The coral holobiont highlights the dependence of cnidarian animal hosts on their associated microbes

Claudia Pogoreutz, Christian R. Voolstra, Nils Rädecker, Virginia Weis, Anny Cardenas, and Jean-Baptiste Raina

Contents

7.1 Introduction: The coral holobiont as an ecosystem engineer and its reliance on associated microbes ................................................................. 92

7.2 The coral–Symbiodiniaceae relationship .............................................. 94

7.2.1 Symbiodiniaceae: Micro-algal engines of the coral holobiont machinery ......................................................................................... 94

7.2.2 Innate immunity, symbiosis sensing, and cell signaling ................... 95

7.2.3 Coral bleaching: The breakdown of the coral–Symbiodiniaceae relationship .................................................................................. 96

7.3 Symbiodiniaceae–bacteria relationships .............................................. 97

7.4 Diversity and function of microbes associated with the coral host ........ 97

7.4.1 The host as a habitat .................................................................. 97

7.4.2 Diversity of coral-associated bacteria and interspecies interactions ......................................................................................... 100

7.4.3 Acquisition of bacterial associates and their roles in early coral life-stages ..................................................................................... 101

7.4.4 Coral probiotics .......................................................................... 102

7.4.5 Contribution of bacteria to holobiont nutrient cycling ................... 102

7.4.6 Archaea associated with the coral holobiont ............................... 103

7.4.7 Protists and fungi associated with the coral holobiont ................. 104

7.5 Summary and Outlook ...................................................................... 105

References ................................................................................................. 106
7.1 Introduction: The coral holobiont as an ecosystem engineer and its reliance on associated microbes

The productivity and biodiversity of coral reefs are unmatched in the marine environment (Hatcher 1990). Surrounded by oligotrophic oceans, coral reefs are buzzing oases of life in a marine desert (Darwin 1842). Tropical coral reefs cover just 0.1% of the seafloor but provide habitat for \( \sim 32\% \) of all marine multicellular species (Fisher et al. 2015) and contribute to the livelihoods of more than 600 million people (Moberg and Folke 1999; Wilkinson 2008; Spalding et al. 2017). This entire ecosystem is supported by its foundation species: reef-building corals (Figure 7.1). These organisms sustain the immense productivity of coral reefs (Wild et al. 2004), contribute to the reef food web, and their calcareous skeletons form the structural basis of the reef framework.

As early as the 19th century, scientists identified the cohabitation of benthic cnidarians with intracellular photosynthetic dinoflagellates, initially termed “zooxanthellae” (Krueger 2017) (Figure 7.2). Building on more than a century of research, the coral–dinoflagellate symbiosis is one of the best characterized eukaryotic endosymbioses, and a powerful model to understanding the functioning of symbioses in general. Corals also harbor a diverse array of other microbes comprised of protists, fungi, bacteria, archaea, and viruses. This collective is called the coral holobiont (Rohwer et al. 2002). The coral metaorganism, by comparison, is a more restricted definition and typically only describes the coral host and associated microbes for which a function has been proposed or is known (Jaspers et al. 2019). This suite of organisms forms a complex network of symbiotic interactions that extend the metabolic repertoire, immunity, and

---

**FIGURE 7.1** Scleractinian corals are the foundation species of coral reef ecosystems. Complex symbiotic interactions facilitate nutrient uptake and recycling, thereby enabling corals to thrive in highly oligotrophic environments and to build the structural framework of coral reefs. Image credit: Anna Roik.
Coral holobionts depend on associated microbes

Microbes can therefore be considered fundamental to the ecological success of corals and the reefs they build (Bang et al. 2018). Reef ecosystems have existed for ∼240 million years, but face unprecedented and accelerating decline: the 2015–2017 global coral bleaching event affected 74% of reefs worldwide, and up to half of the coral cover was lost on the Great Barrier Reef alone, the largest reef system in the world (Hughes et al. 2018b). Future predictions for coral reefs are dramatic: even under a 1.5°C warming scenario, it is expected that coral reefs will decline by a further 70%–90%, with larger losses of up to 99% projected to be highly likely under a 2.0°C warming scenario (IPCC 2018). Such trajectories make the understanding of coral holobiont functioning and the contribution of its various microbes critical, not only to comprehend how symbiotic interactions have shaped the most biodiverse marine ecosystem on Earth, but also to help conserve and protect corals and the reefs they build for future generations. In this chapter, we discuss the state of knowledge of coral–microbe interactions and how they shape the ecology, resilience, and adaptation of the coral holobiont.

FIGURE 7.2 Overview and diversity of cnidarian–dinoflagellate symbioses. (a) Reef-building or stony coral (Anthozoa, Scleractinia: Acropora humilis); (b) Fire coral, a calcifying hydrocoral (Hydrozoa: Millepora platyphylla); (c) Blue Coral, a calcifying “soft” coral (Anthozoa, Octocorallia: Heliopora coerulea); (d) upside-down jellyfish (Scyphozoa: Cassiopea sp.). All of these marine cnidarian holobiont systems are intimately engaged in a mutualistic relationship with dinoflagellates of the family Symbiodiniaceae.
7.2 The coral–Symbiodiniaceae relationship

7.2.1 Symbiodiniaceae: Micro-algal engines of the coral holobiont machinery

The ecological success of the coral–Symbiodiniaceae symbiosis is based on efficient nutrient recycling between host and symbiont (Figure 7.3). The driving force of this bidirectional nutrient exchange ultimately lies in the complementary nutrient limitations of the two symbiotic partners (Shantz et al. 2016; Bang et al. 2018). While the heterotrophic coral host is limited by organic nutrient availability (e.g., glucose), the autotrophic intracellular dinoflagellates are limited by inorganic nutrients (e.g., carbon dioxide or ammonium) (Muscatine et al. 1989; Falkowski et al. 1993; Rädecker et al. 2015). These reciprocal metabolic exchanges are governed by the uptake of limiting nutrients and release of excess nutrients by each symbiotic partner (Muscatine 1990; Cuning et al. 2017). Symbiodiniaceae translocate high rates of excess photosynthetically-fixed carbon to the coral host. The host metabolism, in turn, produce waste compounds such as carbon dioxide through respiration available to the Symbiodiniaceae (Falkowski et al. 1993). This nutrient exchange is so efficient that the translocation of photosynthates can fully meet or even exceed the respiratory requirements of the coral host (Muscatine and Porter 1977; Muscatine 1990; Falkowski et al. 1993), and hence constitutes its primary energy source (Tremblay et al. 2012). As a consequence, symbiotic coral hosts may overcome their carbon limitation and shift instead towards a nitrogen-limited state (Cuning et al. 2017; Rädecker et al. 2018).

In a stable symbiosis, host and symbionts are nitrogen-limited and compete for available environmental ammonium (Pernice et al. 2012). Increasing evidence suggests that this resource competition is critical for maintaining the functioning of the coral–Symbiodiniaceae symbiosis (Cui et al. 2019). Coral hosts use the translocated carbon for ammonium assimilation required for amino acid synthesis (Cui et al. 2019). Consequently, carbon translocation reduces nitrogen availability for the dinoflagellates. The nutrient cycling in the intact symbiosis is thus stabilized by a positive feedback loop: because symbionts translocate carbon, they are nitrogen-limited; and because they are nitrogen-limited, a substantial fraction of photosynthetically fixed carbon cannot be channeled towards their biomass and growth, and is hence released to the host. While this “selfish” interaction between host and symbiont is central to the ecological success of the coral–Symbiodiniaceae relationship, it also renders the symbiosis highly susceptible to environmental disturbance.

Initially considered to be a single species, *Symbiodinium microadriaticum* (Freudenthal 1962), the recently established family Symbiodiniaceae currently encompasses seven distinct genera (*Symbiodinium*—formerly Clade A, *Breviolum* (B), *Cladocopium* (C), *Durusdinium* (D), *Effrenium* (E), *Fugacium* (F), and *Gerakladium* (G)) that have originated and diversified alongside reef-building corals approximately 160 mya ago (LaJeunesse et al. 2018). Coral reefs of the Indo-Pacific are almost exclusively dominated by *Cladocopium* and *Durusdinium* (LaJeunesse et al. 2003, 2004), with at least 50–100 possible “species” currently identified around Australia alone (LaJeunesse et al. 2003; Silverstein et al., 2011). The remarkable diversity of this family influences their host’s susceptibility to environmental fluctuations, such as thermal stress and salinity (Berkelmans and van Oppen 2006; Sampayo et al.
Coral holobionts depend on associated microbes (2008). In the world’s warmest reefs of the Persian/Arabian Gulf, corals predominantly harbor *Cladocopium thermophilum*, an association that is central to the thermotolerance of these coral communities (Hume et al. 2016). Similarly, the stress-tolerant *Durusdinium trenchii* has rapidly taken over Caribbean corals following repeated anthropogenic disturbances and increasing seawater temperatures (Pettay et al. 2015). However, thermal adaptation of reef communities represents a significant physiological tradeoff: corals harboring *D. trenchii* typically grow slower (Little et al. 2004) and incorporate only half the amount of photosynthates compared to those associated with *Cladocopium* (Cantin et al. 2009). Recently, multiple Symbiodiniaceae genomes have become available (Shoguchi et al. 2013; Lin et al. 2015; Aranda et al. 2016), and comparative analyses have revealed that these organisms possess an extensive transporter repertoire for carbon and nitrogen metabolites, which is unique among dinoflagellates and likely underpins their symbiotic lifestyle (Aranda et al. 2016).

### 7.2.2 Innate immunity, symbiosis sensing, and cell signaling

The regulation of the coral–Symbiodiniaceae partnership is complex and only partially understood. There is strong evidence that the host innate immune system plays a large role in mechanisms of recognition, maintenance, and dysbiosis of the association (Weis 2008). In the majority of coral species, the symbiosis is established anew with each host generation. The algae are acquired via phagocytosis by nutritive phagocytes that comprise the host gastrodermal tissue (endoderm) (Fadlallah 1983). However, instead of being digested, the algae specific to a particular host persist and proliferate within host vacuoles (termed symbiosomes) (Colley and Trench 1983; Schwarz et al. 1999). This process is mediated by the host innate immune system (Palmer 2018). The process of phagocytosis is a complex part of innate immunity that is highly conserved across the Metazoa (Underhill and Ozinsky 2002).

Microbes, including Symbiodiniaceae, are arrayed with a variety of microbe-associated molecular patterns (MAMPs), including glycans, that are recognized by host pattern recognition receptors (PRRs) on phagocyte cell surfaces (Weis 2008). These MAMP–PRR interactions launch a variety of signaling cascades that determine the fate of the phagocytized microbe. The model in corals is that tolerogenic pathways allow for the persistence and proliferation of algal symbionts inside symbiosomes, while resistant pathways are launched during dysbiosis and bleaching (see next section) that reject and remove the symbiont. There is overwhelming evidence for the presence of MAMP–PRR interactions and downstream innate immune signaling in corals. The majority of evidence comes from now extensive -omics studies that repeatedly point to elaboration, enhancement, and overexpression of innate immunity genes in corals and other symbiotic cnidarians (Rodriguez-Lanetty et al. 2004; Shinzato et al. 2011; Baumgarten et al. 2015; Mohamed et al. 2016; Voolstra et al. 2017; Cunning et al. 2018; Shumaker et al. 2019). There are also a variety of studies, often in sea anemone model systems, that provide evidence of innate immune pathway function including: host lectin–symbiont glycan interactions (Wood-Charlson et al. 2006; Bay et al. 2011; Parkinson et al. 2018), scavenger receptors (Neubauer et al. 2016), thrombospondin type 1 repeat proteins (Wolfovicz et al. 2016; Neubauer et al. 2017), complement system (Poole et al. 2016), the master immunity gatekeeper NF-κB (Mansfield et al.
Cellular Dialogues in the Holobiont


Also critical to symbiosis regulation is the maintenance of host and symbiont biomass ratios through cell cycle regulation. Host and symbiont biomass ratios reach a dynamic homeostasis after symbionts fully colonize a host (Davy et al. 2012). Symbiont populations in hospite grow much more slowly than those in culture and are arrested at the G1/S transition (Smith and Muscatine 1999). Mechanisms that coordinate the two cell cycles and an understanding of how this coregulation becomes decoupled during dysbiosis have yet to be revealed.

7.2.3 Coral bleaching: The breakdown of the coral–Symbiodiniaceae relationship

Stressful environmental conditions such as temperature anomalies, nutrient enrichment, or pollution can result in so-called coral bleaching, a (general) stress response characterized by whitening of the coral tissue caused primarily by the loss of Symbiodiniaceae endosymbionts from the tissue via expulsion, host cell apoptosis/detachment, digestion, or exocytosis of the symbiont cells (Gates et al. 1992; Douglas 2003; Dunn et al. 2007; Davy et al. 2012). Coral bleaching can also occur via the loss of photosynthetic pigment from the symbionts in hospite (Jones et al. 1998). Several triggers that induce the coral bleaching cascade have been proposed, including oxidative stress (Lesser 1997) and changes in nutrient stoichiometry (Wiedenmann et al. 2012; Morris et al. 2019). However, the underlying cellular mechanisms are still not fully understood.

Corals may recover from a bleaching event by repopulating their tissues with Symbiodiniaceae (Jones et al. 2008; Silverstein et al. 2015). However, as bleaching effectively results in the disruption of carbon fixation by Symbiodiniaceae and subsequent loss of photosynthate translocation to the coral host (Ezzat et al. 2015), coral host starvation may eventually result in mortality as a consequence of prolonged bleaching. The availability of heterotrophic food sources and the heterotrophic capacity of the host, thus, may determine the resilience of corals during heat stress (Grottoli et al. 2006). Mass coral bleaching events at the ecosystem scale have resulted in the loss of entire reefs, or reef systems (Hoegh-Guldberg 2011; Hughes et al. 2018a) and are projected to increase in the future due to climate change driving ocean warming (IPCC 2018).

The majority of contemporary observations of ecosystem-scale coral bleaching coincide with high-temperature anomalies, such as the El Niño Southern Oscillation (ENSO), or marine heatwaves, which can push corals beyond their thermal limits (Hoegh-Guldberg 1999; Hughes et al. 2003, 2017, 2018a). The first observations of coral bleaching were made in 1983 in the Eastern Pacific near the Panama-Costa Rica border (Glynn 1983). Due to the increasing severity and frequency of high-temperature anomalies attributed to global warming, coral bleaching events have become more common in the past decades. Since then, three mass bleaching events have occurred at the global scale, subsequently named the First, Second, and Third Global Bleaching Event, recorded in 1997/1998, 2009/2010, and 2015–2016, respectively (Hughes et al.
Coral holobionts depend on associated microbes. A recent analysis of bleaching records of 100 reefs from 1980–2016 has shown that the average turnaround time between bleaching events has halved in the past thirty years and is now only six years (Hughes et al. 2018a). This concerning trend means that the likelihood of recurring annual mass bleaching events in the coming decade is increasing, and the amount of time in between these bleaching events is no longer sufficient to allow full reef recovery (Hughes et al. 2018a).

### 7.3 Symbiodiniaceae–bacteria relationships

Interactions between bacteria and Symbiodiniaceae are challenging to study in hoste because of the complex nature of the coral holobiont. The physiology, functional diversity, and stress tolerance of Symbiodiniaceae have been studied in cultured strains for decades to disentangle their contribution to the health and functioning of the holobiont. Symbiodiniaceae cultures harbor very diverse bacterial communities with abundances exceeding those of the algal cells by almost two orders of magnitude (Ritchie 2012; Lawson et al. 2018). Recurring bacterial taxa such as *Marinobacter* (Gammaproteobacteria), *Labrenzia*, and other *Roseobacter* (Alphaproteobacteria), have been identified in association with a wide diversity of Symbiodiniaceae genera (Ritchie 2012; Lawson et al. 2018). Strikingly, the same bacterial taxa are also known to positively influence the growth of many phytoplankton species (Seymour et al. 2017). Future studies on Symbiodiniaceae–bacteria interactions should use axenic cultures to characterize the effect of specific bacteria on the growth and physiology of the dinoflagellates. Protocols to set up axenic microalgal cultures exist and have successfully been applied to multiple phytoplankton species (Shishlyannikov et al. 2011; Cho et al. 2013). Establishing axenic Symbiodiniaceae cultures is an important first step towards identifying the functional roles and reciprocal exchanges occurring between Symbiodiniaceae and their bacterial partners.

### 7.4 Diversity and function of microbes associated with the coral host

#### 7.4.1 The host as a habitat

Coral holobionts associate with a range of bacteria, archaea, and viruses, among other microorganisms (Rohwer et al. 2002; Wegley et al. 2007) (Figure 7.3). Coral holobionts can be separated into three distinct compartments: the surface mucus layer (SML), the coral tissue, and the underlying aragonite skeleton. The three compartments are governed by distinct physicochemical properties and environmental gradients (Ferrer and Szman 1988; Wangpraseurt et al. 2016; Pernice et al. 2019) as well as distinct associated microbial communities (Sweet et al. 2011; Pollock et al. 2018). Most coral-associated prokaryotes have not yet been cultivated and the functional contributions of specific taxa are largely unknown. Nonetheless, prokaryotic functions are crucial to understanding microbial contribution to coral health and adaptation. Molecular tools have allowed for the in-depth characterization of bacterial communities associated with corals and revealed specific assemblages of bacteria differing between the SML, tissue, and skeleton. SML-associated bacteria are largely dominated by
FIGURE 7.3  The coral holobiont consists of the animal host and a diversity of eukaryotic and prokaryotic microorganisms, and their potentially numerous metabolic interactions. Microbial community composition and structure within the holobiont readily respond to environmental fluctuations (e.g., of temperature, nutrients, or light availability), or stress. The eukaryotic Symbiodiniaceae are intracellular tissue-associated microalgal symbionts that engage in an intimate and efficient nutrient-exchange mutualism with their coral host. Through their high carbon acquisition, fixation, and translocation rates to the host, Symbiodiniaceae can be considered true unicellular “engines” of the coral holobiont, which are able to provide organic carbon at rates that can fully meet or even exceed the respiratory requirements of the coral animal host. Among the prokaryotic associates of the coral holobiont, bacteria are the best-studied members. Coral-associated bacterial communities are highly diverse, uneven, and exhibit distinct compositions in the coral surface mucus layer, the coral tissues, and the coral skeleton. In the coral skeleton, endolithic algae (including the abundant and diverse Ostreobium spp.) and fungi form dense bands. These endoliths are hypothesized to metabolically interact with the coral host tissue. The metabolic functions of prokaryotes, endolithic algae, and endolithic fungi are potentially highly diverse; whether and how they may contribute to holobiont functioning is an area of active exploration.
Gammaproteobacteria, commonly represented by members of the genus *Vibrio* (Koren and Rosenberg 2006) and by a broad range of taxa presumed to be commensals that can inhibit the growth of pathogens (Ritchie 2006). Shifts in SML-associated bacteria communities towards a dominance of Alphaproteobacteria, Verrucomicrobia, and Cyanobacteria have been reported in mucus sheets of *Porites* corals, in particular related to aging mucus (Glasl et al. 2016), variation in nutrient composition, and heat stress (Lee et al. 2015, 2016), although studies from bleached and healthy coral mucus from the Persian/Arabian Gulf showed no difference between bacterial communities (Hadaidi et al. 2017).

Bacterial assemblages associated with coral tissues have been widely studied. Tissue-associated bacterial communities are strongly driven by coral and Symbiodiniaceae genotypes (Rohwer et al. 2002). *Endozoicomonas*, Alteromonadaceae, and *Ralstonia* are examples of tissue-associated taxa ubiquitously found across many coral species from widely separated locations (Bayer et al. 2013; Ainsworth et al. 2015; Roder et al. 2015; Hernandez-Agreda et al. 2016; Neave et al. 2016a,b; Certner and Vollmer 2018). Endosymbiotic and epibiotic tissue-associated bacterial communities have been characterized by coupling laser microdissection and next-generation sequencing. The orders Rhizobiales, Caulobacterales, and Burkholderiales have been identified in association with Symbiodiniaceae, while members of the family Endozoicomonadaceae, and orders Rickettsiales and Rhodobacterales have been identified in association with coral host cells (Ainsworth et al. 2015; Neave et al. 2016b, 2017). To date, several genomes of coral-associated *Endozoicomonas* are available (Neave et al. 2014, 2017; Ding et al. 2016), as well as protocols for isolation and culture (Pogoreutz and Voolstra 2018).

The coral skeleton contains a rich microbial community, which is often dominated by a diversity of cyanobacteria (Yamazaki et al. 2008). Some of these cyanobacteria have been cultured and characterized, such as *Plectonema terebrans*, *Mastigocoleus testarum*, and *Halomicronema excentricum*. Recent studies revealed that while skeleton-associated cyanobacteria occur at low relative abundances, they can be highly diverse (Marcelino and Verbruggen 2016). Other bacterial functional groups, such as anoxygenic phototrophic bacteria and anaerobic green sulphur bacteria, have also been identified in coral skeletons by spectral signatures of bacteriochlorophylls and via next-generation sequencing (Magnusson et al. 2007; Ralph et al. 2007; Yang et al. 2016).

Besides such broad compartmentalization, localization of microbes within a host can provide information on putative functions. Fluorescence *in situ* hybridization (FISH) approaches have shown that in visibly healthy hosts, bacteria tend to form aggregates (Ainsworth et al. 2006; Bayer et al. 2013; Wada et al. 2016, 2019; Neave et al. 2017), which can contain one or more cell morphologies (Wada et al. 2019). In contrast, diseased and lesioned coral tissues appear “overgrown” and crowded by bacteria (Ainsworth et al. 2006; Wada et al. 2016). Two bacterial genera previously identified and imaged in coral tissues are particularly worthwhile mentioning: (1) the abundant *Endozoicomonas*, which aggregate in the gastroderm of *Stylophora pistillata* (Bayer et al. 2013; Neave et al. 2017) and *Pocillopora verrucosa* (Neave et al. 2017); and (2) the putative parasite *Candidatus Aquarickettsia rohweri*, which is present in the mucocytes of the coral ectoderm (Klinges et al. 2019). This genus
lacks genes for nitrogen metabolism and the synthesis of most sugars but maintains the genetic machinery for sensing and responding to extracellular nitrogen, as well as a complete type IV secretion system, collectively suggesting that Ca. A. rohweri may be a true coral parasite (Klinges et al. 2019).

7.4.2 Diversity of coral-associated bacteria and interspecies interactions

Studies on coral-associated microbes have mostly focused on bacterial diversity associated with coral tissues. Coral bacterial communities are typically diverse, uneven, and consist of hundreds to thousands of bacterial taxa (Rohwer et al. 2002; Bourne et al. 2008; Ziegler et al. 2016). Structure and composition of bacterial communities readily respond to the host’s environment, that is, reflect environmental gradients, fluctuations, and habitat suitability (Thurber et al. 2009; Morrow et al. 2015; Roder et al. 2015; Ziegler et al. 2017). In adult corals, bacterial community shifts due to environmental changes can occur within a few days (Thurber et al. 2009) and in some cases within hours (Ziegler et al. 2017), with older colonies exhibiting delayed microbiome shifts, possibly due to differences in microbial composition, bacteria–bacteria interactions, or host energetics (Sweet et al. 2017).

The tissue-associated Endozoicomonas commonly display substantial changes in relative abundance depending on coral or reef health (Bayer et al. 2013; Roder et al. 2015; Neave et al. 2016a). The relative abundance of this genus is usually high in healthy corals and on reefs with high coral cover, but low on degraded reefs and in stressed, bleached, or diseased corals (Bourne et al. 2008; Meyer et al. 2014; Morrow et al. 2015). It was hence suggested that Endozoicomonas may be important for coral holobiont health, but its potential roles have yet to be identified. Increases in abundance of opportunistic bacteria and putative pathogens, such as Vibrionaceae, Rhodobacteraceae, or Flavobacteriaceae, often occur in chronically or severely stressed corals (Cárdenas et al. 2012; Roder et al. 2014a,b; Ziegler et al. 2016; Gignoux-Wolfsohn et al. 2017; Certner and Vollmer 2018). Stress-associated bacterial community shifts in corals are also reflected in the functional gene repertoire of the microbiome, showing increases in abundance of genes involved in virulence, stress resistance, or sulfur and nitrogen metabolism (Thurber et al. 2009).

Interactions between bacteria exert essential selective forces that sculpt coral microbial assemblages. For instance, the production of antibacterial molecules is one of the most important mechanisms used by native commensal bacteria for shaping the diversity of coral-associated prokaryotes and controlling the presence of pathogens (Sweet et al. 2011). Antibacterial activity has been demonstrated in numerous bacterial taxa (Ritchie 2006; Nissimov et al. 2009; Rypien et al. 2010; Kvennefors et al. 2012; Pereira et al. 2017) and coral extracts (Kelman et al. 2006; Gochfeld and Aeby 2008), but only a few compounds have been identified to date (Raina et al. 2016). Other bacteria–bacteria interactions also occur through quorum sensing (QS), which allows cells to communicate and synchronize gene expression in a concerted density-dependent manner to coordinate population behaviors. A broad spectrum of coral-associated bacteria, including some known coral pathogens, rely
Coral holobionts depend on associated microbes

7.4.3 Acquisition of bacterial associates and their roles in early coral life-stages

Coral sexual reproduction occurs either through spawning where gametes are released into the water column, or through internally fertilizing gametes and brooding larvae inside the coral polyp (Fadlallah 1983). In spawning corals, the establishment of coral–bacteria symbioses is widely believed to happen horizontally (i.e., through acquisition of symbionts from the environment) during the coral pelagic larval phase, or even after settlement and metamorphosis (Apprill et al. 2009; Sharp et al. 2010). Conversely, in brooders, bacteria can be vertically transmitted (i.e., directly passed from the parent colony to the planula before its release). Bacterial communities exhibit dynamic changes between the different ontogenetic stages of coral development (Damjanovic et al. 2019; Epstein et al. 2019), likely reflecting a succession of microbial functions relevant for the holobiont (Bernasconi et al. 2019) and potentially following a winnowing process (Epstein et al. 2019).

A broad phylogenetic range of bacteria can be vertically or horizontally acquired. The most common associates of gametes, embryos, and larvae of brooders and spawners include the genera *Roseobacter*, *Marinobacter*, *Alteromonas*, *Vibrio*, *Bradyrhizobia*, and *Endozoicomonas*. *Roseobacter*- and *Alteromonas*-affiliated sequences are consistently found in very early developmental stages of several coral species (Apprill et al. 2009; Ceh et al. 2012, 2013; Sharp et al. 2012). These taxa are metabolically diverse and have been linked with the production of antibiotic compounds to counter coral pathogens (Piekarski et al. 2009). Furthermore, *Alteromonas* spp., *Vibrio* spp., and the diazotroph *Bradyrhizobia* might provide biologically available nitrogen to coral larvae (Ceh et al. 2013; Lema et al. 2014). The transmission of potentially beneficial bacteria from parent coral colonies to gametes or early ontogenetic stages might be a mechanism to ensure microbial inheritance across generations. In addition, bacterial behavior is likely to play a role in microbiome establishment. Indeed, many environmentally acquired bacterial symbionts use chemotaxis—the ability to direct their movement towards or away from specific chemicals—to locate their hosts (Raina et al. 2019). Chemotaxis may be a particularly prevalent mechanism employed on reefs because the coral surface is characterized by strong gradients of organic compounds that can act as cues for microorganisms (Ochsenkühn et al. 2018). Several coral-associated bacterial families, such as Endozoicomonadaceae, Rhodobacteraceae, or Oceanospirillaceae, exhibit chemotaxis towards constituents of coral mucus (Tout et al. 2015). Chemotaxis and motility, however, can also enable coral pathogens, such as *Vibrio shiloi* and *V. coralliilyticus*, to locate and infect their hosts (Banin et al. 2001;
Meron et al. 2009) using chemical cues such as dimethylsulfoniopropionate (DMSP) present in the coral mucus (Garren et al. 2014).

Bacteria are also directly involved in the transition between pelagic and benthic life stages in the coral life cycle. Indeed, after a pelagic phase (typically ranging from a week to more than 100 days), coral larvae must attach to a suitable reef structure to metamorphose into colony-forming juveniles (Connolly and Baird 2010). Habitat-specific environmental cues, mainly produced by specific bacteria associated with crustose coralline algae (CCA), contribute to coral larval recruitment and metamorphosis (Harrington et al. 2004; Webster et al. 2004; Tebben et al. 2015). Clear shifts in the bacterial community structure of CCA occur following thermal stress, resulting in significant reduction of coral larvae recruitment (Webster et al. 2011). Similarly, antibiotic treatment of larval cultures reduced settlement rates, suggesting that the presence of certain bacteria is essential for settlement induction (Vermeij et al. 2009).

7.4.4 Coral probiotics

Understanding the dynamic interplay of coral-associated microbes in relation to the prevailing environment is deeply interconnected with the setup, maintenance, and inheritance of microbial symbiotic relationships. The underlying premise is that microbial associates can adapt quickly to the surrounding environment and contribute functions that support coral holobiont health and resilience (Reshef et al. 2006; Ziegler et al. 2017, 2019; Bang et al. 2018). In recent years, efforts have been channeled into coral “probiotics” applications. Their ultimate goal is to fast-track ecological adaptation to global climate change by designing physiologically augmented coral holobionts (Peixoto et al. 2017). The research field of coral probiotics includes the isolation and screening of native bacterial associates for functional genes beneficial to coral health, and subsequent physiological assays to determine holobiont performance after inoculation with putatively beneficial bacterial isolates (Rosado et al. 2018). Experimental inoculation with mixed consortia of native coral bacterial isolates harboring dinitrogen fixation (\textit{nifH}), denitrification (\textit{nirK}), and DMSP-degrading (\textit{dmdA}) genes resulted in partial mitigation of coral bleaching compared to controls or corals challenged with the temperature-dependent pathogen \textit{Vibrio coralliilyticus} (Rosado et al. 2018). Open questions to this line of research are the temporal stability of the observed beneficial effects, the underlying mechanistic nature, and the potential for application of coral probiotics at the reef scale.

7.4.5 Contribution of bacteria to holobiont nutrient cycling

The ubiquitous coral symbionts \textit{Endozoicomonas} harbor large numbers of genes involved in amino acid synthesis and carbohydrate cycling, suggesting its involvement in holobiont nutrient cycling (Neave et al. 2017), with different strains potentially exhibiting a different genetic and metabolic makeup (Neave et al. 2017; Pogoreutz et al. 2018). Taxonomy-based functional inference was used recently and suggested a role of \textit{Endozoicomonadaceae} in processes related to nitrate reduction in giant clams (Rossbach et al. 2019). As such, \textit{Endozoicomonadaceae} may provide otherwise inaccessible nitrogen sources, including ammonia, to the coral host.
Nitrogen (N) cycling is a critical component of holobiont health (Cardini et al. 2014; Rädecker et al. 2015; Pogoreutz et al. 2017a). Most research on nitrogen cycling in the coral holobiont has focused on prokaryotic dinitrogen (N$_2$) fixation (Shashar et al. 1994; Lesser et al. 2007; Rädecker et al. 2014; Bednarz et al. 2017), while the assessment of other major nitrogen cycling pathways such as nitrification, denitrification, and ANAMMOX has only received marginal attention to date (Wafar et al. 1990; Tilstra et al. 2019), and is currently limited to describing the presence of functional genes in sequencing datasets (Wegley et al. 2007; Siboni et al. 2008; Neave et al. 2017). Prokaryotic N$_2$ fixation commonly occurs in reef-building corals, helping supply the holobiont with “new” bioavailable nitrogen (Lesser et al. 2007; Cardini et al. 2015; Benavides et al. 2017). The biologically fixed nitrogen is then assimilated by both, coral (Benavides et al. 2016; Bednarz et al. 2017) and Symbiodiniaceae (Lesser et al. 2007; Cardini et al. 2015; Pogoreutz et al. 2017a). Nitrogen assimilation rates, however, appear to depend on environmental nitrogen availability, highlighting the importance of integrating environmental context into the study of coral holobiont function. The particular role of N$_2$ fixation to coral heat stress (bleaching) remains to be determined. Elevated temperatures rapidly cause an increase in the relative abundance and activity of coral-associated N$_2$ fixers (Santos et al. 2014; Cardini et al. 2016). It was previously concluded that excess nitrogen supply has the potential to ameliorate the effects of heat stress caused by global warming in corals (Santos et al. 2014; Cardini et al. 2016). An increase in holobiont N$_2$ fixation, however, can shift the N:P ratio of dinoflagellate symbionts in Red Sea corals (Pogoreutz et al. 2017a), thereby destabilizing the coral–algae symbiosis, resulting in coral bleaching (Wiedenmann et al. 2012). Ultimately, whether increases in N$_2$ fixation during heat stress have beneficial or detrimental effects on coral holobiont health may likely be determined by the environmental context (e.g., ambient nutrient regime), host nutritional state, or heterotrophic capacity (Bednarz et al. 2017; Pogoreutz et al. 2017b), and will require further mechanistic studies considering all major nitrogen cycling pathways.

In addition, symbiotic interactions between corals and bacteria might involve the cycling of essential compounds, for instance vitamins. Indeed, the cnidarian host may rely on bacterial symbionts for the provision of cobalamin, which is required for methionine synthesis by both corals and Symbiodiniaceae (Robbins et al. 2019). Concentrations of cobalamin in the coral gastrovascular cavity (coelenteron) are up to 35 times higher than in surrounding reef waters (Agostini et al. 2009), strongly suggesting that the dense bacterial communities harbored in the gastrovascular cavity are producing this essential molecule. In addition, some species from the genus Acropora are lacking an essential enzyme to synthesize the amino acid cysteine (Shinzato et al. 2011), and likely rely on their associated microbes for its provision.

### 7.4.6 Archaea associated with the coral holobiont

Corals associate with a diversity of archaea including representatives from the Crenarchaeota and Euryarchaeota. Crenarchaeota of the class Thermoprotei often dominate the archaeal community, while most abundant euryarchaeal members are affiliated to the Marine Group II and Thermoplasma (Kellogg 2004; Siboni et al.
Interestingly, SML-associated archaeal sequences are most similar to obligate and facultative anaerobic and uncultivated archaea from anoxic environments, suggesting anaerobic microniches within the SML (Kellogg 2004). In terms of absolute abundance, archaeal cell numbers can comprise up to half of the prokaryotic community with an average of \(>10^7\) cells/cm\(^2\) on the surface of *Porites astreoides* colonies (Wegley et al. 2004).

The diversity of ammonia-oxidizing archaea (AOA) has also been evaluated in coral tissues by amplifying the *amoA* gene encoding the alpha-subunit of the ammonia monooxygenase (Beman et al. 2007; Siboni et al. 2008). In addition, AOA are suggested to be less host-specific and more geographically dependent (Siboni et al. 2012). The presence of *amo* genes in coral-associated archaea was also supported by an integrated genomic approach of Thaumarchaeota genomes assembled from *Porites lutea* metagenomes (Robbins et al. 2019). This study revealed the presence of other relevant key metabolic pathways, including the reductive tricarboxylic acid cycle, cobalamin synthesis, and taurine dioxygenase in the Thaumarchaeota genomes, suggesting these symbionts may contribute to the host’s demand for essential vitamins and carbon metabolism (Robbins et al. 2019).

### 7.4.7 Protists and fungi associated with the coral holobiont

Two photosynthetic alveolates, *Chromera velia* (Moore et al. 2008) and *Vitrella brassicaformis* (Oborník et al. 2012), which are the closest free-living relatives of the large parasitic phylum Apicomplexa, are protists commonly associated with corals worldwide (Janouškovec et al. 2013). A recent transcriptomic study revealed that the coral host response to *C. velia* inoculation was similar to that of a parasite or pathogen infection in vertebrates (Mohamed et al. 2018). This suggests that *C. velia*, despite its photoautotrophic capabilities, is not involved in mutualistic interactions with corals, but rather parasitic or commensal (Mohamed et al. 2018). In addition to these two alveolates, the presence of apicomplexans in coral tissues has been reported for the past 30 years (Upton and Peters 1986; Toller et al. 2002; Šlapeta and Linares 2013; Clerissi et al. 2018) and it was recently revealed that a single apicomplexan lineage is ubiquitously associated with corals, and might be the second most abundant microeukaryote group (after Symbiodiniaceae) associated with coral tissues (Janouškovec et al. 2012; Kwong et al. 2019). Although the nature of the association between these “corallicolids” and the coral host remains unknown, their genomes lack all genes encoding for photosystem proteins, but retained the four ancestral genes involved in chlorophyll biosynthesis (Kwong et al. 2019).

Deeper in the skeleton, endolithic protist algae can form dense bands visible to the unaided eye below the tissues of many coral species and are often dominated by the filamentous green algae *Ostreobium* spp. (Siphonales, Chlorophyta) (Kornmann and Sahling 1980). Recent molecular studies have revealed the astonishing genetic diversity of this group, with up to 80 taxonomic units at the near-species level (Marcelino and Verbruggen 2016; Marcelino et al. 2017, 2018; Verbruggen et al. 2017). These filamentous algae colonize the skeleton of coral juveniles early in their development (Massé et al. 2018) and can interact with the coral tissue through transfers of photosynthates (Schlichter et al. 1995; Fine and Loya 2002; Pernice et al.
Coral holobionts depend on associated microbes. High-throughput amplicon sequencing has also revealed the presence of other, less abundant, boring green microalgae closely related to *Phaeophila*, *Bryopsis*, *Chlorodesmis*, *Cladophora*, *Pseudulvella*, and red algae from the Bangiales order in coral skeletons (Marcelino and Verbruggen 2016).

Fungi are prevalent in corals and have been well studied in the coral skeleton where they penetrate the calcium carbonate microstructures and ultimately interact with *Ostreobium* cells (Le Campion-Alsumard et al. 1995). Along with endolithic algae, fungi are present in the newly deposited coral skeleton (Bentis et al. 2000; Golubic et al. 2005), where they exhibit rapid growth to match skeletal accretion (Le Campion-Alsumard et al. 1995). Fungi were the most abundant microorganisms in the *Porites astreoides* metagenome, contributing to 38% of the microbial sequences (Wegley et al. 2007). These fungi belonged mainly to the phylum Ascomycota, but also members of Basidiomycota and Chytridiomycota were detected. Based on their genomic potential, these organisms might play a role in nitrogen recycling through the reduction of nitrate and nitrite to ammonia and subsequent ammonia assimilation (Wegley et al. 2007).

### 7.5 Summary and Outlook

The coral–dinoflagellate symbiosis is a well-studied system with high potential for understanding mechanisms of host–microbe interactions, controls, and co-evolution. The functional importance of bacteria, archaea, and other protists in this complex system has only recently started to emerge and the resulting picture is that of a holobiont where all partners depend on and interact with each other. These complex interdomain relationships are orchestrated and regulated by immune systems. It is therefore important to adopt a two-pronged approach, elucidating the immune responses of each individual partner (i.e., coral host, Symbiodiniaceae, bacteria, and other microbes), but also gaining a greater understanding of immunity at the holobiont scale. To elucidate this complex network of interactions, the Aiptasia model system may help to functionally interrogate the mechanistic underpinnings of the ecology, stress resilience, and environmental adaptation of cnidarian holobionts. For instance, the ability of genetic and microbiome manipulation of Aiptasia allows for detailed studies into the contribution of specific genes or microbes.

It is understood that productivity, structural complexity, and biodiversity of coral reef ecosystems critically depend on healthy coral holobionts, which in turn are linked to the diversity and identity of the associated microbes. This implies the need for a radically different approach to address the key questions of coral health and resilience in the face of climate change: one must examine the complexity of the coral holobiont in its entirety, that is, considering the diversity and function of all associated organisms. Gaining a holistic understanding of the biology and functioning of coral holobionts is of prime importance, given that coral reefs are at the brink of ecological collapse due to the effects of climate change and local stressors. New strategies to mitigate reef loss are now more important than ever. One such strategy may lie in the manipulation of holobiont immunity or associated microbes to enable ecological adaptation at a rate and scale that matches the pace of environmental change.
References


Coral holobionts depend on associated microbes


Coral holobionts depend on associated microbes


