Chapter 2
Sponge and Coral Microbiomes

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Abstract Coral/sponge holobiont is the stable assemblage of the host and its symbiotic bionts, e.g., microalgae, bacteria, archaea, virus, fungi, and protists. Coral/sponge microbiome means the entire microbial community and genes that reside within a coral/sponge. Sponges host abundant and diverse microbes including bacteria, archaea, and fungi. Corals form a close mutualistic relationship with photosynthetic, endosymbiotic dinoflagellates of the genus *Symbiodinium*, along with microorganisms including bacteria, archaea, fungi, and viruses. These microbiota and algae are thought to have various symbiotic relationships with coral/sponge host including mutualism, commensalism, and parasitism.

Keywords Sponge · Coral · Holobiont · Microbiome · Structure · Function

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2.1 Introduction

Strictly speaking, "microbiota" means a collection or community of microbes, while "microbiome" refers to the full collection of genes of all the microbes in a community. However, now in many cases, "microbiome" is also used to mean all the microbes in a community. Meanwhile, although originally coined to specifically refer to host-associated microbial communities [1], the word "microbiome" is now utilized broadly to refer to any habitats. Therefore, the term "microbiome" used in this book includes two meanings: all microbes and their genes in a community.

"Holobiont" was first used to describe the assemblage of different species that form ecological units, typically symbiosis. According to Lynn Margulis, all individuals who participate in a particular symbiosis are bionts, and the entire organism that is comprised of these bionts is a holobiont [2]. By this definition, nearly every macrospecies is a holobiont because it always lives in symbiosis with some other species. For example, all plants and animals, from lower organisms, e.g., invertebrates to humans, live in close association with microbial organisms.

Coral/sponge holobiont means the stable assemblage of the host and its symbiotic bionts, e.g., microalgae, bacteria, archaea, virus, fungi, and protists. The entire assemblage of genomes in the holobiont is termed a "hologenome" which includes the host's genome and its microbiome. The concept of the holobiont was first used to understand corals' components, ecological functions, and their evolution over time and then expanded to other species, e.g., sponges. There is a similar concept "metaorganism" (an entity formed by the aggregation of a number of individual organisms) or "superorganism" (an organism consisting of many organisms) to mean the association of one macrospecies and its bionts.

In 2007, the Human Microbiome Project (HMP) was launched by the NIH (National Institutes of Health, USA) with the aim to characterize the human microbiota (microbiome) and analyze their role in human health. In 2015, the USA announced a new National Microbiome Initiative (NMI) to foster the integrated study of microbiomes across different ecosystems including marine microbiomes. Microbiome represents a frontier in the microbiology field. In the field of marine microbiology, *Science* journal published a special issue on the marine microbiome in 2015. Coral/sponge microbiome is one of the hotspots of marine microbiome because of its important value in ecology, evolution, and biotechnology. In 2016, using next-generation sequencing, the World Sponge Microbiome Project has been achieved, finding phylogenetically diverse microbes on a global scale [3]. Soon afterward, the dataset of the Sponge Microbiome Project was announced in 2017, which represented a comprehensive resource of sponge-associated microbial communities [4].

2.2 Sponge Microbiome

2.2.1 Sponge Microbial Community

Sponges are complex holobionts or metaorganisms because they host abundant and diverse microbes including at least 46 bacterial phyla, 3 archaeal phyla, 3 fungal phyla, and phylogenetically diverse algae [3, 5–10]. These bionts are thought to have various symbiotic relationships with sponge host including mutualism, commensalism, and parasitism.

The presence of bacteria in the mesohyl of sponges was first confirmed in the early 1960s by the use of electron microscopy (EM) [11]. Scanning electron microscopy (SEM) was subsequently employed to detect cyanobacteria in sponges [12]. Traditional culture-dependent approach has been used to investigate the diversity of microbes in sponge holobionts. For instance, a lot of novel fungi and actinobacteria from sponges, e.g., *Marihabitans*, *Polymorphospora*, and *Streptomonospora*, were isolated from marine sponges for the first time in my group [13–15]. Even now, it is still an effective strategy to study the function of microbes derived from sponges, e.g., natural products with medical potentials for marine drug development. In order to recover novel species, some innovative culture methods have been employed, e.g., through antibiotic administration [16] or floating filter cultivation [17].

Because of the medium and condition limitation in the simulation of in situ environment, only a small percentage of microbial populations, i.e.,<1%, could be isolated from sponges in the laboratory. Therefore, culture-independent methods have become the main strategies for the revelation of community structure of sponge microbial symbionts. For example, polymerase chain reaction (PCR)-based cloning library was first successfully employed to detect the diversity of unculturable sponge-associated bacteria. Denaturing gradient gel electrophoresis (DGGE) fingerprint was successfully used to compare the bacterial components among different species of sponges [18]. The subsequent fluorescence in situ hybridization (FISH) allows to reveal the bacterial spatial distribution within sponge tissues [19]. The great advances in the bacterial symbionts' diversity evaluation come from pyrosequencing which makes the comprehensive descriptions of bacterial community structures possible especially the rare biosphere [20].

Sponges could get their microbial symbionts through horizontal transmission from the environmental seawater and vertical transmission from parents [21]. Sponges are suggested to be capable of differentiating food bacteria from symbionts. Wilkinson et al. [22] suggested that the chemical composition of the bacterial outer layer may play a role in sponge symbionts' recognition. However, the question of how sponges discriminate between food and symbionts remains unsolved. Ankyrin-repeat proteins (ARP) may interact with surrounding cells and proteins and might be involved in the recognition and protection from host phagocytosis that allows the host to discriminate between food and symbiont bacteria [23].

In 2002, Hentschel et al. revealed monophyletic clusters of sponge-derived sequences more closely related to each other than that from non-sponge sources and suggested the sponge-specific microbes [24]. Whereafter, by 16S ribosomal RNA gene amplicon pyrosequencing of 32 sponge species from eight locations around the world's oceans, only a minimal core bacterial community consisting of very few OTUs was found. In contrast, a large species-specific bacterial community, which is represented by OTUs present in only a single sponge species, was detected [25]. The sponge species-specific bacteria represent the unique association of sponge microbes which was in depth proved by the global analysis [3]. The species-specific bacteria are probably vertically transmitted which has been demonstrated in sponge larvae by FISH [26].

Cenarchaeum symbiosum was first reported in association with marine sponge *Axinella mexicana* in 1996 [27]. Till now, three archaeal phyla, i.e., *Crenarchaeota*, *Euryarchaeota*, and *Bathyarchaeota*, have been detected in sponges [6, 9, 10, 28]. Particularly, the vertical transmission of archaea in sponge larvae was demonstrated, suggesting a very close coevolutionary relationship of archaea with sponge host [26, 29].

Prokaryotic symbionts show different distribution characteristics in one sponge, for instance, a significant difference of bacterial phylotypes between the cortex and endosome was revealed in sponge *Astrosclera willeyana* [30]. *Bacteroidetes*, *Frankineae*, and *Propionibacterineae* were detected only in the endosome, whereas *Cyanobacteria*, *Planctomycetacia*, and *Micrococcineae* were only associated with the cortex. Some branches of a-*Proteobacteria*, c-*Proteobacteria*, *Corynebacterineae*, *Acidimicobidae*, *Crenarchaeota*, and *Euryarchaeota* also showed distribution difference in *Astrosclera willeyana*.

Compared with the knowledge of sponge-associated bacterial diversity, the diversity of eukaryotic symbionts in sponges remains largely unknown. Phylogenetically diverse eukaryotic symbionts were detected in the *N. huxleyi* metagenome [31]. Using 454 pyrosequencing of the V4 region of 18S ribosomal ribonucleic acid gene of eukaryota associated with 11 species of South China Sea sponges, 2 phyla of fungi (*Ascomycota* and *Basidiomycota*) and 9 phyla of protists including 5 algal phyla (*Chlorophyta*, *Haptophyta*, *Streptophyta*, *Rhodophyta*, and *Stramenopiles*) and 4 protozoal phyla (*Alveolata*, *Cercozoa*, *Haplosporidia*, and *Radiolaria*) including 47 orders (12 fungi, 35 protists). Entorrhizales of fungi and 18 orders of protists were detected in sponges, and sponge species-specific eukaryotic symbionts were suggested [5]. Particularly, the in situ active fungi in sponges *T. swinhoei* and *X. testudinaria* were revealed using 18S rRNA gene transcripts [32].

2.2.2 Sponge Microbial Function

Sponges probably represent one of the most complex symbioses on earth with a core microbial community and sponge-specific or sponge species-specific microbial lineages. After learning more about the diversity of sponge microbial symbionts, the

function evaluation of the microbial symbionts represents the frontier and hot issue of sponge symbioses; however, to date, the function of sponge microbiomes lags behind the understanding of taxonomic affiliation. The primal strategy for the function investigation of sponge microbes is culture dependent. Particularly, the potentials in producing biologically active natural products have been carried out for sponge-derived microbes especially actinobacteria and fungi.

Compound isolation-based activity assay and functional gene-/genome-based evaluation are two main ways to analyze the microbial functions [31, 33–36]. In particular, omics provides a promising strategy for understanding the metabolism and function of sponge microbiomes and has revealed previously unknown diversity and functions of sponge symbionts [37–40]. In 2010, Thomas et al. first explored the functional genomic signature of bacteria associated with the sponge *Cymbastela concentrica* [37]. Thereafter, Liu et al. analyzed the bacterial functional proteins in the sponge *Cymbastela concentrica* using metaproteogenomic technique [38]. Fan et al. investigated the metabolisms of the bacterial communities of six sponges using metagenomics and suggested the functional equivalence and evolutionary convergence in complex microbial communities of sponge symbionts [39]. To date, the well-known function of sponge microbiomes mainly includes an element cycle for providing nutrients for the sponge hosts and removing metabolic wastes [41–43] and chemical defense by producing bioactive compounds [35, 36].

The nitrogen cycle is a critical biogeochemical process of the oceans. Marine sponges have been suggested to play an important role in the marine nitrogen cycle. Mohamed et al. [44] provided the first molecular evidence for the presence of potential anammox bacteria in sponges. Using functional genes (*amoA*, *nirS*, *nirK*, and *nxrA*) involved in ammonia oxidization and denitrification and 16S rRNA genes for specific bacterial groups as markers, phylogenetically diverse prokaryotes including bacteria and archaea, which may be involved in the ammonia oxidization and denitrification processes in sponges, were revealed in seven South China Sea sponge species [45, 46]. Sulfate-reducing bacteria (SRB) are known to play a key role in the cycling of marine elements. Phylogenetically diverse SRB, which mainly belonged to the genus *Desulfovibrio* in the class *Deltaproteobacteria*, in three sponges *Arenosclera heroni*, *Dysidea arenaria*, and *Astrosclera willeyana* from the South China Sea were detected [47].

In 2010, López-Legentil et al. detected the expression of ammonia monooxygenase genes in ammonia-oxidizing archaea associated with the barrel sponge *Xestospongia muta* [48]. Liu et al. proved the expression of the ammonia monooxygenase membrane-bound subunits (*AmoB* and *C*) and an ammonia transporter (*AmtB*) in the microbial community of *C. concentric* by metaproteogenomic analysis [38]. Nitrifying community with transcriptional activity in sponge microbiomes was observed in South China Sea sponges. For example, the expression of *ureC* genes from *Proteobacteria*, which were the predominant component in sponge *X. testudinaria*, suggested the function of bacterial symbionts in urea utilization [49]. In addition, the inhabitancy and transcriptional activity of *Nitrosopumilus*-like AOA (ammonia-oxidizing archaea) and *Nitrospira* NOB (nitrite-oxidizing bacteria) in this sponge *T. swinhoei* from the South China Sea were confirmed [50].

The metabolic analysis of sponge holobionts at the whole community level including prokaryotes and eukaryotes is helpful for understanding the biology and ecology of sponge symbioses. In 2014, phylogenetically diverse prokaryotes and eukaryotes were detected in deep-sea sponge *N. huxleyi* using metagenomics in my group. MEGAN and gene-enrichment analyses indicated different metabolic potentials of prokaryotic symbionts from eukaryotic symbionts, especially in nitrogen and carbon metabolisms [31].

As the oldest multicellular animals lack active defense ability and developed immune system, still sponges have survived in the complex sea environment for almost 630 million years, mainly because of their chemical defense against predator, other colonial organisms, and pathogenic microbes besides their strong regenerative capacity. The secondary metabolites produced by marine sponges include steroids, isoprenoids, non-isoprenoids, quinones, nitrogen and nitrogen-sulfur heterocyclic compounds, alkaloids, peptides, and terpenes, and most of them show higher biological activities, e.g., cytotoxicity, anti-pathogens, enzymic inhibition, etc. [51–54]. Sponges are currently the most important marine sources of biologically active natural products [55], since the number of natural products isolated from sponges, *ca*. 6000, accounts for nearly one-third of the total marine natural products. Among the seven marine drugs in the market before 2016, three are derived from sponges, e.g., anticancer drug cytarabine (Ara-C) and eribulin mesylate (E7389) and antivirus vidarabine (Ara-A). Thus, bioactive compounds isolated from marine sponges have become a starting point for developing new marine drugs.

It is worth mentioning that some of these compounds isolated from marine sponges are only synthesized in symbiotic relationships with fungi, microalgae, archaea, cyanobacteria, and bacteria [56, 57]. In 1996, macrolide swinholide A was limited to unicellular heterotrophic bacteria in sponge *Theonella swinhoei*, and an antifungal cyclic peptide was found to occur only in the filamentous heterotrophic bacteria [58], providing the first chemical evidence for the uncultured bacterial origin of sponge-derived compounds. Afterward, Piel et al. found the bacterial gene cluster which was responsible for biosynthesizing onnamides and proved the producer was uncultured *Entotheonella* spp., providing gene evidence for bacterial origin of sponge-derived compounds [59, 60].

2.3 Coral Microbiome

2.3.1 Coral Microbial Community

Corals are holobionts or “metaorganisms,” e.g., in a mutualistic relationship with photosynthetic, endosymbiotic dinoflagellates of the genus *Symbiodinium*, which can provide >90% of a coral’s nutritional requirements, along with microorganisms including bacteria, archaea, fungi, and viruses.

Coral microbiome means the entire microbial community (and associated genes) that resides on or within a coral. Extensive phylogenetic surveys of coral microbiomes have revealed that the dominant symbionts reside within the *Proteobacteria* (particularly *Gammaproteobacteria* and *Alphaproteobacteria*) as well as *Actinobacteria*, *Bacteroidetes* (especially *Flavobacteria*), and *Cyanobacteria* [61]. For example, in *Porites astreoide*, the most prominent bacterial groups were *Proteobacteria* (68%), *Firmicutes* (10%), *Cyanobacteria* (7%), and *Actinobacteria* (6%) [62]. In particular, Roder et al. showed that bacterial diversity of fungid host species *Ctenactis echinata* is primarily structured by one bacterial taxon (genus *Endozoicomonas*) representing more than 60% of all bacteria [63].

The coral microhabitats include coral mucus, tissues, skeleton, and gastric cavity. The surface mucopolysaccharide layer, produced by endosymbiotic *Symbiodinium* spp., is composed of glycoproteins and provides an ideal habitat for microbes, e.g., 10^6–10^8 microbial cells per milliliter [61]. However microbial community in the coral mucus is not very stable because of the environmental effects. In contrast, microbes inhabiting in the coral epithelium and gastrodermal tissues are always specific to coral host, though with a generally low microbial abundance. Coral skeleton provides a unique habitat for coral symbiotic microbes.

Similar with sponges, the establishment of coral-microbes symbioses includes two strategies, inheritance, i.e., vertical transmission, and acquisition from the surrounding environment, i.e., horizontal transmission. However, the molecular mechanisms that allow for the establishment, recognition, and maintenance of microbial symbionts are still unknown. Meanwhile, coral microbiome research that mainly focus on bacteria, archaea, virus, while fungi are rarely involved.

The coral holobiont is a dynamic assemblage of the coral host, zooxanthellae, endolithic algae, fungi, bacteria, archaea, and viruses. The coral animal can adapt to differing ecological niches by "switching" its microbial associates. Zooxanthellae and some bacteria form relatively stable and species-specific associations with coral hosts. Other associations are less specific, e.g., coral-associated archaea [61]. According to Roder et al. [63], the content and structure of the coral microbiome aligns with environmental differences and denotes habitat adequacy. On the other hand, an inflexible bacterial community under different environmental conditions was also suggested [64]. Compared to the changes in the *Symbiodinium* community, the associated bacterial community remains remarkably stable even under conditions of coral bleaching. Totally, coral holobionts might occupy structural landscapes ranging from a highly flexible to a rather inflexible composition with consequences for their ability to respond to environmental change [64].

2.3.2 Coral Microbial Function

Symbiodinium spp. fixes carbon by photosynthates and transfer nutrients to the coral host. The coral microbes play an important role in the element cycling, e.g., carbon, nitrogen, phosphorus, and sulfur [61].

It is known that coral microbiota is involved in the carbon fixation by the Calvin cycle, a reductive acetyl-CoA pathway, or reverse Krebs cycle, and carbon degradation. Coral microbiota undertakes nitrogen cycling via nitrogen fixation, nitrification, and denitrification. Metagenomics analysis suggested that the coral-associated bacteria possessed a large number of genes for the uptake and processing of sugars and proteins [62]. Ceh et al. revealed that coral larvae acquire nutrients previously taken up from the environment by bacteria, which may increase the survival rate and fitness of the developing coral and therefore contribute to the successful maintenance of coral reefs [65].

Corals are one of the largest producers of dimethylsulfonipropionate (DMSP) in the oceans. Some coral microbes, e.g., *Endozoicomonas* spp., have been proved to be capable of DMSP metabolism [66]. In addition, inorganic sulfur can also be cycled via sulfate-reducing bacteria (SRB). Dissolved organic phosphorus may be recycled by coral microbes, e.g., *Vibrio* spp. is capable of phosphorus degradation [67].

Take reef-building coral *Porites astreoide* as an example, functionally, the bacterial community is primarily heterotrophic and includes a number of pathways for the degradation of aromatic compounds, and the most abundant is the homogentisate pathway. Particularly, a wide diversity of fungal genes involved in carbon and nitrogen metabolism were detected, which suggested that endolithic fungi could be converting nitrate and nitrite to ammonia within the coral holobionts [62].

It was confirmed (both spatially and temporally) that a nitrogen fixer (*Prosthecochloris*, a green sulfur bacteria) in the green layers of coral skeletons, played an essential role in providing nutrients for the coral holobiont in the nutrient-limited reef ecosystem [68].

Archaea associated with the surface mucus of corals include marine group II, anaerobic methanotroph, anaerobic nitrate reducers (i.e., denitrification), and marine group III (8%). Coral-associated archaea may contribute to nitrogen recycling in the holobiont, presumably by acting as a nutritional sink for excess ammonium trapped in the mucus layer, through nitrification and denitrification processes [69].

Marine viral assemblages within the coral holobionts probably have important but currently unknown functions in the coral stress response, coral disease, and the adaptive potential of the coral holobionts with respect to climate change [70].

Using *Porites* spp. as a case study, Sogin et al. presented evidence that the relative abundance of different subclades of *Symbiodinium* and bacterial/archaeal families were linked to positive and negative metabolomic signatures. Consequently, coral partner choice likely influences cellular metabolic activities and, therefore, holobiont nutrition [71].

The shifts in the prokaryotic community composition during mucus aging may lead to the prevalence of opportunistic and potentially pathogenic bacteria. Particularly, microbe-depleted corals started exhibiting clear signs of bleaching and necrosis. Thus, it could be concluded that the natural prokaryotic community inhabiting the coral SML contributes to coral health [72].

Coral microbiome responds and quickly adapts to disturbance and has central roles in the coral reef ecosystem. Prosser [73] recently stated that quantitative information on the links between microbial community structure, populations, and activities will allow predictions on the impacts of climate change to ecosystem processes. Thus, theoretically, coral microbes may be used for predicting responses of reef ecosystems to climate changes, if important linkages occurring between the microbial communities and macroecological change. Ultimately, this microbial perspective will improve our ability to accurately predict the resilience of specific reefs and contribute to the conservation of these important ecosystems [74].

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