie, 2012; Bourne et al., 2013; Ainsworth et al., 2015; Peixoto et al., 2017; Silveira et al., 2017; Bernasconi et al., 2018); Table 1). For example, some coral-associated bacteria can rapidly take up organosulfur compounds released by Symbiodiniaceae cells, such as dimethylsulfoniopropionate (DMSP), to sustain their growth and produce an antimicrobial compound active against common coral pathogens (Raina et al., 2016; Raina et al., 2017). The stability of coral-associated bacterial communities during thermal stress is correlated to the Symbiodiniaceae spp. harboured by the coral host, which potentially affect the susceptibility of the holobiont to bleaching, disease, and colonisation by opportunistic potential pathogens (Littman et al., 2010). Global network analysis of coral holobiont composition has demonstrated that Symbiodiniaceae types co-occur with specific bacterial taxa with phylogenetic affiliation with groups involved in key nutritional pathways (i.e. nitrogen fixation, phosphate production and solubilisation, degradation of DMSP) and/or production of antimicrobial/anti-pathogenic compounds, further supporting the existence of a mutualistic relationship between these microorganisms in the coral holobiont (Ceh et al., 2013; Bernasconi et al., 2018). Furthermore, diazotrophic bacteria provide fixed nitrogen to endosymbiotic Symbiodiniaceae, whereby increased nitrogen availability stimulates high cell division rates by intercellular Symbiodiniaceae, resulting in inorganic carbon sequestration (Lesser et al., 2007; Pernice et al., 2012; Rädecker et al., 2015). Increased transfer of diazotrophically-derived nitrogen to endosymbiotic Symbiodiniaceae and endolithic algae particularly occurs during low availability of external nutrients or during bleaching (Bednarz et al., 2017; Bednarz et al., 2019). Collectively, these insights provide growing evidence that the ecological and metabolic interactions between Symbiodiniaceae and associated bacterial assemblages may be an important feature in maintaining the stability and function of the coral holobiont.

Bacteria-Symbiodiniaceae interactions could be the hidden key for coral reef resilience

Climate change is causing a pandemic of catastrophic “coral bleaching”, whereby endosymbiotic Symbiodiniaceae are expelled by their hosts during stressful conditions (Weis, 2008). The capacity of reef-building corals to adapt to accelerating global climate change and localised environmental stressors is critical to future coral reef survival (Hoegh-Guldberg, 1999; Torda et al., 2017). While the cnidarian host can exhibit stress tolerance through physiological plasticity, the functional capacity of the symbionts also influences holobiont survival. Photo-oxidative mechanisms of bleaching occurs via the physiological collapse of ‘heat sensitive’ Symbiodiniaceae due to severe photosystem damage and the production of harmful reactive oxygen species (ROS), whereby ‘heat tolerant’ Symbiodiniaceae possess a superior antioxidants system (Warner and Suggett, 2016). In a recent study, the most abundant core member of the Symbiodiniaceae-associated bacterial communities was a member of the *Labrenzia*, a genus of α-proteobacteria also associated with corals and other microalgae cultures (Lawson et al., 2018). A notable function of *Labrenzia* is its ability to produce ROS scavenging-DMSP, and therefore the consistent presence of this bacterium in Symbiodiniaceae cultures potentially supports the DMSP-based antioxidant-system of Symbiodiniaceae. Moreover, the relative abundance of *Labrenzia* differs across Symbiodiniaceae cultures at the genus level (Lawson et al., 2018), and corals harbouring thermally sensitive Symbiodiniaceae (ITS2 type C21a) experienced a reduced abundance of *Labrenzia* in the bacterial community following thermal stress, while corals containing thermally tolerant *Durusdinium trenchii* (ITS2 type D1a) showed an increased in the relative abundance of *Labrenzia* (Grottoli et al., 2018). It is therefore plausible that *Labrenzia* sp. contribute to the observed diversity in antioxidant capacity across Symbiodiniaceae. Furthermore, bacterial communities are able to regulate the coral holobionts’ external levels of the reactive oxygen species superoxide which may contribute to pathogen resistance (Diaz et al., 2016); symbiotic bacteria associated with Symbiodiniaceae could therefore provide a similar mechanism of protection.

These results raise a provocative hypothesis that bacteria might in fact be partially responsible for some traits previously solely ascribed to Symbiodiniaceae, and more importantly, that some of these traits may play a role in the stability of coral-symbiont associations. Again, such a notion emphasizes the critical need to consider bacterial dynamics and resource exchange when examining Symbiodiniaceae functional diversity.

Concluding Remarks

Unravelling the significance of the associated bacteria on Symbiodiniaceae health and functional diversity provides a conceptual shift in the factors determining the resilience of coral reef organisms to survive in a changing ocean, with wider implications for understanding algal–microbial interactions in extreme environments. We argue that systematic study of bacterial-Symbiodiniaceae interactions is crucial, not least because a detailed understanding of the associated bacterial functions is imperative for conservation and restoration approaches leveraging the microbiome to alter host phenotype (van Oppen et al., 2015; Peixoto et al., 2017; Morgans et al., 2019). Addressing these critical gaps in our knowledge is only now possible as a result of technological advances allowing for the manipulation of Symbiodiniaceae-bacterial assemblages, and for examination of microorganism metabolic interactions. For example, localisation and identification of the bacteria associated with Symbiodiniaceae in the free-living and endosymbiotic state could be achieved using a combination of fluorescent *in situ* hybridisation (FISH), laser microdissection, pure culturing, and 16S rRNA gene sequencing. Imaging techniques, such as transmission electron microscopy, have been applied to investigate viral-mediated coral bleaching and disease (Wilson et al., 2001; Davy et al., 2006) and have the potential to be applied to observe Symbiodiniaceae-bacteria associations. Molecular exchange can be measured and visualised at the cellular level using stable isotopic labelling combined with nanoscale secondary-ion mass spectrometry (NanoSIMS) (Raina et al., 2017). Identification and quantification of key metabolites and metabolic pathway activity could be achieved with the integration of metabolomic and transcriptomics analyses, as was recently applied to elucidate mechanisms underlying symbiont compatibility in the Aiptasia-Symbiodiniaceae symbiosis (Matthews et al., 2017). Phylogenetic and gene expression analyses of coral-associated fungi revealed a diverse and metabolically active community associated with a coral holobiont, and these analytical approaches could be applied to Symbiodiniaceae-bacteria associations (Amend et al., 2012). Metagenomics and interaction networks could be used to help predict Symbiodiniaceae-bacteria interactions, as was successfully applied to infer phage-bacteriophage interactions in bleached and diseased corals (Soffer et al., 2015). Metabolic pathway analysis can be used to study the flexibility in metabolic networks and has been applied to reveal nutritional provisioning requirements in the *Planococcus citri* (mealybug)-bacteria symbiosis (Hanson et al., 2014); thus, there is enormous potential for employing metabolic pathway analysis of the coral holobiont to predict a system-wide integrated response to changes in the environment (Suggett and Smith, 2019). However, several important challenges remain, including identifying the genomes of the bacteria in cultured Symbiodiniaceae, and the generation of robust axenic Symbiodiniaceae cultures that can be used for co-growth experiments in order to characterise the functional interactions of specific bacterial strains. Another challenge, when investigating interactions under laboratory conditions is that the composition of bacteria communities of cultured Symbiodiniaceae strains may not reflect wild populations, because of experimental procedures or acclimatisation to laboratory conditions. Overcoming these challenges will potentially usher a critical new era that will enable resolving the metabolic interactions at the heart of coral reef symbioses. Research directed to exploring Symbiodiniaceae-bacterial interactions will provide desperately needed new grounds with which to understand how multi-partner resource exchange amongst the coral holobiont regulates fitness and resilience of reef ecosystems to rapidly changing environmental conditions.

Competing interests

We declare we have no competing interests.

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Symbiodiniaceae-bacteria interactions: rethinking metabolite exchange in reef-building corals as multi-partner metabolic networks.

Running Title: Corals as multi-partner metabolic networks.

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Summary

The intimate relationship between scleractinian corals and their associated microorganisms is fundamental to healthy coral reef ecosystems. Coral-associated microbes (Symbiodiniaceae and other protists, bacteria, archaea, fungi, and viruses) support coral health and resilience through metabolite transfer, inter-partner signalling, and genetic exchange. However, much of our understanding of the coral holobiont relationship has come from studies that have investigated either coral-Symbiodiniaceae or coral-bacteria interactions in isolation, while relatively little research has focused on other ecological and metabolic interactions potentially occurring within the coral multi-partner symbiotic network. Recent evidences of intimate coupling between phytoplankton and bacteria have demonstrated that obligate resource exchange between partners fundamentally drives their ecological success. Here, we posit that similar associations with bacterial consortia regulate Symbiodiniaceae productivity and are in turn central to the health of corals. Indeed, we propose that this bacteria-Symbiodiniaceae-coral relationship underpins the coral holobiont's nutrition, stress tolerance, and potentially influences the future survival of coral reef ecosystems under changing environmental conditions. Resolving Symbiodiniaceae-bacteria associations is therefore a logical next step towards understanding the complex multi-partner interactions occurring in the coral holobiont.

Keywords: Symbiodiniaceae; microbiome; bacteria; coral; metabolism; symbiotic interactions

Originality-Significance Statement

Complex interspecific interactions between coral hosts and the multitude of symbiotic microorganisms they harbour underpins the health and survival of coral reefs. However, research to date has focused on coral host-unicellular algae (Symbiodiniaceae) or coral host–prokaryote interactions, while other relationships within the coral holobiont have been relatively overlooked. We propose to view the coral holobiont as a multi-partner metabolic network, providing a paradigm shift from a bipartite symbiosis to a community-based integration of factors that determine the resilience of coral reef organisms. We review emerging evidence of the importance of the ecological relationships occurring between marine phytoplankton and bacteria, and review Symbiodiniaceae-bacteria interactions that could underpin Symbiodiniaceae ecological success and contribute to coral holobiont fitness. Understanding these interactions is of critical importance for predicting the resilience and adaptability of coral reefs to environmental change.

Introduction

Reef-building corals are holobionts, comprising the cnidarian host and its associated microbes: dinoflagellates [Symbiodiniaceae, (LaJeunesse et al., 2018)] and other protists, bacteria, archaea, fungi, and viruses (Rohwer et al., 2002; Kwong et al., 2019; Pernice et al., 2019). The cnidarian host relies on the resources from these numerous symbionts to support its energy budget, growth rate, fecundity and metabolism (Peixoto et al., 2017; Torda et al., 2017). Indeed, coral-microorganism interactions have influenced the genomic evolution of both the host and microorganism partners, shaped coral development, and defined the ecological success of the coral holobiont (Thompson et al., 2015). Yet, of the multiple potential interactions within the coral holobiont, research has generally focused on the coral-dinoflagellate and coral-bacteria interactions e.g. (Webster et al., 2016; Hillyer et al., 2018), and relatively few studies have investigated other potential inter-partner interactions (Fig. 1; but see Table 1).

Numerous studies have demonstrated that both endosymbiotic Symbiodiniaceae and associated bacteria support the persistence of corals through the exchange of metabolites and bioactive compounds (Rohwer et al., 2002; Cantin et al., 2009; Ainsworth et al., 2010; Bourne et al., 2013; Glasl et al., 2016; Peixoto et al., 2017; Hillyer et al., 2018; Matthews et al., 2018). Yet, remarkably, the role of bacteria in regulating Symbiodiniaceae resource acquisition, competitive performance, and functional diversity (as both free-living and endosymbionts) is relatively unexplored (Ritchie, 2012; Bourne et al., 2013; Ainsworth et al., 2015; Peixoto et al., 2017; Silveira et al., 2017; Bernasconi et al., 2018); Table 1). This fundamental gap in knowledge wholly constrains our understanding of how microbes act in concert to regulate the health of coral holobionts, especially given the importance of bacterial-algal interactions for nutrient cycling, signal transduction and gene transfer as demonstrated for other microalgal taxa (Seymour et al., 2017). Emerging evidence suggests that interactions with bacterial associates may be important for Symbiodiniaceae nutrition and survival in their free-living state (Jeong et al., 2012; Frommlet et al., 2015; Lawson et al., 2018). Furthermore, global co-occurrence of specific bacterial taxa and Symbiodiniaceae in corals (Bernasconi et al., 2018) and co-localisation of distinct bacterial taxa with Symbiodiniaceae in cnidarian host tissues (Ainsworth et al., 2015) suggest specific Symbiodiniaceae-bacterial interactions may be crucial to support holobiont metabolic functioning. Exploring Symbiodiniaceae-bacteria associations is therefore a logical next step towards fully understanding the complex symbiotic interactions occurring in the coral holobiont, and assists development of conservation strategies for reefs under global climate change (van Oppen and Blackall, 2019).

Unlocking the significance of these interactions in Symbiodiniaceae ecological success could help answer fundamental questions surrounding coral reef function and resilience, including: 1) do obligate associations with bacterial symbionts enable Symbiodiniaceae to thrive as both free-living cells and endosymbionts?; 2) do interactions with bacteria promote the immense functional diversity observed for Symbiodiniaceae?; and 3) what role, if any, could Symbiodiniaceae-bacterial interactions play in the ecological success of the coral holobiont? Here, we consider that Symbiodiniaceae may hold an innate dependency on specific bacteria for resource exchange, thus forming an intimate association that may support optimum metabolic fitness of the coral holobiont. We present the current data supporting these relationships in the coral symbiotic network and explore how evidence from other algal-bacterial systems suggest Symbiodiniaceae-bacteria interactions may be a hidden key in determining the overall health and resilience of the coral holobiont.

Are bacteria obligate symbionts and “resource surrogates” for free-living Symbiodiniaceae?

Metabolic exchange between autotrophic organisms and bacteria underpin the ecological success of many plant and algal species across marine and terrestrial systems (Ramanan et al., 2016). Bacterial and archaeal associates protect many eukaryotic hosts against pathogens, supply and recycle essential nutrients (Ramanan et al., 2016), and even contribute to the acclimatisation and adaptation of their host to environmental change (Schönknecht et al., 2013). Phytoplankton-bacteria interactions drive the major oceanic biogeochemical cycles, contribute to energy transfers to higher trophic levels, and to the emission of climate-regulating gasses (Seymour et al., 2017). More specifically, bacteria can enhance the growth of dinoflagellates through specific nutrient exchange (Croft et al., 2005), while dinoflagellate-bacteria interactions may play a role in algal toxin production, e.g. neurotoxin production by *Alexandrium tamarense* (Zhang et al., 2015). Furthermore, specific bacterial taxa are consistently present in dinoflagellate cultures, including Symbiodiniaceae (Frommlet et al., 2015; Lawson et al., 2018), which strongly suggests that associated bacteria carry out important functions for these microalgae (Zhang et al., 2015).

The functional diversity of Symbiodiniaceae in terms of stress tolerance provides corals with the capacity to respond to environmental change, and thus acquiring new Symbiodiniaceae species from the environment or undergoing changes in the relative abundance of species already present in the coral tissues can enable corals to acclimatise to increasing environmental threats (Baker, 2003; Berkelmans and Van Oppen, 2006; Loram et al., 2007; Stat and Gates, 2011; Boulotte et al., 2016; Hume et al., 2016). Recent evidence has demonstrated the uptake of exogenous, thermally tolerant Symbiodiniaceae species by corals in response to repeated temperature stress (Boulotte et al., 2016). Furthermore, molecular studies have now confirmed that most Symbiodiniaceae genetic variants forming symbioses spend part of their life-cycle as free living cells before colonising new coral recruits (Cunning et al., 2015). Yet despite over 50 years of research examining Symbiodiniaceae biology and ecology, relatively little is known about the free-living life stage of Symbiodiniaceae (Stat et al., 2006; Takabayashi et al., 2012; Cunning et al., 2015; Suggett et al., 2017; LaJeunesse et al., 2018). For instance, it is still unclear how Symbiodiniaceae thrive outside of the coral tissues in oligotrophic waters and sediments without the concomitant provision of essential nutrients.

Cultured Symbiodiniaceae predate on bacteria under both nutrient-replete and -depleted conditions, demonstrating that heterotrophic feeding may be a survival strategy for Symbiodiniaceae cells when free-living (Jeong et al., 2012). However, dependency upon bacteria for resource acquisition by free-living Symbiodiniaceae likely extends far beyond predation, and may also involve exchange of key metabolites (Seymour et al., 2017). For example, *Sulfitobacter* species promote diatom growth through the secretion of indole-3-acetic acid synthesised from diatom-derived tryptophan, demonstrating how mutual exchanges of metabolites can influence the success of bacterial and phytoplankton partners (Amin et al., 2015). Evidence for algal acquisition of vitamins (e.g. vitamin B12; (Croft et al., 2005)), and enhanced micronutrient bioavailability (e.g. Fe (Amin et al., 2015), Zn (Ferrier-Pagès et al., 2005)) through symbiotic relationships with specific bacteria further highlights the important nutritional links between these groups of aquatic microorganisms.

Most dinoflagellates are difficult to grow axenically, which suggests that associated bacteria provide necessary metabolic resources for successful dinoflagellate growth outside of the host, and that these bacterial partners might operate as “resource surrogates” (Ritchie, 2012; Suggett et al., 2017). Indeed, changes in bacterial community composition modify the growth of the dinoflagellate *Gymnodinium catenatum* by approximately four-fold, which is equivalent to growth enhancements triggered by a 5°C difference in temperature or a six-fold difference in light intensity under laboratory conditions (Bolch et al., 2017). A recent study confirmed that bacteria are abundant and diverse within most Symbiodiniaceae laboratory cultures, and that some bacterial taxa are conserved across Symbiodiniaceae genera (Lawson et al., 2018). Notably, members of the Rhodobacterales, a family of α-proteobacteria commonly associated with many phytoplankton taxa, have been found in Symbiodiniaceae cultures all over the world, suggesting these bacteria are conserved mutualists of Symbiodiniaceae (Ritchie, 2012; Lawson et al., 2018). In culture, Symbiodiniaceae spp. and associated bacteria form calcifying structures known as symbiolites (i.e. aragonitic microbialites in which Symbiodiniaceae are encased) (Frommlet et al., 2015). Evidence suggests symbiolite formation is a photosynthesis-induced bacterial-Symbiodiniaceae calcification, which likely occurs in reef sediments as well (Frommlet et al., 2018). This is the first evidence of metabolic coupling between Symbiodiniaceae and associated bacteria, and identify an entirely new endolithic niche for free-living (benthic) Symbiodiniaceae. The formation of symbiolites is likely ecologically important, as it can protect Symbiodiniaceae from grazers and UV radiation, while still permitting photosynthesis (Friedmann, 1982; Shashar et al., 1997; Jeong et al., 2012; Frommlet et al., 2018). These novel insights into bacterial-Symbiodiniaceae associations reinforce how this coupling may be essential to the fitness and life-cycle viability of the dinoflagellates, and may ultimately influence their availability to new coral recruits. However there is as yet very little understanding of the metabolic exchange between Symbiodiniaceae and their bacterial partners in the water column, sediments and in endosymbiosis (Fig. 3).

Genome reduction and the exchange of metabolic intermediates between hosts and symbionts are common phenomena of obligate symbioses, and the absence of important metabolic pathways within an organism can point towards dependencies of symbiotic partners. For example, the abundant coral genus *Acropora* lacks the enzyme responsible for cysteine biosynthesis, implying this coral is dependent on its microbial symbionts for provision of this amino acid (Shinzato et al., 2011). While still large compared to other microalgae, as a result of idiosyncratic features such as multiple gene copies, Symbiodiniaceae genomes are small relative to other dinoflagellates (Fig. S1), suggesting this family could have also lost necessary components, such as key enzymes, due to obligate interactions with bacterial symbionts, coral hosts, or other symbionts. Symbiodiniaceae-associated bacteria may provide metabolic capabilities that are missing in the Symbiodiniaceae genome. Examples of potential obligate bacterial contributions observed in other microalgae-bacteria interactions include the ability to degrade complex polysaccharides, to reduce competition (Ramanan et al., 2016) or to acquire bioavailable iron (Hopkinson and Morel, 2009)). Iron is essential for a range of physiological processes in microalgae, including photosynthesis, respiration and nitrogen fixation (Behrenfeld et al., 2009). Although, Symbiodiniaceae in a free-living state can acquire bioavailable trace metals from ambient seawater using specific transporters (Morel and Price, 2003), the concentrations of bioavailable iron in much of the global ocean are too low to support microalgal growth (Hopkinson and Morel, 2009). Recent culturing work examining trace metal availability demonstrated that the free-living *Fugacium kawagutii* (previously *Symbiodinium kawagutii*) requires high concentrations of bioavailable iron to achieve optimum growth (Rodriguez and Ho, 2018). Marine bacteria produce siderophores, which bind and concentrate iron into bioavailable forms, enabling the uptake of this limiting micronutrient by phytoplankton; for example, the production of siderophores by the γ-proteobacterium *Marinobacter* promotes the growth of its dinoflagellate partner, *Scrippsiella trochoidea* (Amin et al. 2009). Intriguingly, *Marinobacter* was also part of the “core” bacterial-associates shared between diverse Symbiodiniaceae cultures (Lawson et al., 2018). It is therefore likely that Symbiodiniaceae depend on bacterial associates to fulfil their needs for bioavailable iron (Fig. 2).

Another important micronutrient is Vitamin B_{12} (cobalamin), a cofactor involved in the production of the amino acid methionine, which is needed to synthesize every protein as well as in diverse metabolic pathways including the generation of antioxidants glutathione and DMSP (Croft et al., 2005). Cobalamin is synthesised by many heterotrophic bacteria and nearly all oxygenic photosynthetic cyanobacteria but as with many algal protists, Symbiodiniaceae require exogenous cobalamin for growth as they do not possess the genetic machinery to generate the active form of this vitamin (Supplemental Information S1). Genomic evidence suggests Symbiodiniaceae possess cobalamin-dependent methionine synthase and not cobalamin-independent methionine synthase (Supplemental Information S1), implying that free-living Symbiodiniaceae depend on bacterial symbionts to gain access to this important cofactor.

Do bacteria drive Symbiodiniaceae functional diversity?

Substantial genetic and functional diversity of Symbiodiniaceae provides capacity for the coral holobiont to respond to a broad range of environmental conditions (Sampayo et al., 2009; Pettay et al., 2015; Boulotte et al., 2016; Hume et al., 2016; Silverstein et al., 2017; Suggett et al., 2017; LaJeunesse et al., 2018), which is of immense ecological importance to the survival and growth of coral reefs. There is high genetic divergence between Symbiodiniaceae species (even those in the same genus) (Liu et al., 2018; González-Pech et al., 2019); however, many of the drivers of the large functional diversity of Symbiodiniaceae remain largely unresolved. Gene duplication and horizontal gene transfer are two drivers of genome evolution, which promote the emergence of novel traits. Long-term symbiosis can lead to the sharing of genetic traits between algae and their associated microbiome, which has been proposed to have had a major impact on the gene content of algae (Keeling, 2009; Schönknecht et al., 2014; Husnik and McCutcheon, 2018). For example, emerging genomic evidence has shown that horizontal gene transfer from bacteria and archaea facilitates the survival of the extremophilic, unicellular red alga, *Galdieria sulphuraria,* which lives in hot, metal-rich, toxic and acidic environments (Schönknecht et al., 2013). A growing body of evidence indicates that horizontal gene transfer is an important source of gene innovation in dinoflagellates (Wisecaver et al., 2013). Gene transfer from associated bacteria may therefore provide a similar adaptive mechanism for Symbiodiniaceae and may explain the large functional diversity evident across the Symbiodiniaceae family. For example, members of the Rhodobacterales, which have been found in close association with corals (Apprill, 2017) and are an important component of the Symbiodiniaceae bacterial-associates (Lawson et al., 2018), have a large propensity for transferring genes to other microbes in the marine environment (McDaniel et al., 2010; Webster and Reusch, 2017). It is therefore plausible that Symbiodiniaceae could acquire foreign genes from associated bacteria via bacteriophages or similar genetic elements such as gene transfer agents (Solioz and Marrs, 1977; Shumaker et al., 2019). Furthermore, microbes in coral reef environments show a much higher rate of novel-trait acquisition by horizontal gene transfer than by spontaneous mutation, suggesting an alternate mode of adaptation via swapping of potentially beneficial genes between marine microbes (McDaniel et al., 2010), and possibly Symbiodiniaceae.

Horizontal symbiont acquisition, where offspring acquire symbionts from the environment, could provide an opportunity for free-living Symbiodiniaceae and/or bacterial communities to establish novel, potentially advantageous, associations with a cnidarian host. This mode of Symbiodiniaceae transmission is observed in the majority of coral species and could allow offspring to select a symbiont community that confers advantages locally. The role of bacteria in facilitating functional diversity in Symbiodiniaceae is entirely unexplored, but raises intriguing questions about whether the variability of Symbiodiniaceae fitness (and stress resistance) is determined purely by genetic differences between Symbiodiniaceae species (Sampayo et al., 2009; Pettay et al., 2015; Boulotte et al., 2016; Hume et al., 2016; Silverstein et al., 2017; Suggett et al., 2017; LaJeunesse et al., 2018; Liu et al., 2018; González-Pech et al., 2019) or whether other mechanisms play a role.

Dynamic resource exchange supports coral holobiont health and survival

Obligate associations between the cnidarian host and Symbiodiniaceae are essential for coral reef growth and survival. The host coral provides the endosymbionts with substrates for photosynthesis, which in turn fuels the autotrophic production of metabolites that the host cannot synthesise independently (e.g. palmitoleic acid in *Exaiptasia pallida*, (Matthews et al., 2018)). This resource exchange is one of the defining features allowing reef-building corals to flourish in otherwise nutrient-poor environments. Bacteria are also key ecological partners of cnidarians and are increasingly recognised as crucially important for the health of the holobiont (Bourne et al., 2016; Raina et al., 2016; Hernandez-Agreda et al., 2017; Peixoto et al., 2017; Brener-Raffalli et al., 2018) (Fig. 1). Gammaproteobacteria in the genus *Endozoicomonas* are a ubiquitous endosymbiont for corals (Bayer et al., 2013), and comparative genomic analysis of different strains of *Endozoicomonas* from different hosts suggests a common role in carbohydrate cycling and potential specificities in amino acid synthesis (Neave et al., 2017). Recent advances have allowed a better understanding of the functional significance of coral-associated bacterial communities, their roles in resource provision and nutrient cycling (Rädecker et al., 2015; Bourne et al., 2016), their responses to shifts in environmental conditions (Brener-Raffalli et al., 2018), and their potential “probiotic” role in mediating holobiont health and survival (Damjanovic et al., 2017; Peixoto et al., 2017; Webster and Reusch, 2017; Rosado et al., 2019; van Oppen and Blackall, 2019).

Only a few studies have specifically considered Symbiodiniaceae-bacterial interactions in the coral holobiont, with their results pointing to the potentially critical role that these partnerships might play in regulating holobiont nutrient cycling and competitive fitness (Ritchie, 2012; Bourne et al., 2013; Ainsworth et al., 2015; Peixoto et al., 2017; Silveira et al., 2017; Bernasconi et al., 2018); Table 1). For example, some coral-associated bacteria can rapidly take up organosulfur compounds released by Symbiodiniaceae cells, such as dimethylsulfoniopropionate (DMSP), to sustain their growth and produce an antimicrobial compound active against common coral pathogens (Raina et al., 2016; Raina et al., 2017). The stability of coral-associated bacterial communities during thermal stress is correlated to the Symbiodiniaceae spp. harboured by the coral host, which potentially affect the susceptibility of the holobiont to bleaching, disease, and colonisation by opportunistic potential pathogens (Littman et al., 2010). Global network analysis of coral holobiont composition has demonstrated that Symbiodiniaceae types co-occur with specific bacterial taxa with phylogenetic affiliation with groups involved in key nutritional pathways (i.e. nitrogen fixation, phosphate production and solubilisation, degradation of DMSP) and/or production of antimicrobial/anti-pathogenic compounds, further supporting the existence of a mutualistic relationship between these microorganisms in the coral holobiont (Ceh et al., 2013; Bernasconi et al., 2018). Furthermore, diazotrophic bacteria provide fixed nitrogen to endosymbiotic Symbiodiniaceae, whereby increased nitrogen availability stimulates high cell division rates by intercellular Symbiodiniaceae, resulting in inorganic carbon sequestration (Lesser et al., 2007; Pernice et al., 2012; Rädecker et al., 2015). Increased transfer of diazotrophically-derived nitrogen to endosymbiotic Symbiodiniaceae and endolithic algae particularly occurs during low availability of external nutrients or during bleaching (Bednarz et al., 2017; Bednarz et al., 2019). Collectively, these insights provide growing evidence that the ecological and metabolic interactions between Symbiodiniaceae and associated bacterial assemblages may be an important feature in maintaining the stability and function of the coral holobiont.

Bacteria-Symbiodiniaceae interactions could be the hidden key for coral reef resilience

Climate change is causing a pandemic of catastrophic “coral bleaching”, whereby endosymbiotic Symbiodiniaceae are expelled by their hosts during stressful conditions (Weis, 2008). The capacity of reef-building corals to adapt to accelerating global climate change and localised environmental stressors is critical to future coral reef survival (Hoegh-Guldberg, 1999; Torda et al., 2017). While the cnidarian host can exhibit stress tolerance through physiological plasticity, the functional capacity of the symbionts also influences holobiont survival. Photo-oxidative mechanisms of bleaching occurs via the physiological collapse of ‘heat sensitive’ Symbiodiniaceae due to severe photosystem damage and the production of harmful reactive oxygen species (ROS), whereby ‘heat tolerant’ Symbiodiniaceae possess a superior antioxidants system (Warner and Suggett, 2016). In a recent study, the most abundant core member of the Symbiodiniaceae-associated bacterial communities was a member of the *Labrenzia*, a genus of α-proteobacteria also associated with corals and other microalgae cultures (Lawson et al., 2018). A notable function of *Labrenzia* is its ability to produce ROS scavenging-DMSP, and therefore the consistent presence of this bacterium in Symbiodiniaceae cultures potentially supports the DMSP-based antioxidant-system of Symbiodiniaceae. Moreover, the relative abundance of *Labrenzia* differs across Symbiodiniaceae cultures at the genus level (Lawson et al., 2018), and corals harbouring thermally sensitive Symbiodiniaceae (ITS2 type C21a) experienced a reduced abundance of *Labrenzia* in the bacterial community following thermal stress, while corals containing thermally tolerant *Durusdinium trenchii* (ITS2 type D1a) showed an increased in the relative abundance of *Labrenzia* (Grottoli et al., 2018). It is therefore plausible that *Labrenzia* sp.

contribute to the observed diversity in antioxidant capacity across Symbiodiniaceae. Furthermore, bacterial communities are able to regulate the coral holobionts' external levels of the reactive oxygen species superoxide which may contribute to pathogen resistance (Diaz et al., 2016); symbiotic bacteria associated with Symbiodiniaceae could therefore provide a similar mechanism of protection.

These results raise a provocative hypothesis that bacteria might in fact be partially responsible for some traits previously solely ascribed to Symbiodiniaceae, and more importantly, that some of these traits may play a role in the stability of coral-symbiont associations. Again, such a notion emphasizes the critical need to consider bacterial dynamics and resource exchange when examining Symbiodiniaceae functional diversity.

Concluding Remarks

Unravelling the significance of the associated bacteria on Symbiodiniaceae health and functional diversity provides a conceptual shift in the factors determining the resilience of coral reef organisms to survive in a changing ocean, with wider implications for understanding algal–microbial interactions in extreme environments. We argue that systematic study of bacterial-Symbiodiniaceae interactions is crucial, not least because a detailed understanding of the associated bacterial functions is imperative for conservation and restoration approaches leveraging the microbiome to alter host phenotype (van Oppen et al., 2015; Peixoto et al., 2017; Morgans et al., 2019). Addressing these critical gaps in our knowledge is only now possible as a result of technological advances allowing for the manipulation of Symbiodiniaceae-bacterial assemblages, and for examination of microorganism metabolic interactions. For example, localisation and identification of the bacteria associated with Symbiodiniaceae in the free-living and endosymbiotic state could be achieved using a combination of fluorescent *in situ* hybridisation (FISH), laser microdissection, pure culturing, and 16S rRNA gene sequencing. Imaging techniques, such as transmission electron microscopy, have been applied to investigate viral-mediated coral bleaching and disease (Wilson et al., 2001; Davy et al., 2006) and have the potential to be applied to observe Symbiodiniaceae-bacteria associations. Molecular exchange can be measured and visualised at the cellular level using stable isotopic labelling combined with nanoscale secondary-ion mass spectrometry (NanoSIMS) (Raina et al., 2017). Identification and quantification of key metabolites and metabolic pathway activity could be achieved with the integration of metabolomic and transcriptomics analyses, as was recently applied to elucidate mechanisms underlying symbiont compatibility in the Aiptasia-Symbiodiniaceae symbiosis (Matthews et al., 2017). Phylogenetic and gene expression analyses of coral-associated fungi revealed a diverse and metabolically active community associated with a coral holobiont, and these analytical approaches could be applied to Symbiodiniaceae-bacteria associations (Amend et al., 2012). Metagenomics and interaction networks could be used to help predict Symbiodiniaceae-bacteria interactions, as was successfully applied to infer phage-bacteriophage interactions in bleached and diseased corals (Soffer et al., 2015). Metabolic pathway analysis can be used to study the flexibility in metabolic networks and has been applied to reveal nutritional provisioning requirements in the *Planococcus citri* (mealybug)-bacteria symbiosis (Hanson et al., 2014); thus, there is enormous potential for employing metabolic pathway analysis of the coral holobiont to predict a system-wide integrated response to changes in the environment (Suggett and Smith, 2019). However, several important challenges remain, including identifying the genomes of the bacteria in cultured Symbiodiniaceae, and the generation of robust axenic Symbiodiniaceae cultures that can be used for co-growth experiments in order to characterise the functional interactions of specific bacterial strains. Another challenge, when investigating interactions under laboratory conditions is that the composition of bacteria communities of cultured Symbiodiniaceae strains may not reflect wild populations, because of experimental procedures or acclimatisation to laboratory conditions. Overcoming these challenges will potentially usher a critical new era that will enable resolving the metabolic interactions at the heart of coral reef symbioses. Research directed to exploring Symbiodiniaceae-bacterial interactions will provide desperately needed new grounds with which to understand how multi-partner resource exchange amongst the coral holobiont regulates fitness and resilience of reef ecosystems to rapidly changing environmental conditions.

Competing interests

We declare we have no competing interests.

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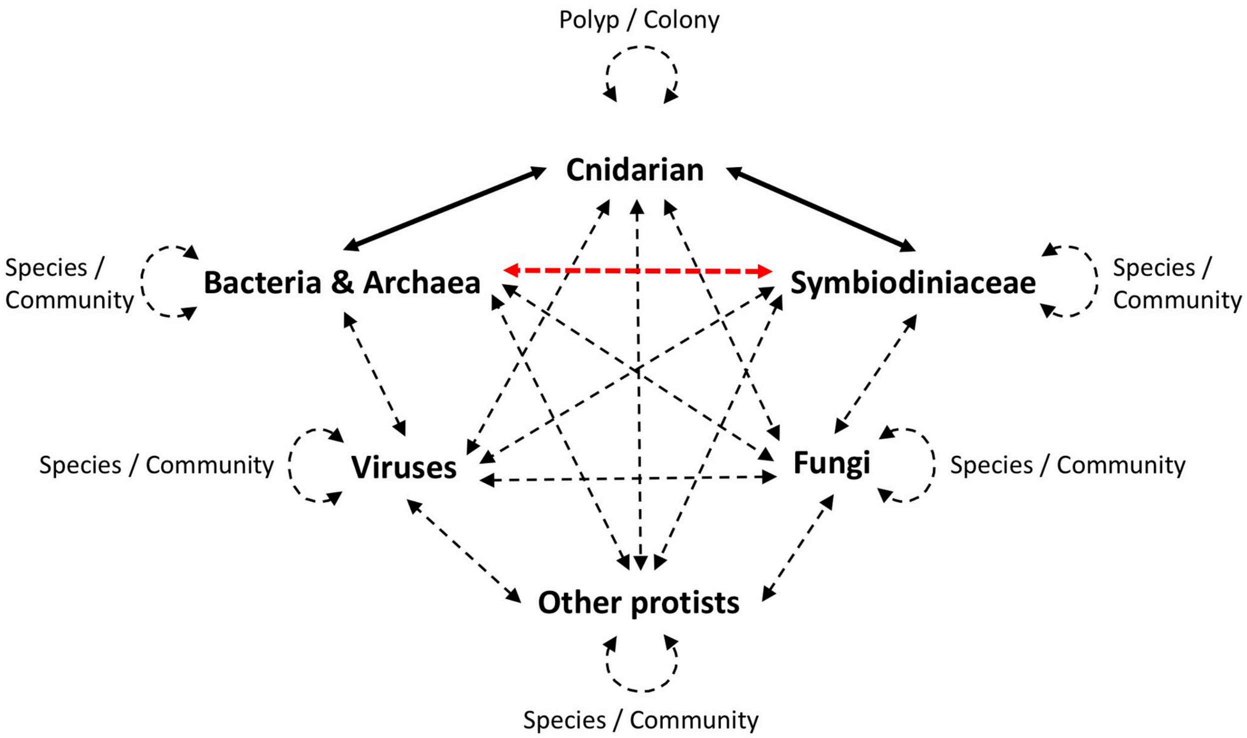

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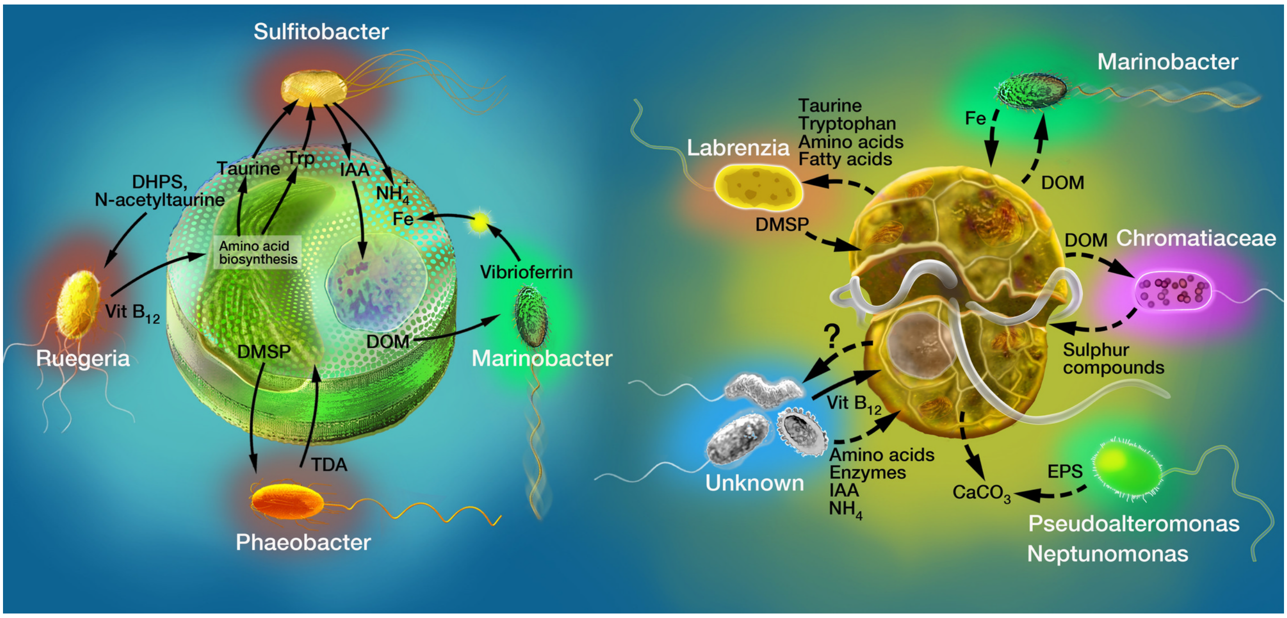

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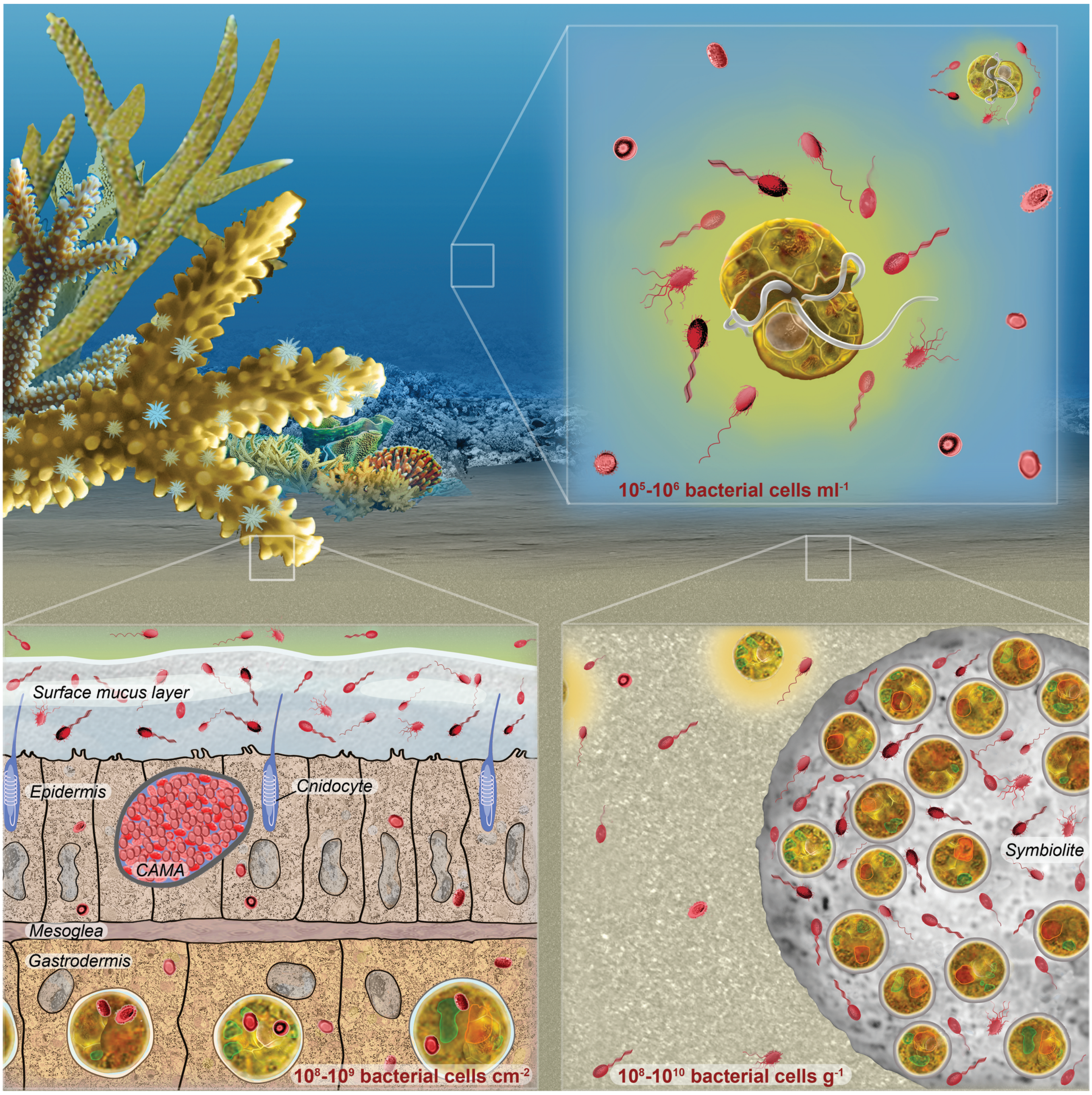

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Table 1. List of publications exploring interactions within the coral holobiont beyond coral-Symbiodiniaceae and coral-bacteria interactions.

Organisms	Type of study	Subject	Reference
Holobiont	• Review	• Interactions in the coral holobiont, including nutrient cycling and response to environmental stressors	(Bourne et al., 2016)
Holobiont	• Review	• Review of data on coral core microbiome and potential ecological significance	(Hernandez-Agreda et al., 2017)
Holobiont	• Review	• Nitrogen cycling in the coral holobiont	(Rädecker et al., 2015)
Holobiont	• Review	• Sulphur cycling in the coral holobiont	(Raina et al., 2010)
Holobiont	• Research article	• Metagenomic analysis of coral-associated microbiota under stable conditions	(Wegley et al., 2007)
Holobiont	• Research article	• Metagenomic analysis of coral-associated microbiota under different stressors	(Thurber et al., 2009)
Holobiont	• Research article	• Metagenomic analysis of the coral holobiont under heat stress	(Littman et al., 2011)
Holobiont	• Research article	• Metatranscriptome analysis of holobiont responses to coral disease	(Closek et al., 2014)
Holobiont	• Research article	• Metatranscriptome analysis of holobiont responses to coral disease	(Daniels et al., 2015)
Holobiont	• Research article	• Metatranscriptome analysis of holobiont responses to temperature and pathogen	(van de Water et al., 2018)
Holobiont	• Research article	• Integrated genomic analysis of Symbiodiniaceae, bacteria and archaea in holobiont	(Robbins et al., 2019)
Symbiodiniaceae-bacteria (within coral)	• Review	• Review of potential mechanisms for coral holobiont health and resilience to environmental changes	(Peixoto et al., 2017)
Symbiodiniaceae-bacteria (within coral)	• Short communication	• Symbiodiniaceae presence influenced the composition (but not species richness, evenness or phylogenetic diversity) of bacteria associated with the coral host	(Bourne et al., 2013)
Symbiodiniaceae-bacteria (within coral)	• Review	• Nutritional interactions between Symbiodiniaceae and bacteria in the coral holobiont, including carbon and nitrogen cycling, and DMSP interactions	(Silveira et al., 2017)
Symbiodiniaceae-bacteria (within coral)	• Research article	• Characterise baseline patterns of bacteria-Symbiodiniaceae co-occurrence across host genera and spatial distributions	(Bernasconi et al., 2018)
Symbiodiniaceae-bacteria (within coral)	• Research article	• Visualisation of bacteria in the same cellular space as Symbiodiniaceae in coral tissues	(Ainsworth et al., 2015)
Symbiodiniaceae-bacteria (Free-living and within coral)	• Review	• Explores potential bacteria-Symbiodiniaceae interactions e.g. antibiotic activity, quorum sensing and disease defence	(Ritchie, 2012)
Symbiodiniaceae-bacteria (Free-living)	• Research article	• Characterisation of bacteria in Symbiodiniaceae cultures to identify potential core bacterial members	(Lawson et al., 2018)

Symbiodiniaceae-bacteria (Free-living)	• Research article	• Free-living Symbiodiniaceae feed on heterotrophic bacteria in nitrogen-depleted water	(Jeong et al., 2012)
Symbiodiniaceae-bacteria (Free-living)	• Research article	• Evidence for symbiolite formation in Symbiodiniaceae cultures as a result of nutritional links	(Frommlet et al., 2015)
Symbiodiniaceae-bacteria (Free-living)	• Research article	• Evidence that symbiolite formation is bacterial-Symbiodiniaceae calcification and photosythesis driven, and likely occurs under natural conditions	(Frommlet et al., 2018)
Symbiodiniaceae-viruses (within coral)	• Review	• Viral lysis of Symbiodiniaceae cells as a mechanism of bleaching	(Buerger and van Oppen, 2018)
Symbiodiniaceae-viruses (within coral)	• Research article	• Evidence of viruses associated with Symbiodiniaceae within corals	(Correa et al., 2013)
Symbiodiniaceae-viruses (within coral)	• Research article	• Viral infection of corals under thermal stress	(Davy et al., 2006)
Symbiodiniaceae-viruses (within coral)	• Research article	• Symbiodiniaceae-derived viral infection contributes to temperature-induced bleaching	(Wilson et al., 2001)
Viruses	• Review	• Virus-host interactions and their roles in coral bleaching and disease, microbial community dynamics, and reef biogeochemical cycling	(Thurber et al., 2017)
Bacteriophage	• Research article	• Bacteriophage interactions with coral microbes in stressed and diseased tissues	(Soffer et al., 2015)
Fungi	• Research article	• Taxonomic diversity, stability and function of fungi in coral	(Amend et al., 2012)
Microeukaryote	• Review	• The ecological significance of microeukaryote interactions in coral reefs habitats	(Ainsworth et al., 2017)
Endolithic algae	• Review	• The role of endolithic communities in holobiont health and function	(Pernice et al., 2019)
Archaea	• Research article	• The global distribution and diversity of coral-associated archaea and role in holobiont nitrogen cycling	(Siboni et al., 2008)