




Characterization of bacterial community structure in two alcyonacean soft corals (*Litophyton* sp. and *Sinularia* sp.) from Chuuk, Micronesia

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Abstract Microbes in the coral holobiont play important roles in nitrogen fixation, carbon supply, antibiotic production, mucus recycling, and food supply to maintain homeostasis in corals. However, microbes can also induce coral diseases in response to environmental changes under non-optimal conditions. Therefore, studies of microbial communities are needed to understand the health statuses of corals in response to environmental changes. In this study, we performed 16S rDNA metabarcoding to investigate the bacterial communities in two healthy alcyonacean soft coral species (*Sinularia* sp. and *Litophyton* sp.) inhabiting the coast of Weno Island (Chuuk, Micronesia) and in ambient seawater. We identified 18 bacterial phyla, 24 classes, 54 orders, 109 families, and 222 genera associated with the two corals and seawater. The bacterial communities differed in the corals and seawater. The bacterial community in *Sinularia* sp. was dominated

by the genus *Spirochaeta* in Spirochaetaceae (63.9% relative abundance), followed by *Endozoicomonas* (10%). In *Litophyton* sp., the bacterial community also contained *Spirochaeta* (19.5%) and *Endozoicomonas* (4.7%), although Cellvibrionaceae (23.7%) was dominant and other groups such as Rhizobiales (11.5%) and Rhodospirillales (8.7%) were evenly distributed. In ambient seawater, the predominant bacteria were *Pelagibacter* (29.2%), Rhodobacteraceae (15.5%), *Prochlorococcus* (11.3%), and *Vibrio* (5.8%), which are distinct from the species in the two coral species. The microbial communities between the two alcyonacean soft corals and seawater were different, and the microbial community differences were coral species-specific.

Keywords Alcyonacean soft coral · Holobiont · Symbiont · Bacterial community · rDNA metabarcoding

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Introduction

Coral reefs are among the most biologically diverse ecosystems on Earth, providing niches for numerous marine organisms (Blackall et al. 2015). However, coral reefs and their associated organisms have become increasingly vulnerable to disturbances from climate change and pollution caused by human activities in recent years. Particularly, climate change causes coral bleaching, a stress response in corals, resulting in the loss of their symbiotic algae and causing the corals to turn white (Lesser 2007). In some cases, these stress responses lead to the death of the corals and devastation of coral reefs.

Generally, coral-associated microbes maintain coral health by influencing the structure and function of coral holobionts. This includes adaptation of coral to

environmental changes and recycling of nutrients such as sulfur, carbon, and nitrogen compounds (Webster and Reusch 2017; Robbins et al. 2019; Pernice et al. 2020). In addition, the health of the corals depends on their specific bacterial colonization (Morrow et al. 2012; Krediet et al. 2013). In general, bacterial communities associated with corals are among the most diverse and complex communities in the microbial biosphere (Sunagawa et al. 2010; Bourne and Webster 2013; Hernandez-Agreda et al. 2016). Coral-related bacterial communities vary in their response based on intrinsic and extrinsic factors, such as the coral species, habitat, and environmental conditions (Thompson et al. 2014), and differ from those in ambient seawater (Sunagawa et al. 2010; Barott et al. 2011; Roder et al. 2014). Specifically, coral metabolism affects the associated bacterial community structure. Environmental factors, such as geographic location, water depth, nutrient concentration, and temperature, also have significant effects (Apprill et al. 2009; Vega Thurber et al. 2009; Littman et al. 2011). Therefore, the relationship between bacterial communities and their hosts or the environment have been described in terms of bacterial diversity associated with corals (Rohwer et al. 2002), host specificity (Ceh et al. 2011), changes in bacterial communities in response to competition between corals and macroalgae (Vega Thurber et al. 2012), and coral disease (Rohwer et al. 2002; Ceh et al. 2011; Vega Thurber et al. 2012; Wilson et al. 2012). However, changes in the temporal and spatial diversity of bacterial communities in response to environmental changes have not been widely studied. In addition, the composition of the microbial community associated with corals and their health in response to environmental changes and human activities are unclear. Therefore, it is important to investigate the composition of coral-associated microbial communities.

The alcyonacean soft coral genera *Litophyton* and *Sinularia* are two of the most widely distributed soft corals (Chen et al. 2012; Abou El-Kassem et al. 2018). They are abundant in the tropical western Pacific regions, including the islands of the Federated States of Micronesia (FSM) (GBIF.org 2020). The FSM are surrounded by coral reefs that support rich biodiversity. Thus, these reefs are beneficial to the Micronesian people, supporting their survival by providing food and revenue from fish sales and tourism. In addition, these corals have drawn increased attention because they contain pharmacological compounds and have potential medicinal properties originating from their various secondary metabolites such as sesquiterpenes, diterpenes, polyhydroxylated steroids, and polyamines (Grote et al. 2008; Chen et al. 2012). Although biodiversity remains high within the FSM reefs, there are threats from a variety of human activities, such as coastal development, pollution, tourism, and fishing. Indeed, a Status Report of 2020 on the FSM (Hall 2020) showed that the coastal areas

of Chuuk State, FSM, were more affected by rapid population growth, environmental pollution, and climate change than the other islands around Micronesia, such as the Palau, Kiribati, and Marshall Islands. However, few studies have examined the bacterial community associated with the soft corals *Sinularia* and *Litophyton*. Understanding the bacterial associations in these two soft corals may provide insight into the relationship between bacteria and coral in the reefs of Chuuk State.

In this study, we analyzed the relative abundance of bacterial taxa in the microbial communities in two different leather soft corals, *Litophyton* sp. (Palau green tree leather coral) and *Sinularia* sp. (smooth leathery soft coral), and in the ambient coastal seawater from the Weno Island, Chuuk State, FSM, using the 16S rDNA metabarcoding approach. Overall, the potential functional roles of common bacteria were explored, and their possible functional roles were assessed based on their occurrence by comparing these with the published reports.

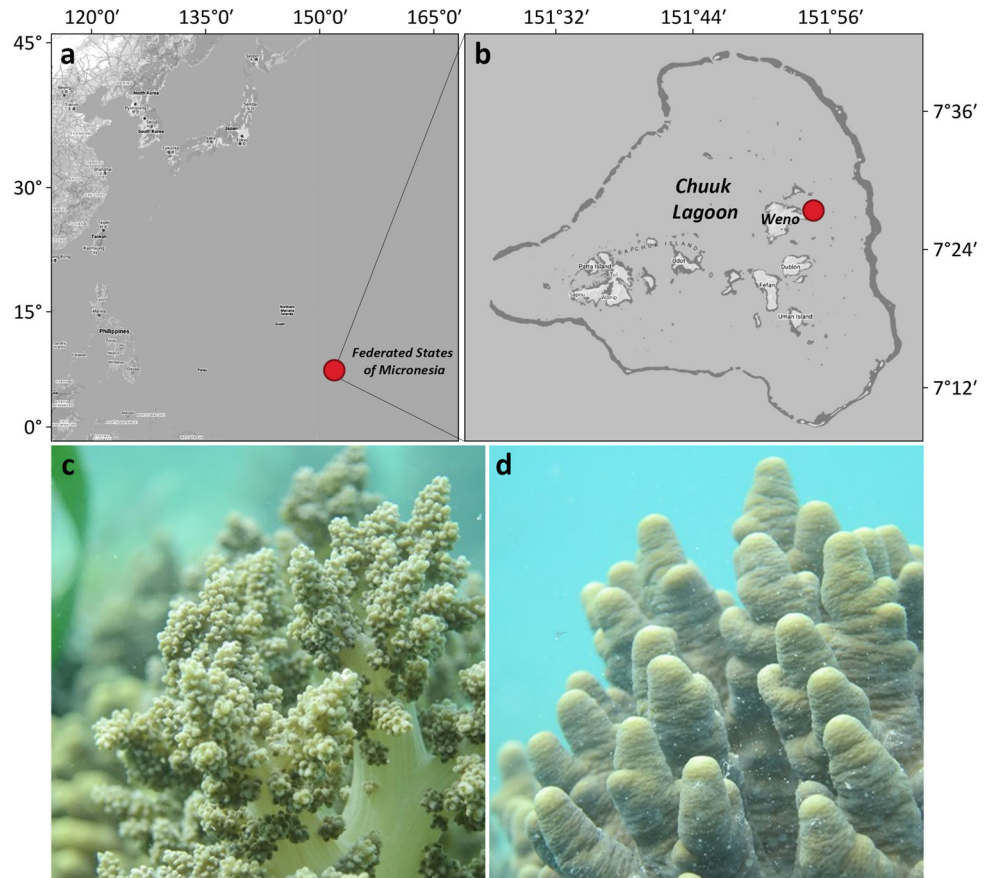
Materials and methods

Sample collection and harvest of bacterial communities from two alcyonacean soft corals and ambient seawater

Ten fragments of a single colony in two visually healthy alcyonacean soft coral species, *Litophyton* sp. and *Sinularia* sp., were collected from a depth of 3–10 m along the Coast of Weno Island by scuba diving on May 13, 2014 (7°27'22.9" N, 151°54'21.2" E, Fig. 1). Each coral subsample was immediately placed in a plastic bag and transported to the ship. On the ship, the coral samples were washed twice with sterilized seawater, placed in sterilized plastic bags, and stored on ice until they were transported to the laboratory. The samples of two alcyonacean soft coral species, *Litophyton* sp. (No. B_S_HI_00001111) and *Sinularia* sp. (No. B_S_HI_00001112), were stored in the Library of Marine Samples of the Korea Institute of Ocean Science & Technology. The identification and characterization of these coral species were confirmed using mitochondrial DNA gene cytochrome oxidase I (*COI*) and phylogenetic analyses (Suppl. Figure 1).

Ambient seawater samples (5 L each, sampling at a depth of 3–10 m using a Niskin water sampler and placed in a sterilized 5-L polyethylene bottle) were collected in duplicate. Bacterial cells on the surface mucus layer of each coral fragment were detached by performing repeated sonication (VibramCell; Sonic & Materials, Inc., Newtown, CT, USA) at 15 A and 4 °C for 5 min in sterilized seawater (Jung et al. 2013). The detached large-sized particles and organic matter were pre-filtrated (or removed)

Fig. 1 Corals and seawater sampling site, and photographs of the two alcyonacean soft corals. **a** Red circle shows the location of Chuuk Lagoon in Northeastern Pacific Ocean. **b** Red circle shows the coral and seawater sampling site ($7^{\circ}27'22.9''$ N, $151^{\circ}54'21.2''$ E) near Weno Island in Chuuk Lagoon. **c** *Litophyton* sp. **d** *Sinularia* sp.



using a 3- μ m polycarbonate membrane (TSTP04700; Millipore, Billerica, MA, USA). The bacterial communities were harvested from the flow-through by re-filtering using a 0.2- μ m polycarbonate membrane (TTTP04700, Millipore). The filters were washed three times with approximately 50 mL distilled water at 50–60 $^{\circ}$ C to remove environmental DNA debris (Jung et al. 2018). The bacterial communities from seawater were also harvested using a 0.2- μ m polycarbonate membrane. The filters were stored at -80 $^{\circ}$ C until genomic DNA (gDNA) extraction.

DNA extraction and 16S rDNA metabarcoding analyses

The filters with the harvested bacterial communities were cut into several pieces for gDNA extraction. gDNA was extracted using a DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions; DNA was diluted to a final concentration of 20 ng/ μ L. The quantity and quality of the total gDNA were determined using a NanoDrop (Nano-MD-NS, SCINCO Ltd., South Korea). The V1–V3 hypervariable regions of the bacterial 16S rDNA genes were amplified using the universal tagged forward (27F: 5'-GAG TTT GAT CMT GGC TCA G-3') and reverse (518R: 5'-ATT ACC GCG

GCT GG-3') primers (Kim et al. 2016). PCR was performed using ExTaq polymerase (Takara, Shiga, Japan) on a T100 PCR Thermal Cycler (Bio-Rad, Hercules, CA, USA). The PCR protocol consisted of an initial denaturation step at 95 $^{\circ}$ C for 3 min, followed by 35 cycles of denaturation at 95 $^{\circ}$ C for 10 s, annealing at 52 $^{\circ}$ C for 45 s, extension at 72 $^{\circ}$ C for 1 min, and a final extension step at 72 $^{\circ}$ C for 5 min. The amplified products were individually purified using a QIAquick PCR Purification Kit (Qiagen). Although we performed duplicate experiments, we attempted to overcome the experimental bias and obtain accurate results by performing three PCRs in distinct tubes and mixing the PCR products to obtain more accurate metabarcoding results (Jung et al. 2018). Prior to obtaining the metabarcoding results, the amplified PCR products were individually purified using an UltraClean PCR Cleanup Kit (MoBio; Folsom, CA, USA). DNA concentrations of the cleaned PCR products were measured using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Equal concentrations of the PCR products were analyzed using a 454 automated sequencer (GS Junior Sequencing System, Roche, Basel, Switzerland) using titanium chemistry. All sequences obtained from this study have been deposited in the National Center for

Biotechnology Information Sequence Read Archive under BioProject accession number PRJNA610452.

Bioinformatics, statistical analyses, and functional annotation of common bacteria

Bioinformatics analyses were performed as described by Kang et al. (2021). The sequences were subjected to a quality check; short sequence reads (length < 150 bp), low-quality sequences (score < 25), singletons and chloroplast sequences, and any non-bacterial ribosome sequences and chimeras were removed using Roche GS20 software (Gontcharova et al. 2010). Using the Basic Local Alignment Search Tool, the sequence reads were compared to the Silva rRNA database (Altschul et al. 1990; Quast et al. 2013). Sequence reads with an *E* value less than 0.01 were considered as partial 16S rDNA sequences. 16S rDNA sequences with at least 97% similarity were considered for species identification, and their complete taxonomic hierarchy was assigned, which consisted of the phylum, class, order, family, genus, and species.

Alpha diversity indices were estimated by calculating the Chao1, Shannon, and Simpson indices using the R vegan package (Oksanen et al. 2010). All visualizations except Venn diagrams were created in the R ggplot2 package. Venn diagrams were prepared using Venny 2.1.0 (<https://bioinfogp.cnb.csic.es/tools/venny/>). Operational taxonomic units (OTUs) with an abundance of < 1% in at least one sample were filtered out. The relative abundances of the filtered bacterial OTUs were normalized through logarithmic transformations. Cluster analyses were performed on the data from the filtered bacteria using the group average clustering in the Bray–Curtis similarity method. To test the null hypothesis (no significant difference between the groups discriminated according to the agglomerative clustering analysis), similarities were analyzed with ANOSIM in PRIMER version 6.1.13 (Clarke et al. 2006).

To predict the functional roles of the common bacterial associates, functional annotation of prokaryotic taxa (FAPROTAX) was performed using python collapse_table.py with the normalized OTU table (Louca et al. 2016). The FAPROTAX dataset (available at <http://www.zoology.ubc.ca/louca/FAPROTAX>) is a manually constructed database based on cultured representatives of marine and freshwater microbiomes. The functional group abundances in each module were calculated by multiplying the calculated values (“function tables”) and the total sum of OTUs belonging to each major module.

Results

Bacterial communities in the two alcyonacean soft corals (*Litophyton* sp. and *Sinularia* sp.) and ambient seawater were identified using 16S rDNA metabarcoding. A mean of 6437 reads was retrieved after quality trimming (Table 1), among which 6108 were detected in ambient seawater samples, whereas 7233 and 5971 reads were found in *Litophyton* sp. and *Sinularia* sp., respectively. The mean number of observed OTUs with 97% quantified reads was lowest for *Sinularia* sp. (270), followed by *Litophyton* sp. (327) and ambient seawater (384). Chao 1, Shannon diversity, and Simpson evenness are presented in Fig. 2a; ambient seawater samples had the highest alpha diversity index (Chao1: mean 597; Shannon: 3.9; Simpson: 0.9), whereas *Sinularia* sp. had the lowest index (Chao 1: mean 443; Shannon: 2.1; Simpson: 0.6). The Venn diagram indicated that bacterial species in ambient seawater, *Litophyton* sp., and *Sinularia* sp. represented 519, 424, and 531 taxa, respectively; in all samples, common bacteria overlapped in 39 taxa (3.3% of the total number of bacteria) (Fig. 2b). *Sinularia* sp. and *Litophyton* sp. showed the largest OTU overlap of 178 taxa and *Litophyton* sp., and ambient seawater showed the smallest OTU overlap of 68 taxa.

Taxonomic classification revealed 18 bacterial phyla, 24 classes, 54 orders, 109 families, and 222 genera. Except for the most dominant Proteobacteria, which were assigned at the class level, the other bacterial groups were classified at the phylum level (Fig. 3). The next most dominant phylum was Cyanobacteria (13.3%), followed by Bacteroidetes (3.8%). The bacteria associated with *Sinularia* sp. were dominated by the genus *Spirochaeta* in Spirochaetaceae, with a mean of 63.2% relative abundance, followed by Gammaproteobacteria (13.0%), Alphaproteobacteria (6.1%), and Cyanobacteria (3.4%). In *Litophyton* sp., the associated bacteria differed from those of *Sinularia* sp. and included Gammaproteobacteria (32.1%), Alphaproteobacteria (26.6%), Spirochaetes (19.7%), and Tenericutes (6.2%). In seawater, Alphaproteobacteria and Gammaproteobacteria belonging to the phylum Proteobacteria were dominant, with relative abundances of 57.9% and 22.4%, respectively.

Common bacterial OTUs displaying a mean relative abundance of > 1% in the two corals and ambient seawater included 36 taxa. The mean of the total relative abundance of common OTUs was 85.7% in *Sinularia* sp., 84.0% in *Litophyton* sp., and 69.4% in ambient seawater. The bacterial communities from the two corals were clustered with 35% similarity and contained four common bacterial OTUs (OTU1161, OTU1162, OTU1002, and OTU1008); the first two OTUs (*Spirochaeta*) were assigned to Spirochaetaceae,

Table 1 Observed OTUs and alpha diversity index for the bacterial communities in ambient seawater and the two corals

Alpha diversity	Ambient seawater			<i>Litophyton</i> sp.			<i>Sinularia</i> sp.		
	1st	2nd	mean	1st	2nd	Mean	1st	2nd	mean
No. of reads	6411	5805	6108	8497	5969	7233	3823	8118	5971
OTUs	404	363	384	366	288	327	220	319	270
Chao1	661	534	597	506	421	464	408	477	443
Simpson	0.946	0.940	0.943	0.878	0.895	0.887	0.616	0.663	0.639
Shannon	3.915	3.850	3.883	2.944	3.077	3.010	1.968	2.149	2.058

The observed OTUs (> 97% sequence similarity) and alpha diversity indices (Chao1, Shannon, and Simpson index) of bacteria communities are displayed

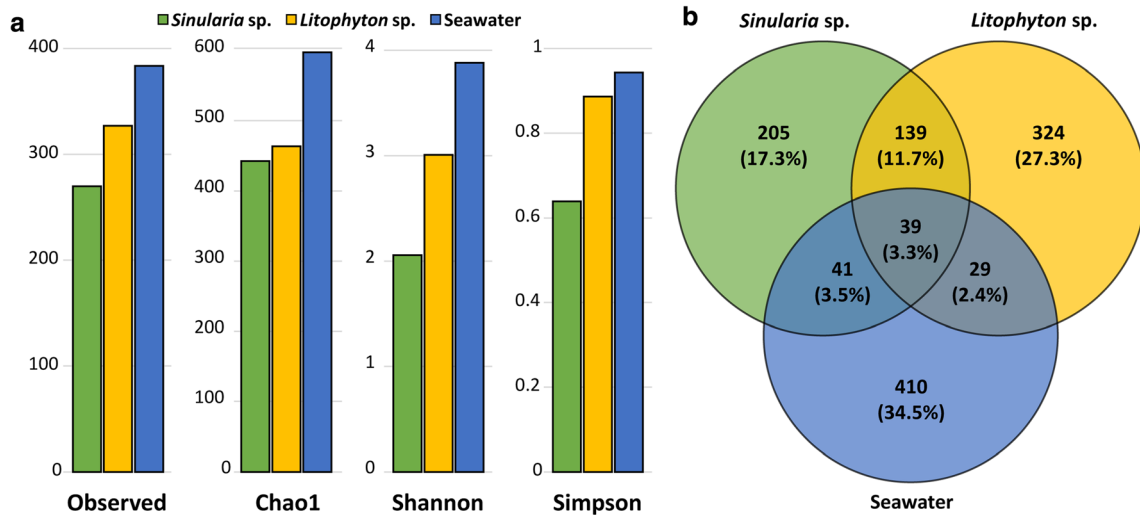


Fig. 2 Alpha diversity of two alcyonacean soft corals (*Sinularia* sp. and *Litophyton* sp.) and ambient seawater. **a** Observed OTUs, Chao 1, Shannon, and Simpson index, **b** Venn diagram showing the shared and unique bacterial OTUs at 97% similarity cutoff

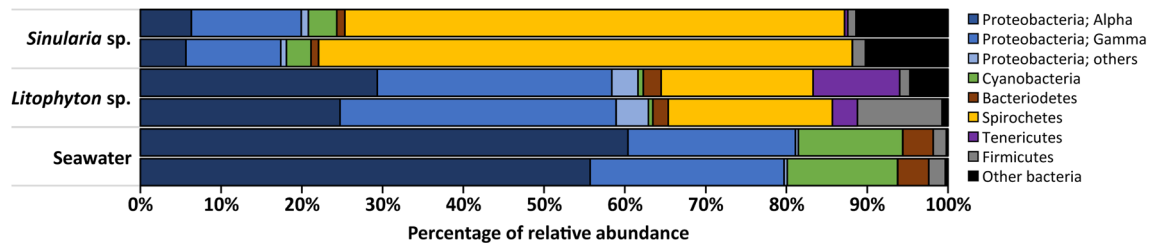


Fig. 3 Taxonomic composition of the bacterial communities in the two alcyonarian corals and ambient seawater

and the other two OTUs to *Endozoicomonas*. Interestingly, the common bacterial associates showing > 1% relative abundance were not common between the corals and seawater (Fig. 4). *Spirochaeta* and *Endozoicomonas* were predominant in *Sinularia* sp. with relative abundances of 63.9% and 10.0%, respectively. In addition to the two bacterial groups that were common between the two corals, *Litophyton* sp. had other associates, such as Cellvibrionaceae (23.7%), Rhizobiales (11.5%), and Rhodospirillales (8.7%). In ambient seawater, the predominant bacterial communities completely differed from those in the two coral species, namely *Pelagibacter* in SAR11

(29.2%), Rhodobacteraceae (15.5%), *Prochlorococcus* in Cyanobacteria (11.3%), *Vibrio* (5.8%), Alteromonadaceae (3.8%), and Oceanospirillaceae (2.5%).

Thirty-six common bacterial OTUs were identified using the pipeline of FAPROTAX to evaluate the potential functional differences among samples (Fig. 5). The functional profiles of bacteria in *Sinularia* sp. were mainly associated with heterotrophy such as aerobic chemoheterotrophy and chemoheterotrophy, autotrophy such as in Cyanobacteria, oxygenic photoautotrophy and phototrophy, carbon cycling such as fermentation, and nitrogen cycling such as nitrogen fixation. Particularly,

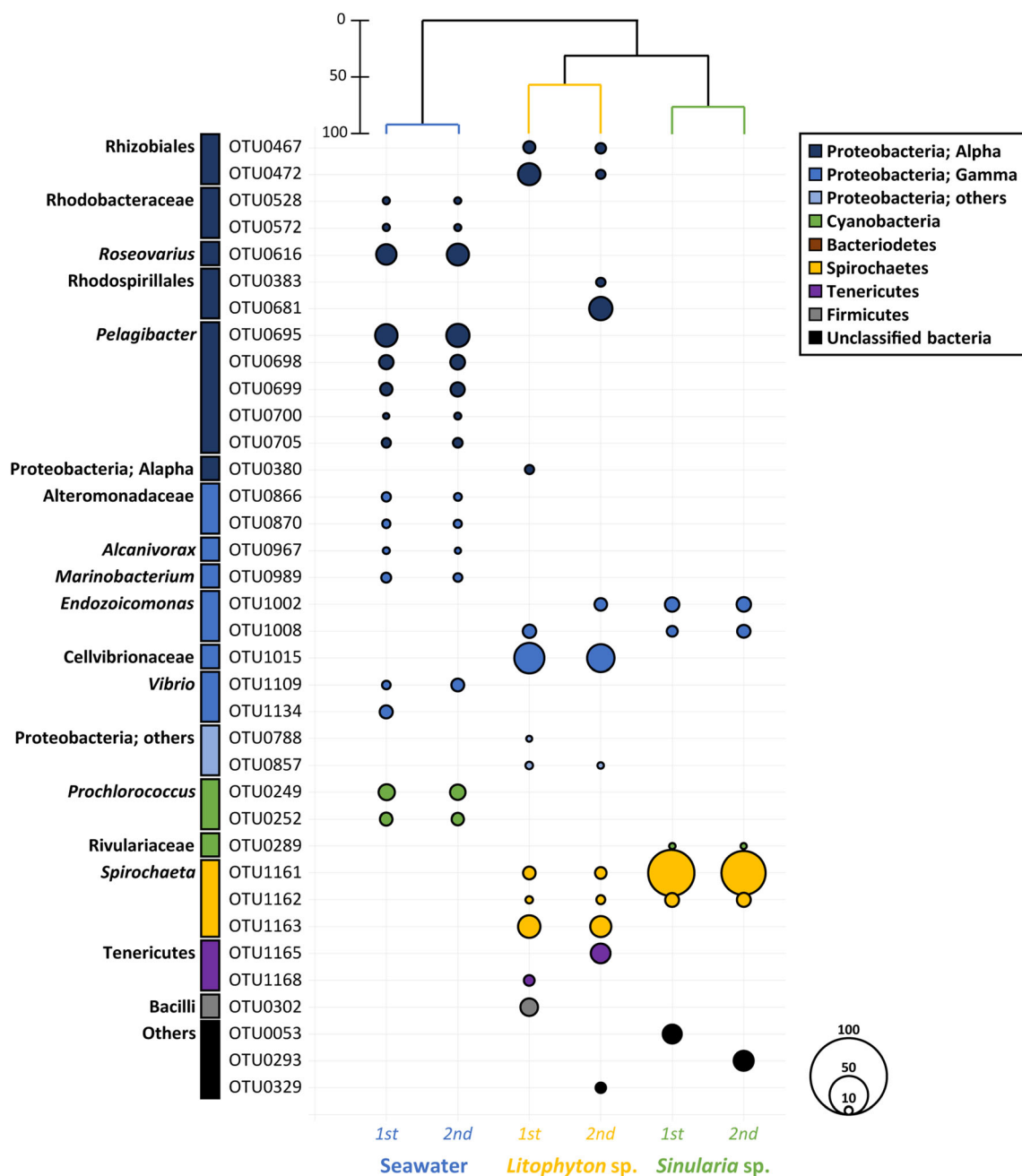
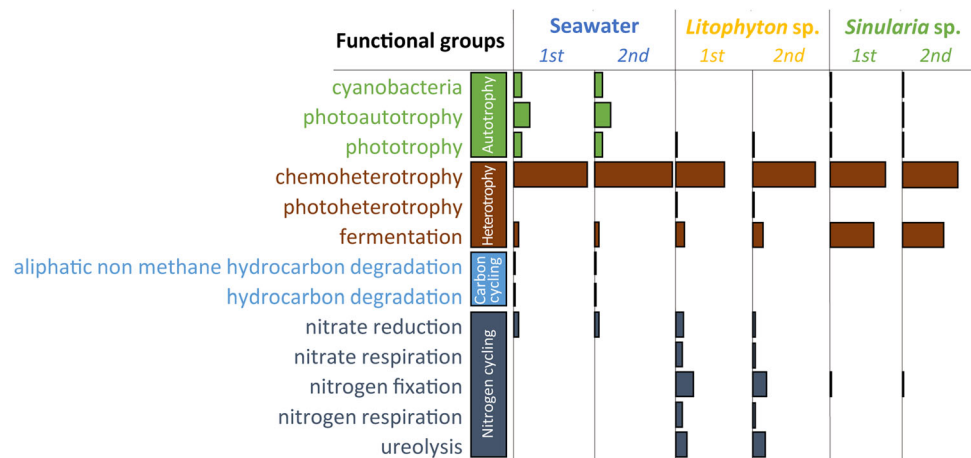


Fig. 4 Bubble chart showing the most abundant bacterial OTUs. Scale indicates the relative abundance. Common bacterial OTUs were clustered using the Bray–Curtis similarity method

chemoheterotrophy and fermentation were the most prominent functions, averaging 48.4% and 41.8% of the total functional categories, respectively. In *Litophyton sp.*, the functional categories predominant were heterotrophy (52.4%; chemoheterotrophy, aerobic chemoheterotrophy, and photoheterotrophy), carbon cycling associated with fermentation (9.0%), nitrogen cycling (24.9%; nitrogen fixation, ureolysis, nitrogen reduction, nitrate respiration, and nitrogen respiration), and autotrophy (1.5%). In ambient seawater, the functional groups of bacteria were

autotrophy (33.0%; photoautotrophy, phototrophy, oxygenic phototrophy, Cyanobacteria, and phototrophy), heterotrophy (69.0%; chemoheterotrophy, aerobic chemoheterotrophy, and fermentation), carbon cycling (1.2%; hydrocarbon degradation and aliphatic non-methane hydrocarbon degradation), and nitrogen cycling (11.2%; nitrogen reduction, nitrate respiration, and nitrogen respiration).

Fig. 5 Functional groups of 36 common bacteria in two corals and ambient seawater. These data were obtained through functional annotation of prokaryotic taxa (FAPROTAX) analyses



Discussion

Generally, coral-associated bacteria communities play crucial roles in coral holobionts and are essential for coral biology by performing complementary metabolism. Therefore, identifying and studying these communities can provide insights into the interactions between coral and microbiota and predict the coral health condition in response to environmental changes. In this study, we compared the bacterial communities in two co-occurring and ecologically important tropical alcyonacean soft coral species, *Sinularia* sp. and *Litophyton* sp., with those in ambient seawater. We found that the microbial communities between the two alcyonacean soft corals and seawater were different and observed microbial community differences in coral species-specific (Frias-Lopez et al. 2002; Apprill et al. 2009; Ochsenkühn et al. 2018). Specifically, the microbial community in *Sinularia* sp. was dominated by the genus *Spirochaeta* in Spirochaetaceae, followed by *Endozoicomonas*. In *Litophyton* sp., the microbial community was dominated by *Spirochaeta* and *Endozoicomonas*, but other associates, such as Cellvibrionaceae, Rhizobiales, and Rhodospirillales, were also common. However, in ambient seawater, the predominant bacteria were *Pelagibacter*, *Rhodobacteraceae*, *Prochlorococcus*, and *Vibrio*, which are distinct from the species in the two alcyonacean coral species. Particularly, Spirochaetaceae was the predominant taxon in approximately half the *Sinularia* sp. and was present in *Litophyton* sp. but not in ambient seawater. Generally, some bacteria, such as Spirochaetaceae and *Endozoicomonas* in *Sinularia* sp., are frequently found in various corals worldwide, whereas Cellvibrionaceae, Rhizobiales, and Rhodospirillales bacteria in the *Litophyton* sp. are rarely detected near other corals (Lesser et al. 2018; Weber and Apprill 2020; Rosales et al. 2020). Indeed, previous studies reported that Spirochaetaceae is associated with various corals in different

regions, such as the *Muricea* coral species (Holm and Heidelberg 2016), *Anthothela* coral species from deep sea (Lawler et al. 2016), *Lophelia pertusa* from cold water (Kellogg et al. 2009), gorgonians from deep sea (Gray et al. 2011), and *Corallium rubrum* (van de Water et al. 2016), suggesting that Spirochaetaceae are commonly found in coral reefs including *Sinularia* sp. and *Litophyton* sp. Although Spirochaetaceae are a coral symbiont found across different coral species, regions, and water depths, we could not confirm the specific environmental factors that determine the occurrence of these bacteria as a coral association.

Additionally, previous studies reported that *Spirochaetes* are likely associated with the breakdown of lignocellulose and nitrogen fixation in termite guts (Lilburn et al. 2001; Brune 2014). Although Spirochaetaceae was found in two alcyonacean soft corals, the role of Spirochaetaceae remains unclear, as they have been found in diseased and healthy corals (Frias-Lopez et al. 2002; Sekar et al. 2008; Closek et al. 2014; Ainsworth et al. 2015). Therefore, functional prediction using FAPROTAX and the database of metagenomics of bacterial community-identified ecosystem functions were searched, although the database is not exhaustive. The putative roles of the identified common bacteria are summarized in Table 2. Recently, FAPROTAX and metagenomic sequencing have been developed as powerful tools for predicting metabolic and ecological relevant functions of bacterial communities from 16S rRNA gene sequencing data (Jung et al. 2021; Sansupa et al. 2021). In this study, the functional profiles revealed that differences in the bacterial communities between the two corals and ambient seawater can be attributed to nitrogen functions in coral bacteria and phototrophic functions in seawater.

Generally, distinct bacterial communities in corals may play functional roles in sustaining nutrient cycling and promoting coral acclimatization to environmental changes

Table 2 Putative contribution of the two alcyonacean soft coral-associated common bacteria

Bacterial influence	Putative roles and effect	<i>Sinularia</i> sp.	<i>Litophyton</i> sp.
Biogeochemical cycling	Nitrogen fixation	Spirochaetaceae (Kimes et al. 2010; Holm and Heidelberg 2016; Lawler et al. 2016; van de Water et al. 2016)	Spirochaetaceae (Kimes et al. 2010; Holm and Heidelberg 2016; Lawler et al. 2016; van de Water et al. 2016), Rhizobiales and Rhodospirillales (Lema et al. 2012; Wiegel 2015)
	DMSP degradation	<i>Endozoicomonas</i> (Tandon et al. 2020)	<i>Endozoicomonas</i> (Tandon et al. 2020)
	Carbon fixation	Spirochaetaceae (Baker et al. 2015)	Spirochaetaceae (Baker et al. 2015)
	Polysaccharide degradation	–	Cellvibrionaceae (Raina et al. 2009; Kellogg 2019)
Potential pathogen	Bleaching	–	Rhizobiales (Rosales et al. 2020)

(Reshef et al. 2006; Ziegler et al. 2017; Bang et al. 2018). The roles of bacterial associations in coral have been estimated in terms of their contribution to symbiont functions, including nitrogen (Rohwer et al. 2002; Lesser et al. 2004; Siboni et al. 2008; Lema et al. 2012; Rädicker et al. 2015), sulfur (Raina et al. 2013; Zhang et al. 2015), and carbon cycling (Baker et al. 2015; van de Water et al. 2018). In addition, they protect the host coral from potential pathogens in ambient seawater by producing antibiotics (Reshef et al. 2006; Ritchie 2006; Shnit-Orland and Kushmaro 2009). Indeed, previous studies reported that coral-associated microbial communities are involved in nitrogen fixation, carbon supply, antibiotic production, mucus recycling, and food supply in corals (Rohwer et al. 2002; Lesser et al. 2004; Wild et al. 2004; Ritchie 2006). For example, the functional characteristics of Spirochetes as coral associations have been studied in the context of nitrogen (Lilburn et al. 2001; Kimes et al. 2010) and carbon fixation (Baker et al. 2015), as well as in chemotactic responses to chemical stimulants (Charon et al. 1992). Recently, van de Water et al. (2016) reported that Spirochetes were abundant in red coral *Corallium rubrum* and may have a symbiotic function similar to nitrogen-fixing bacteria in tropical regions, indicating that Spirochaetaceae are involved in nitrogen fixation, carbon supply, antibiotic production, mucus recycling, and food supply in corals, which may have important roles in the holobiont health of the two soft corals.

Spirochaetaceae and *Endozoicomonas* species were found to be common bacterial associates in our two soft corals and are frequently found in shallow waters globally. They are considered as conserved “core” microbial associates (van de Water et al. 2016; Neave et al. 2017; Brener-Raffalli et al. 2018; Kellogg 2019). The core bacteria in coral holobiont may contribute to dimethylsulfoniopropionate (DMSP) breakdown in the sulfur cycle (Raina et al.

2009, 2010). However, this functional role in *Endozoicomonas* has been questioned because of the lack of genes related to DMSP metabolic pathways (Neave et al. 2017). Recently, Tandon et al. (2020) corroborated the functional role of *Endozoicomonas* in sulfur cycles by confirming the presence of DMSP degradation-related genes. A genomic study of *Endozoicomonas* revealed functional genes related to the transport of molecules that potentially influence the transfer of organic molecules between diverse animal hosts and the bacteria (Neave et al. 2014, 2017). Therefore, *Endozoicomonas* bacteria species play important roles in the coral sulfur cycle in the two alcyonacean soft corals. In addition to Spirochaetaceae and *Endozoicomonas*, *Litophyton* sp. also has other common bacterial associates, including Cellvibrionaceae, Rhizobiales, and Rhodospirillales. Cellvibrionaceae are marine bacteria found in surface seawater (Lucena et al. 2020) and as a symbiont in the gills of mollusks (Spring et al. 2015; Brito et al. 2018). Cellvibrionaceae has also been reported from other corals, such as *Porites astreoides* (Weber and Apprill 2020) and stony coral (Rosales et al. 2020), although they were not abundant. Most species in this family possess a large variety of polysaccharide-degrading enzymes (Spring et al. 2015; Lucena et al. 2020). The abundant presence of Cellvibrionaceae in *Litophyton* sp. may contribute to carbon cycling through polysaccharide degradation. Rhizobiales and Rhodospirillales are diazotrophic bacteria that contribute to nitrogen fixation (Lodwig et al. 2003; Lesser et al. 2018). These bacteria are frequently found in other corals in a high relative abundance (Lesser et al. 2018; Rosales et al. 2020). Widely known as diazotrophs, the functional role of Rhizobiales and Rhodospirillales in corals may be related to nitrogen cycling (Lema et al. 2012; Olson and Lesser 2013). In addition, Rhizobiales are potential causative agents for stony coral tissue loss disease (Rosales et al. 2020).

However, the Rhizobiales in *Litophyton* sp. may play functional roles in nitrogen cycling and not as disease-causing agents, as we observed no lesions in this study. Taken together, the dominant Spirochaetaceae bacteria may contribute to nitrogen and carbon cycling and *Endozoicomonas* to sulfur cycling in the coral host *Sinularia* sp. In addition, in *Litophyton* sp., Spirochaetaceae, Rhizobiales, and Rhodospirillales may play functional roles in nitrogen fixation, *Endozoicomonas* in sulfur cycling, and Cellvibrionaceae in carbon cycling.

We found that the microbial communities in ambient seawater significantly differ from those in the two corals, as they were dominated by *Pelagibacter*, *Prochlorococcus*, Rhodobacteraceae, and *Vibrio* bacteria. The bacteria in the ambient seawater of Chuuk, FSM, were similar to those in our previous studies (Suh et al. 2014). Generally, Proteobacteria, Bacteroidetes, and Cyanobacteria are common bacteria in the ocean environment, and the most common genera are *Pelagibacter*, *Roseovarius*, *Prochlorococcus*, and *Vibrio*. In particular, *Pelagibacter* and *Roseovarius* are most abundant in the oligotrophic ocean environment (Morris et al. 2002). *Prochlorococcus* is the smallest solitary Cyanobacteria and most abundant picophytoplankton in tropical waters (Partensky et al. 1999), dominating nutrient-depleted tropical regions (Biller et al. 2015). The occurrence of these oligotrophic bacterial associates matches well with the oligotrophic seawater conditions in Chuuk, suggesting that seawater-associated bacteria in Chuuk are widely distributed in the ocean environment, particularly in nutrient-depleted tropical regions. We also confirmed that *Vibrio* spp. is common in seawater. Generally, *Vibrio* species are widely distributed in aquatic environments and have been extensively studied in several coral diseases (Mouchka et al. 2010; Sweet et al. 2014). Therefore, its presence in the ambient seawater indicates that the corals can be infected by opportunistic outbreaks of these potential pathogenic bacteria following environmental changes caused by anthropogenic pollution and climate changes. However, these potential pathogenic bacteria are only rarely present in the corals, possibly because of the defense mechanisms used by corals (van de Water et al. 2018). In general, coral metabolites play a role in defending against infection by potential pathogenic bacteria via inhibition of the growth and attachment of pathogens (Gochfeld et al. 2015). In addition, the absence of *Vibrio* sp. in the corals' microbiome may result from the defense mechanism, such as shedding of the surface mucus layer, despite the presence of potentially pathogenic *Vibrio* sp. in ambient seawater. Taken together, the bacteria community may play an important role in the defense mechanisms in the two alcyonacean soft corals.

In conclusion, the two alcyonacean soft corals (*Litophyton* sp. and *Sinularia* sp.) from Chuuk, Micronesia,

have different microbial associates, and the most common bacterial associates are related to the biogeochemical functional roles of the coral holobiont. This study improves the understanding of the composition and functions of the bacterial communities in the two corals and provides a foundation for further investigating the health status of the two corals in response to environmental changes in the Chuuk State.

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Declarations

Conflict of interest There are no conflicts of interests to declare.

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