frontiers | Frontiers in Marine Science

ORIGINAL RESEARCH
published: 11 May 2022
doi: 10.3389/fmars.2022.864872

Composition and Potential Functions of Bacterial Communities Associated With *Aurelia* Polyps

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Jellyfish and their associated microbes form an ecological unit called the holobiont. Changes in the composition of dominant microbial assemblages may influence the environmental resilience and function of the holobionts. Differentiating the microbial communities from diverse jellyfish is essential for characterizing the functional contributions of microorganisms but has not been fully explored. In this study, based on 16S rRNA gene sequencing, we investigated the composition of microbial communities associated with two *Aurelia* polyp species (*Aurelia coerulea* and *Aurelia solida*) obtained from seven locations, which were maintained under the same environmental conditions. Sequence analysis showed that the genera *Sphingomonas*, *Phyllobacterium*, and *Ralstonia* were the most abundant members of the *Aurelia*-associated microbial communities and dominated the core microbiome of the *Aurelia* polyps in this study. Functional prediction revealed that chemoheterotrophy and aerobic chemoheterotrophy, based on the FAPROTAX dataset, were the primary functions of the associated microbes of *Aurelia* polyps. In addition, the comparison of microbial communities from different *Aurelia* polyp populations revealed interspecific instead of intraspecific variation, indicating a correlation between the composition of the symbiotic microbiota and genetic background of *Aurelia* polyps.

Keywords: microbiome, *Aurelia*, host genotypes, high-throughput sequencing, jellyfish blooms

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Specialty section:
This article was submitted to Microbial Symbioses, a section of the journal Frontiers in Marine Science

Received: *28 January 2022*
Accepted: *14 April 2022*
Published: *11 May 2022*

Citation:
Li Y, Hao W, Peng S, Sun T, Zhao J and Dong Z (2022) Composition and Potential Functions of Bacterial Communities Associated With Aurelia Polyps. Front. Mar. Sci. 9:864872. doi: 10.3389/fmars.2022.864872

INTRODUCTION

Interactions between organisms are factors determining the coexistence of species and maintenance of biodiversity. It is well established that animals act as hosts for multilineage consortia of microbial communities (i.e., bacteria, archaea, eukaryotes, and viruses) (McFall-Ngai et al., 2013), and microbial assemblages are an important factor in the regulation of host biology. Host-associated microbial communities exert nonnegligible forces on physiological metabolism regulation, immune function, and complex host behaviors (Rook et al., 2017). For instance, the gut microbial

communities of humans and insects assist in inhibiting the invasion and colonization of pathogenic bacteria and provide essential amino acids and vitamins for the physiological metabolism of hosts (Esser et al., 2019). The intestinal microbial communities of several insects significantly improve the environmental adaptability of hosts (Zhang and Leadbetter, 2012). Therefore, understanding the composition and structure of host-associated microbial communities is important in enabling insights into the functional contributions of microbes to hosts.

The colonization of a symbiotic consortium is influenced by many factors, such as the transmission of microbes from the external environment to hosts (Martinson et al., 2017), among-microbe interactions (Martinson et al., 2017), and ecological drift (Costello et al., 2012). Studies have found obvious dissimilarities between the microbiomes associated with aquatic animals (Stevens and Olson, 2015), amphibians (Walke et al., 2014), and terrestrial animals (Ren et al., 2017) from different regions. Additionally, the hosts act as ecological filters to selectively assemble specific species from the regional species pool based on their requirements and resources (Adair et al., 2020), causing the microbiomes of different species living in the same environment to be significantly different. Likewise, hosts of the same species often retain similarities in their microbial communities across various environmental conditions (Cheng et al., 2020). This retained microbiome, termed the "core microbiome", may play an important role in supporting the basic physio-chemical metabolism of hosts (Dietz et al., 2020).

As bacterial life had already existed for approximately three billion years when animals first evolved, microbe-animal interactions likely coevolved with the hosts over millions of years (Knoll, 2015). Based on their early appearance on the evolutionary scene, Cnidarians were likely among the first animals to establish associations with microorganisms (Bosch, 2013). The moon jellyfish *Aurelia* belongs to the phylum Cnidaria (class: Scyphozoa) and is the most common scyphozoan jellyfish in global coastal waters (Dong et al., 2018). The diphasic life cycle of *Aurelia* alternates between free-living pelagic medusa and sessile benthic polyp phases (Lucas, 2001). The composition and structure of microbial communities associated with the jellyfish *Aurelia* have been well investigated. Weiland-Braüuer et al. found that the composition of the microbiota associated with *Aurelia aurita* changed with the compartments of the adult medusae (mucus versus gastric cavity) and the life stages, particularly during the transition from the benthic to the pelagic stages (Weiland-Bräuer et al., 2015). Kramar et al. reported that the composition of *Aurelia solida*-associated microbial communities changed in relation to the period of the bloom (Kramar et al., 2019). Moreover, the presence of potentially pathogenic bacteria (i.e., *Vibrio* and Mycoplasmataceae) in the *Aurelia*-associated microbiome regarded *Aurelia* as a vector of pathogens (Tinta et al., 2019; Peng et al., 2021).

In the present study, we focused on the polyps of two moon jellyfish species, *Aurelia coerulea* and *A. solida*, which were incubated in the same environmental conditions with the same food source for 6 months to explore the relationship between polyps and symbiotic microbes. *Aurelia* polyp is a suitable model organism for the interaction between microbes and animals because of its simple body construction (basic immune and nervous systems), easy culture in the laboratory, high regeneration output, and short asexual reproduction cycle (Chiaverano and Graham, 2017). Based on the genetic and evolutionary relationships of the species, we hypothesized that the microbial community structure and functional characteristics of the species would vary under the same environmental conditions. In addition, the potential ecological functions of bacterial communities associated with *Aurelia* polyps were also discussed in the present study.

MATERIALS AND METHODS

Animal Collection and Culture

The polyps of *A. coerulea* and *A. solida* were originally obtained from the laboratory of Agustin Schiariti (Instituto Nacional de Investigación y Desarrollo Pesquero). *A. coerulea* were collected from the USA, China, Japan, Spain, and France and abbreviated as AC_{USA}, AC_{CHI}, AC_{JAP}, AC_{SPA} and AC_{FRA}, respectively. *A. solida* were collected from Israel and Slovenia and abbreviated as AS_{ISR} and AS_{SLO}, respectively, as detailed in **Table 1**. The polyps of the different *Aurelia* populations were kept separately in tanks. All polyps were cultured at 15°C in ambient fresh seawater with a salinity of 30 practical salinity units and kept on a day:night lighting rhythm of 12:12 h for 6 months of incubation time. Freshly hatched *Artemia salnia* were fed to the *Aurelia* polyps as their sole food source once every two days during the incubation period. Homogenization of cultivation conditions was performed to ensure stability and monovariability (i.e., host genotypes) of the symbiotic microbial communities of polyps from different *Aurelia* species and populations. The polyps were incubated in sterile seawater (0.22-μm filtered) baths for 1 day to clear the digestive system before DNA extraction. Species were identified based on 16S mtRNA pairwise sequence alignment technology.

TABLE 1 | The native locations of *Aurelia* polyps.

Sample	Location	Country	Species
AC_{FRA}	Roscoff	France	*Aurelia coerulea*
AC_{JAP}	Shirahama	Japan	*Aurelia coerulea*
AC_{CHI}	Fenghuang Lake	China	*Aurelia coerulea*
AC_{USA}	Monterery Bay Aquarium	USA	*Aurelia coerulea*
AC_{SPA}	Catalunya	Spain	*Aurelia coerulea*
AS_{ISR}	Red sea	Israel	*Aurelia solida*
AS_{SLO}	Koper port	Slovenia	*Aurelia solida*

DNA Extraction and 16S rRNA Gene Amplification Sequencing

Bacterial DNA of *Aurelia* polyps from 7 populations was isolated using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer's protocols. Each *Aurelia* population encompassed 5 replicates (10 polyps each) to yield read libraries sufficient for analysis. PCR amplification of the V4 hypervariable region of the 16S rRNA gene was performed with the primers 515F (5'-GTGCCAGCMG CCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTA AT-3') (Hugerth et al., 2014). All PCRs were carried out in 30 μL reactions with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs, USA), 0.2 μM forward and reverse primers, and approximately 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s and elongation at 72°C for 30 s, with a final elongation at 72°C for 5 min. PCR products were detected by electrophoresis in a 2% (w/v) agarose gel. PCR amplicons of each sample with bright bands were mixed in equal-density ratios and purified with a GeneJET™ Gel Extraction Kit (Thermo Scientific, USA). Sequencing libraries were generated using an Ion Plus Fragment Library Kit 48 Rxns (Thermo Scientific) following the manufacturer's recommendations. Then, the library concentration was assessed with a Qubit@ 2.0 Fluorometer (Thermo Scientific). The amplicon libraries were sequenced on the Ion S5™ XL platform at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

Sequence Assembly, Quality Control, and Taxonomic Assignment

Single-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Quality filtering of the raw reads was performed under specific filtering conditions to obtain high-quality clean reads according to the Cutadapt (v1.9.1, http://cutadapt.readthedocs.io/en/stable/) quality control process (Kechin et al., 2017). The reads were compared with the reference database (Silva 132 database, https://www.arb-silva.de/) (Quast et al., 2013) using the UCHIME algorithm (UCHIME algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) (Edgar et al., 2011) to detect chimera sequences and then the chimera sequences were removed to obtain clean reads (Haas et al., 2011). The singleton and non-target sequences were removed from the analysis.

Sequence analyses were performed using UPARSE software (UPARSE v7.0.100 http://drive5.com/uparse/) (Edgar, 2013). First, sequences with ≥ 97% similarity were assigned to the same operational taxonomic unit (OTU), and a representative sequence for each OTU was screened for further annotation. Then, the Silva 132 database (https://www.arb-silva.de/) (Quast et al., 2013) was used to annotate each representative sequence with taxonomic information based on the Mothur algorithm, and a representative sequence for each OTU was assigned to a taxonomic level using the RDP classifier (Edgar, 2013). Furthermore, multiple sequence alignments were conducted using MUSCLE software (version 3.8.31, http://www.drive5.com/muscle/) (Edgar, 2004) to study the phylogenetic relationships among different OTUs and the divergence in the dominant species among different samples (groups).

Definition of Rare, Conditionally Rare, Abundant, and Core Taxa

Microbial communities normally consist of a few abundant and many rare species (Easson et al., 2020). In this study, the thresholds for rare, conditionally rare, and abundant taxa were defined based on relative sequence abundance cutoffs, with reference to recent publications (Liu et al., 2017). "Rare taxa" were defined as OTUs with a relative sequence abundance < 0.01% in all samples. "Conditionally rare taxa" were defined as OTUs that were rare (relative sequence abundance < 0.01%) in some but not all samples and were never abundant (relative sequence abundance ≥ 1%). "Abundant taxa" were defined as the OTUs that did not fall in either the rare or conditionally rare categories. "Core taxa" was defined as the OTUs present in all *Aurelia* polyps in this study.

Statistical Analysis and Visualization

The OTU abundance data were normalized corresponding to the sample with the fewest sequences for further analysis of alpha diversity and beta diversity. The Shannon and CHAO1 diversity indices were calculated based on the normalized OTU matrix with QIIME (version 1.7.0) and visualized with the ggplot2 package of R software (version 2.15.3). Nonparametric Kruskal–Wallis tests, nonparametric Mann–Whitney U tests, and t tests were performed to identify significant differences in functional and relative bacterial abundance between different samples. The beta diversity of samples was calculated based on Bray–Curtis dissimilarity at the OTU level and used to perform principal coordinate analysis (PCoA), which was visualized with the ggplot2 and vegan packages of R software (version 2.15.3). In addition, Wilcoxon match-pair tests and permutational analysis of molecular variance (PERMANOVA) were constructed to test for significant differences in microbial alpha and beta diversity between *Aurelia* polyp species using the vegan package of R software (version 2.15.3). Furthermore, the relative sequence abundance of samples at diverse classification levels was visualized by the ggplot2 and reshape2 packages of R software (version 2.15.3). The relative sequence abundance of functional bacterial communities was calculated based on the FAPROTAX database and visualized by the pheatmap package of R software (version 2.15.3).

RESULTS

Bacterial Community Profiling

After filtering the raw data, a total of 2,456,681 clean reads from 35 *Aurelia* polyp samples were obtained, with an average sequence length of 372 bp. A total of 1,213 OTUs were present in the *Aurelia* polyp samples, clustered into 36 phyla, 49 classes, 107 orders, 190 families, and 400 genera.

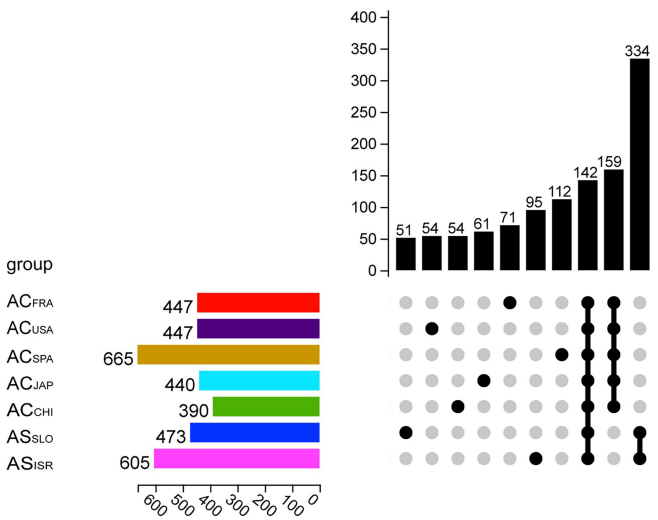

FIGURE 1 | Upset plot showing OTU information of different *Aurelia* polyp groups. The horizontal column on the left represents the average number of OTUs in the microbiome of each group (n = 5). Each single point in the matrix below indicates that the vertical column above it showd the number of OTUs unique to the corresponding *Aurelia* polyp group, while the points on the matrix grouped by a line indicate that the vertical column shows the number of OTUs common to those groups.

Variation in Microbial Composition

A total of 159 OTUs were present in all *A. coerulea* polyp groups, accounting for 82.06% (AC_{SPA})–98.58% (AC_{CHI}) of the total relative sequence abundance (**Figure 1**). In the *A. coerulea polyps*, 36 bacterial phyla, 48 classes, 98 orders, 179 families, and 366 genera were detected. The microbiomes of *A. solida* polyps contained 25 phyla, 40 classes, 84 orders, 145 families, and 257 genera. A total of 334 OTUs were shared between both groups of *A. solida* polyps and accounted for 96.64% and 98.97% of the total relative sequence abundance in AS_{ISR} and AS_{SLO}, respectively (**Figure 1**). Each *Aurelia* polyp group contained unique OTUs, which all had low relative sequence abundance (< 1%, **Figure 1**).

At the phylum and class levels, bacterial communities associated with both *Aurelia* polyp species were dominated by Proteobacteria (mainly classes Alphaproteobacteria and Gammaproteobacteria), Firmicutes (mainly class Clostridia), Bacteroidetes, Acidobacteira and Actinobacteria, together comprising 97.01% (AC_{SPA}) – 99.71% (AC_{JAP}) of the total richness (**Figure 2**). The relative abundances of the other 31 phyla in each *Aurelia* polyp population were <1%, together comprising 0.29% (AC_{JAP}) – 2.99% (AC_{SPA}) of the total richness (**Figure 2**). At the family and genus levels, the polyp microbiomes were dominated by Sphingomonadaceae (mainly the genera *Sphingomonas* and *Sphingobacterium*), Rhizobiaceae (mainly the genus *Phyllobacterium*) and Burkholderiaceae (mainly the genus *Ralstonia*), comprising 53.2% (AC_{SPA})–95.5% (AC_{CHI}) of the total bacterial abundance associated with *A. coerulea* and *A. solida* (**Figure 2**).

At the family level, unidentified Clostridiales (Mann–Whitney U test, $p = 0.039$) were significantly more abundant in *A. solida* than in *A. coerulea*, while Sphingomonadaceae (t test, $p = 0.038$) and Rhizobiaceae (t test, $p = 0.024$) were more abundant in *A. coerulea*. At the genus level, the *A. coerulea* polyps had significantly higher relative sequence abundance of the genera *Sphingomonas* (t test, $p = 0.043$) and *Phyllobacterium* (t test, $p = 0.023$) than *A. solida* polyps.

Variation in Microbial Diversity

To further reveal the inter- and intraspecific differences in the microbiomes of *Aurelia* polyps, the microbes of the two *Aurelia* polyp species were compared based on microbial alpha diversity and beta diversity, as detailed in **Figure 3**.

Regarding interspecific differences in microbial alpha diversity, *A. coerulea* polyps had a lower average Shannon index (2.998) than *A. solida* polyps (3.119), as well as a lower average CHAO1 index (219 and 237 in *A. coerulea* and *A. solida* polyps, respectively, **Figure 3**). However, no significant difference between the *A. coerulea* and *A. solida* groups was detected in either the Shannon indices or CHAO1 indices (Wilcoxon match-pairs test, $p = 0.5566$, **Figure 3**). Regarding intraspecific differences, AC_{CHI} had the highest average Shannon indices of the *A. coerulea* groups (3.367), followed by AC_{SPA}, AC_{FRA}, AC_{USA}, and AC_{JAP} had the lowest (2.635). The highest average CHAO1 index of the *A. coerulea* polyps was found in AC_{SPA} (285), followed by AC_{USA}, AC_{FRA} and AC_{JAP}, and the lowest was found in AC_{CHI} (186). Within the *A. solida* groups, AS_{ISR} had a higher average CHAO1 index (263) than AS_{SLO} (212) but had a lower mean Shannon index (2.449 in AS_{ISR} and 2.831 in AS_{SLO}). Nonetheless, no significant difference was found between the Shannon and CHAO1 indices of polyps in the *A. coerulea* and *A. solida* groups (Wilcoxon match-pairs test, $p > 0.05$, **Figure 3**).

In terms of the interspecific comparison, PCoA based on Bray–Curtis distance demonstrated a significant difference in microbial beta diversity between the microbiomes of the *A. coerulea* and *A. solida* polyps (PERMANOVA test, $p = 0.016$, **Figure 3**).

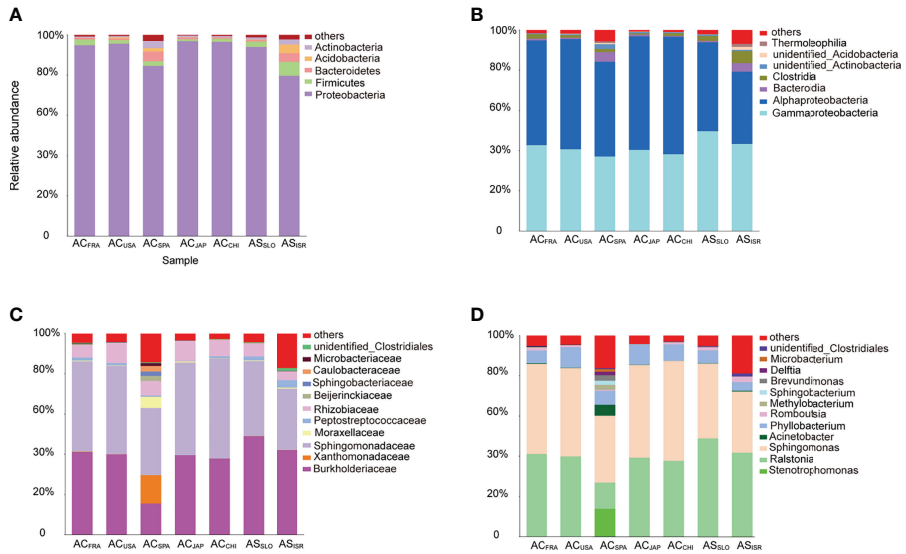

FIGURE 2 | Stacked bar plots of bacterial taxa with relative sequence abundance higher than 1% at the **(A)** phylum level, **(B)** class level, **(C)** family level, and **(D)** genus level in the *Aurelia*-associated bacterial communities. The less abundant taxa are grouped under "others".

Furthermore, significant differences were identified between the abundant and core microbial taxa associated with *A. coerulea* polyps and *A. solida* polyps (PERMANOVA test, $p = 0.044$ and $p = 0.03$, respectively), but there was no significant difference between the rare microbial taxa associated with the two species (PERMANOVA test, $p = 0.403$, **Table 2**). Similarity percentage analysis (Simper) illustrated that the genera *Stenotrophomonas*, *Ralstonia*, and *Sphingobacterium* were the main contributors to the difference in

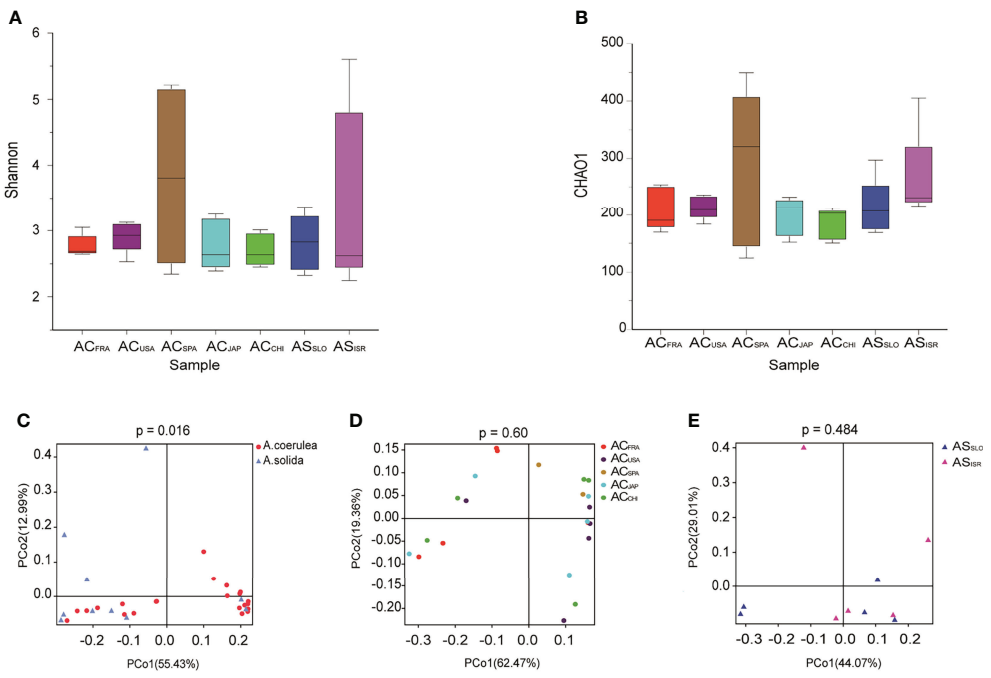

FIGURE 3 | Variation in microbial diversity of different polyp groups. Alpha diversity and richness are represented by Shannon indices **(A)** and CHAO1 **(B)** of *Aurelia* polyp microbiomes. PCoA (Principal coordinates analysis) visualization of bacterial beta - diversity of **(C)** all samples grouped by different types without outline, **(D)** *A. coerulea* polyps samples grouped by location without Outliers, and **(E)** *A. solida* polyp samples grouped by location. PERMANOVA tests were used to analyze significant differences between microbial diversity in different groups.

TABLE 2 | PERMANOVA tests of bacterial community structure of *Aurelia* polyps based on Bray–Curtis dissimilarities of OTU abundance.

Comparison group	Taxa types	OTU numbers	df	F	Pr (>F)
A. coerilea vs *A. solida*	core	20	34	2.698	0.030*
	rare	627	34	1.051	0.403
	conditionally rare	0	NA	NA	NA
	abundant	586	34	2.287	0.044*
A. coerulea	core	20	24	1.618	0.074
	rare	250	24	1.019	0.420
	conditionally rare	772	24	1.172	0.148
	abundant	38	24	1.426	0.087
A. solida	core	20	9	0.947	0.384
	rare	193	9	0.964	0.530
	conditionally rare	519	9	0.779	0.884
	abundant	32	9	0.850	0.438

"NA" represented not available. The values of $p < 0.05$ were marked with "".*

the microbial community between the two species, which contributed 26.8%, 26.1%, and 10.6% to the difference value, respectively (**Figure 4**). In terms of the intraspecific comparison, PCoA showed no significant intraspecific variation among the groups of *A. coerulea* polyps (PERMANOVA test, $p = 0.60$, **Figure 3**) or *A. solida* polyps (PERMANOVA test, $p = 0.484$, **Figure 3**). Furthermore, no significant difference was detected in the rare, conditionally rare, abundant taxa, or core taxa of the intraspecific groups (PERMANOVA test, $p > 0.05$, **Table 2**).

Core Bacterial Microbiome

In total, 20 OTUs were found in all *Aurelia* polyp samples. These 20 OTUs were defined as the "core microbiome" of *Aurelia* polyps, accounting for 57.19% (AC_{SPA})–96.86% (AC_{CHI}) of the total microbial richness (**Figure 5**). The genera *Sphingomonas*, *Ralstonia*, and *Phyllobacterium* were prominent in the core microbiome of the *Aurelia* polyps, accounting for 79.76% (AS_{ISR})–92.64% (AS_{SLO}) and 57.19% (AC_{SPA})–96.13% (AC_{CHI}) of the total core microbial richness in *A. coerulea* polyps and *A. solida* polyps, respectively (**Figure 5**). Notably, 6 OTU members of the core microbiome had significantly different abundances between *A. coerulea* and *A. solida* polyps: OTU_2 (Mann–Whitney U test, $p = 0.034$), OTU_1043 (Mann–Whitney U test, $p = 0.026$), and OTU_1244 (Mann–Whitney U test, $p = 0.037$) all belonging to the genus *Ralstonia*, were significantly more abundant in *A. solida* polyps than in *A. coerulea* polyps. However, OTU_3 (genus *Phyllobacterium*, t test, $p = 0.026$), OTU_1100 (genus *Sphingomonas*, t test, $p = 0.026$), and OTU_1287 (genus *Sphingomonas*, Mann–Whitney U test, $p = 0.011$) were significantly more abundant in *A. coerulea* polyps than in *A. solida* polyps.

Predicted Functions of *Aurelia*-Associated Microbiomes

Ecological functional annotation of polyp-associated microbial communities was conducted based on the FAPROTAX database. A total of 1,247 functional assignments for 8, 326 OTUs were

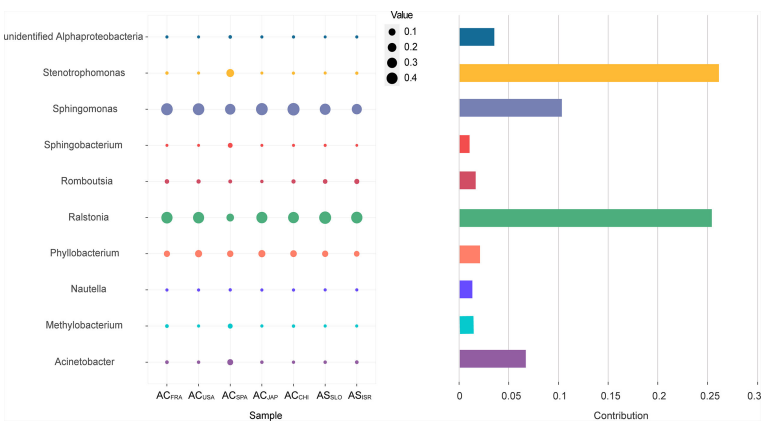

FIGURE 4 | The top 10 genera contributing to the interspecific difference in the microbial community. The vertical axis represents the species. The horizontal axis represents the samples. The bubble size represents the relative sequence abundance of the species. Contribution represents the contribution of the genus in the difference between the *A. coerulea* and *A. solida*.

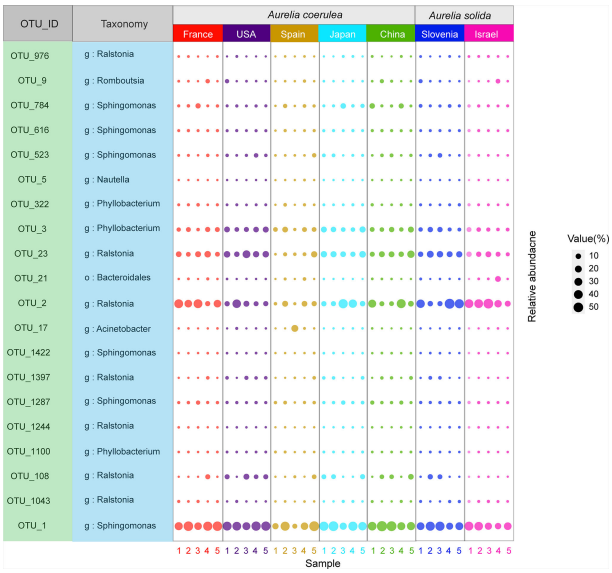

FIGURE 5 | Bubble plot of the core microbiome of *Aurelia* polyps, with bubble size indicating the relative sequence abundance of bacterial taxa.

obtained. OTUs without any functional annotation were excluded from the analysis. Functional group abundances in each sample were calculated as the cumulative relative sequence abundance of OTUs assigned to each functional group after normalizing by the cumulative abundances of OTUs associated with at least one function. Generally, chemoheterotrophy (42.7~70.0% of total OTUs) and anaerobic chemoheterotrophy (36.8~65.5% of total OTUs) were the primary functions in both *A. coerulea* and *A. solida* polyps, followed by functions related to the N cycle, such as nitrate reduction, nitrogen respiration, and denitrification (**Figure 6**). Furthermore, microbial communities associated with *A. coerulea* polyps had significantly higher abundances of

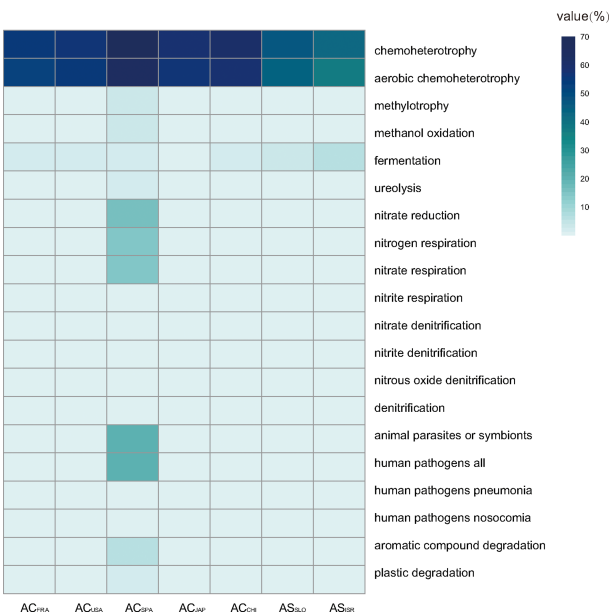

FIGURE 6 | Heatmap matrix on the top 20 functional groups with the highest cumulative OTU relative sequence abundance in *Aurelia* polyp samples based on analysis of FAPROTAX.

chemoheterotrophic bacterial taxa than those associated with *A. solida* polyps (Mann–Whitney U test, $p = 0.004$).

DISCUSSION

Composition of Bacterial Communities Associated With *Aurelia* Polyps

Bacterial communities associated with hosts are shaped by host selection and environmental conditions (Gould et al., 2018). Previous researchers have highlighted environmental factors shaping the structure of microbial communities in hosts such as coral (Zhang et al., 2014; Osman et al., 2020), sponge (Zhang et al., 2014; Easson et al., 2020), and sea anemone (Mortzfeld et al., 2016; Morelan et al., 2019). In this study, despite long-term cultivation in the same environment and feed, we found interspecific variation in beta diversity among *Aurelia* polyps and intraspecific similarity among *Aurelia* polyps. Therefore, our results suggest that the genotype of *Aurelia* polyps is an important factor in the structure of the symbiotic bacterial communities. Weiland-Bräuer et al. concluded that there were large differences in the microbial compositions of *Aurelia* polyps between North Sea/Roscoff and Baltic Sea subpopulations, similar to the present study (Weiland-Bräuer et al., 2015).

Animal hosts are known to be able to modulate their associated microbiome in terms of genotype regulation, such as *via* genetic expression or innate immune response activation (Borges, 2017). For example, in research using hydras as model animals, the variation in antimicrobial peptide genes in different species led to variation in the microbiomes among those species (Bosch, 2013). As part of the evolutionarily ancient marine phylum Cnidaria, *Aurelia* jellyfish may influence the structure of their microbiomes by producing various proteins or antimicrobial peptides *via* gene expression, which interferes with bacterial quorum sensing (selecting microbes from the environment or promoting the colonization and enrichment of targeted microbes) and inhibits bacterial colonization (Weiland-Bräuer et al., 2019). In addition, vertical transmission of bacterial communities from parents to offspring could be another reason for the differences between the bacterial communities of *A. coerulea* and *A. solida* polyps.

The Core Microbiota of *Aurelia* Polyps

Hosts can conditionally shape the structure of microbial communities by interacting to maintain an overall stable state, especially cnidarians (Ainsworth et al., 2015). In the present study, the families Sphingomonadaceae (i.e., genera *Sphingomonas*), Rhizobiaceae (i.e., genera *Phyllobacterium*), and Burkholderiaceae (i.e., genera *Ralstonia*) were detected in all *Aurelia* polyp samples with high relative sequence abundances. Similarly, Weiland-Bräuer et al. proposed that the relative sequence abundance of Sphingomonadaceae was high in *Aurelia* collected in Roscoff, the North Sea, and the Baltic Sea throughout the life stages and decreased with the transition of *Aurelia* polyps to strobila to ephyra to juvenile medusae (Weiland-Bräuer et al., 2015). Peng et al. also detected a high abundance of Sphingomonadaceae and Burkholderiaceae in the associated bacterial community of wild *Aurelia* medusae (Peng et al., 2021). Several studies have suggested that cnidarian-associated bacterial communities are potentially involved in functional interactions and play a positive role in host-environment adaptation (Ziegler et al., 2017; Podell et al., 2020; Roach et al., 2020; Tong et al., 2020). Hence, the family Sphingomonadaceae may be a member of the core microbial communities associated with *Aurelia* populations worldwide. Further studies on the function of Sphingomonadaceae would help to gain insight into the impact of microorganisms on hosts.

Bacteria are functional components of most marine multicellular organisms, especially cnidarians (Morelan et al., 2019). Previous studies have agreed that the core microbiome was closely related to the health, growth, environmental adaptability, and production of the hosts (Bosch, 2013; Ainsworth et al., 2015; Brown et al., 2017; Weigel and Erwin, 2017; Weiland-Bräuer et al., 2020). In our study, a total of 20 core OTUs were identified in the bacterial communities of the scyphozoan body parts, predominantly the genera *Sphingomonas*, *Ralstonia*, and *Phyllobacterium*. The genus *Sphingomonas* (family Sphingomonadaceae), a dibenzofuran- and dibenzodioxin-degrading bacterium with potentially interesting properties for bioaugmentation of contaminated sites (Roggo et al., 2013), was the most abundant taxon in the microbiomes of all *Aurelia* polyps. Furthermore, Feng et al. (2017) identified that certain *Sphingomonas* spp. were potentially able to degrade chlorpyrifos, indicating that *Sphingomonas* could enhance the survival rate of *Aurelia* polyps in contaminated environments. The genus *Phyllobacterium*, which can produce exopolysaccharide (Li et al., 2017), was also dominant in *Aurelia* polyps. The exopolysaccharide could form protective barriers between cells and the environment, regulate cell growth and senescence, and affect cell division and differentiation (Flores-Felix et al., 2018), suggesting that the genus *Phyllobacterium* may be closely related to the transformation process between *Aurelia* life stages and host-environment adaptation.

Potential Functions of Bacterial Communities Associated With *Aurelia* Polyps

Functional prediction is the first step in determining how microbiome biochemical processes affect the ecological functions of hosts (Ross et al., 2018). FAPROTAX is a promising tool for predicting ecologically relevant functions of bacterial and archaeal taxa derived from 16S rRNA amplicon sequencing (Louca et al., 2016). In this study, according to the FAPROTAX database, aerobic chemoheterotrophy in relation to C cycling was the primary function of *Aurelia* polyp-associated bacteria associated with numerous bacteria, such as Sphingomonadaceae and Microbacteriaceae. This demonstrated that *Aurelia* polyps were the main foundation of essential nutrients to support the microbial growth of the associated bacterial communities. Ross et al. (2018) reported that *Aurelia* medusae are potential bacterial vectors and may harm aquaculture activities, as their microbiomes harbor potential fish pathogens. Similarly, in this study, some potential animal pathogens (i.e., Coxiellaceae) or parasites (i.e.,

Bdellovibrionaceae and Haliangiaceae) were present in both *A. coerulea* and *A. solida* polyps. Moreover, nitrification and denitrification, two functions related to the N cycle that have been reported to be involved in waste removal in host coral (Beman et al., 2007), were potential functions that were abundant in both *Aurelia* polyp species. Hence, these bacteria may have similar N cycle-related functions in *Aurelia* polyps, assisting with the adaptation of polyps to nitrate stress in the ambient environment.

Unique Microbes Associated With *Aurelia* Polyps

Each intraspecific group had unique bacterial taxa, which accounted for less than 1% of the relative sequence abundance. These unique bacteria are often overlooked because of their low abundance but may be critical to the functional maintenance of hosts (Shade et al., 2014). For example, *Actinobacteria* sp. and *Ralstonia* sp., located in zooxanthellae and coral intestinal epithelial cells, have low abundance and perform a vital role in the metabolism of the coral *Acropora granulosa* (Ainsworth et al., 2015). Unlike the relative stability of the core microbiome in *Aurelia* polyps (**Figure 1**; **Table 2**), the unique microbes were less controlled and more sensitive to environmental variation. We speculated that unique microbes were preserved by the hosts from the native environment and potentially contributed to the environmental resilience of *Aurelia* polyps. Further study on unique microbes could comprehensively elucidate the mechanism of host selection for microbes in the environment.

CONCLUSION

In this study, we investigated the bacterial communities associated with two moon jellyfish species (*A. coerulea* and *A. solida*) obtained from seven locations and incubated under the same environmental conditions. We found that the genera *Sphingomonas*, *Phyllobacterium*, and *Ralstonia* dominated the core microbial communities of the *Aurelia* polyps. These *Aurelia*-associated microbes may potentially play an important role in host's fitness and host's environmental adaptation by promoting nutrient uptake. Furthermore, the comparison of microbial communities from different *Aurelia* polyp populations revealed interspecific variation, indicating a correlation between the composition of the associated bacterial community and genetic background of *Aurelia* polyps.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/bioproject/ PRJNA799918.

AUTHOR CONTRIBUTIONS

YL: Data analysis, writing - original draft, writing - review & editing. WH: writing - review & editing. SP: Data analysis. TS: Sample process. JZ: Conceptualization, funding acquisition. ZD: Conceptualization, investigation, data analysis, funding acquisition, writing - review & editing. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by grants from the NSFC-Shandong Joint Fund (No. U2106208), National Natural Science Foundation of China (No. 41876138), the National Key Research and Development Program of China (2018YFC1406501), and the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA23050301).

ACKNOWLEDGEMENTS

We wish to thank Marine Biology Station Piran, National Institute of Biology, for making the collection possible, and to Dr. Agustin Schiariti from the Instituto Nacional de Investigación y Desarrollo Pesquero for keeping the cultures and providing us with *Aurelia* polyps.

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