Laboratory-Cultured Strains of the Sea Anemone *Exaiptasia* Reveal Distinct Bacterial Communities

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Exaiptasia is a laboratory sea anemone model system for stony corals. Two clonal strains are commonly used, referred to as H2 and CC7, that originate from two genetically distinct lineages and that differ in their *Symbiodinium* specificity. However, little is known about their other microbial associations. Here, we examined and compared the taxonomic composition of the bacterial assemblages of these two symbiotic *Exaiptasia* strains, both of which have been cultured in the laboratory long-term under identical conditions. We found distinct bacterial microbiota for each strain, indicating the presence of host-specific microbial consortia. Putative differences in the bacterial functional profiles (i.e., enrichment and depletion of various metabolic processes) based on taxonomic inference were also detected, further suggesting functional differences of the microbiomes associated with these lineages. Our study contributes to the current knowledge of the *Exaiptasia* holobiont by comparing the bacterial diversity of two commonly used strains as models for coral research.

Keywords: 16S rRNA gene, coral, core microbiome, host-specific, model organism

INTRODUCTION

Microbes are an essential part of life; they interact with their hosts in such a way that it not only affects their fitness and survival but also defines them as a unique biological entity (McFall-Ngai et al., 2013). Increasing research has focused on understanding these interactions, looking at organisms that range from basal metazoans like *Hydra* sp. (Fraune and Bosch, 2007) to humans (Ursell et al., 2012). Contribution of the microbiota to the evolution, development, and molecular and physiological functioning of animals has been studied for different groups (McFall-Ngai et al., 2013; Rosenberg and Zilber-Rosenberg, 2013); but particularly for corals (Thompson et al., 2015; Bourne et al., 2016), as they form the basis of one of the most productive and diverse ecosystems on earth.

Much work has been done on identifying coral-associated bacteria that can cause or prevent bleaching (Rosenberg et al., 2009) and diseases (Rosenberg et al., 2007; Bourne et al., 2009), highlighting how the coral microbiome can disrupt the holobiont equilibrium. Ultimately, the health and persistence of coral reef ecosystems depends on the symbiotic partnership between the coral animal, its photosynthetic dinoflagellates and a specific consortium of microbes (Bourne et al., 2016). A more comprehensive approach that takes into account the whole coral holobiont is therefore critical to understand the adaptation and resilience of these organisms (Voolstra et al., 2015). However, working with corals is challenging; they are difficult to culture in aquariums and *in situ* studies are not always feasible (Voolstra, 2013).

Advancements in molecular, cell, and developmental biology have been achieved through the establishment of different model systems (Davis, 2004), from which the sea anemone *Exaiptasia* (Grajales and Rodríguez, 2014) has been used as a laboratory model organism for corals (Weis et al., 2008). *Exaiptasia* lives in a symbiotic relationship with dinoflagellates of the genus *Symbiodinium*, but unlike scleractinian corals, it can also live in a symbiont-free (i.e., aposymbiotic) state. Further, it grows fast and propagates easily; its undemanding culturing conditions have facilitated coral research for more than 30 years now (Muller-Parker, 1984; Weis et al., 2008; Voolstra, 2013).

Recently, two genetically distinct populations of *Exaiptasia* have been characterized, a globally distributed lineage (found in Japan, Mexico, Hawaii, the Mediterranean, and Australia) and a local lineage (found in the United States South Atlantic coast as defined in Thornhill et al., 2013), that harbor different *Symbiodinium* species (Thornhill et al., 2013; Grawunder et al., 2015). This specificity and stability of the *Exaiptasia-Symbiodinium* association has been suggested to be the result of coevolutionary processes (Thornhill et al., 2013; Voolstra, 2013), raising the question of how specific the relationship with their microbes is. Microbial community relationships can be highly conserved as observed in the cnidarian *Hydra* sp., where species-specific bacterial phylotypes were maintained over long periods of time and across different environments (Fraune and Bosch, 2007). Bacterial associations of *Exaiptasia* have, however, not been examined until recently (Röthig et al., 2016a), showing that despite its simple body organization, this sea anemone can be associated with diverse bacterial communities. Here, we characterized the bacterial assemblage of symbiotic *Exaiptasia* H2 (originally isolated from Hawaii, USA) and compared it to the previously published microbiome of *Exaiptasia* CC7 (originally obtained from North Carolina, USA; Röthig et al., 2016a), which belong to the "global" and "local" lineages, respectively (Grawunder et al., 2015). Knowledge on the bacterial composition of *Exaiptasia* will be vital for its establishment as a model system for coral research.

MATERIALS AND METHODS

Exaiptasia Culture Conditions

Symbiotic *Exaiptasia* of the clonal strain H2 (Xiang et al., 2013) were cultured in clear polycarbonate containers (2 L capacity; Cambro Camwear, USA) filled with autoclaved natural seawater collected from the Red Sea (∼39 PSU salinity) as described in Röthig et al. (2016a). Tanks were kept in Intellus Control System Incubators (Model I-22LLVL, Percival Scientific, USA) at 25°C on a diurnal 12 h light: 12 h dark cycle under white-light (20–40 µmol photons m^{-2} s^{-1} of photosynthetically active radiation). Anemones were fed 3 times per week with freshly hatched *Artemia* brine shrimps, and seawater in the tanks was stagnant and exchanged 1–2 times per week.

DNA Isolation and 16S rRNA Gene Sequencing

Five anemones and one water sample were processed as described in Röthig et al. (2016a). Briefly, polyps of ∼1.0 cm

size were collected and transferred into 1.5 mL microtubes, and remaining water was carefully removed. DNA was extracted using a spin column DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Animals were not fed 1 week prior to the DNA extraction in order to avoid potential *Artemia* contamination. For the water sample, 500 mL water was collected from the tank in which the animals were reared and filtered through a 0.22 µm Durapore PVDF filter (Millipore, Billerica, MA, USA). The filter was then cut in strips using a sterile razorblade, transferred into a 1.5 mL microtube with 400 µL AP1 buffer (DNease Plant Mini Kit, Qiagen, Hilden, Germany) and incubated on a rotating wheel for 20 min before subsequent DNA extraction procedures. DNA concentrations of all samples were quantified on a NanoDrop 2,000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Primers 784F and 1061R (Andersson et al., 2008) were used to amplify the V5–V6 hypervariable regions of the 16S rRNA gene. PCRs were performed in triplicate per sample (using 15–30 ng of DNA from each anemone sample and ∼5 ng DNA of the water sample) with a Qiagen Multiplex PCR Kit (Qiagen, Hilden, Germany) and a final primer concentration of 0.5 µM for a final volume of 15 µL. Thermal profiles consisted of an initial activation step of 15 min at 95°C, followed by 27 cycles each of 30 s at 95°C, 90 s at 55°C, and 30 s at 72°C, and a final extension step of 10 min at 72°C. Amplified PCR products were pooled for each sample and subsequently cleaned using the Agencourt AMPure XP magnetic bead system (BeckmanCoulter, Indianapolis IN, USA), indexed with Nextera XT sequencing adapters (Illumina, San Diego, CA, USA), and quality checked on a BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). The final library was sequenced at 8 pM with 10% phiX on the Illumina MiSeq, 2 × 300 bp paired-end v3 chemistry according to the manufacturer's instructions.

Bacterial Community Analysis

Sequencing data was analyzed using the software mothur (v1.36.1; Schloss et al., 2009). To allow direct comparisons between the microbiomes of *Exaiptasia* H2 (this study) and *Exaiptasia* CC7 (Röthig et al., 2016a), data obtained in this study was processed following the same procedure described by Röthig et al. (2016a) for the previously published CC7 data. Sequences were demultiplexed, quality trimmed, preclustered (2 bp difference; Huse et al., 2010), and aligned against the SILVA database (release 119; Pruesse et al., 2007). Chimeric sequences were removed using UCHIME (Edgar et al., 2011), and sequences assigned to chloroplasts, mitochondria, archaea, eukaryotes, and other unwanted sequences, were excluded. Phylogenetically classified sequences (Greengenes database release gg_13_8_99; bootstrap = 60; McDonald et al., 2012) were then used to evaluate the bacterial taxonomic composition of samples and plotted with Krona (Ondov et al., 2011). For further analyses, we subsampled to 20,816 sequences per sample, as this was the total number of sequences obtained for the water sample. Sequences were then clustered into Operational Taxonomic Units (OTUs) using a 97% similarity cutoff. Alpha diversity indices and principal coordinate analysis (PCoA) were performed as implemented in mothur. A

list of all determined OTUs and their distribution over samples was created using the commands *make.shared*, *classify.OTU*, and *get.OTUrep*. Based on this we obtained a putative core microbiome (i.e., OTUs that were present in 100% of the *Exaiptasia* samples). For each of these OTUs, the representative sequence was BLASTed against GenBank nr database to identify previous occurrences of identical or highly similar bacterial sequences. Furthermore, shared OTUs between symbiotic H2 and symbiotic CC7 core microbiomes (Röthig et al., 2016a) were identified. Differences in the average relative abundance of these OTUs were assessed using *t*-tests.

Taxonomy-based functional profiling of the bacterial communities of H2 and CC7 was done using METAGENassist (Arndt et al., 2012), an automated taxonomic-to-phenotypic mapping package. Input files were created using the relative abundances of all OTUs present in both core microbiomes combined. These were further filtered based on interquartile range (Hackstadt and Hess, 2009) and normalized over samples by sum and over taxa by Pareto scaling. Data was then analyzed for "metabolism by phenotype" using Spearman distance measure and average clustering algorithm to visualize the results in a heatmap.

RESULTS

The Microbiome of *Exaiptasia* H2

We sequenced 16S rRNA gene amplicon libraries from five *Exaiptasia* H2 anemones and one water sample, yielding a total of 788,277 sequences. Quality filtering steps removed 423,408 sequences, while subsequent chimera detection removed further 23,675 sequences. Lastly, removal of undesirable sequences matching non-bacterial targets (18,311) resulted in a final set of 322,883 sequences with an average length of 292 bp that annotated to bacteria, and were used for further analyses. Rarefaction analysis showed that a subsample of 20,816 sequences from each sample was sufficient to account for the majority of bacterial diversity (Figure S1). Clustering at the 97% similarity level resulted in 211 OTUs (Table S1), from which 176 were associated with *Exaiptasia* and 106 with water. From these, 105 and 35 OTUs were exclusively found in the anemone and water samples, respectively. A specific microbiome was observed for *Exaiptasia* H2 (Figure S2), showing a clear separation from the water sample (Figure S3).

At least 10 bacterial phyla, 18 classes, and 41 orders were recovered from the symbiotic H2 microbiome (**Figure 1**). Of these phyla, Proteobacteria (52.6%) and Bacteroidetes (36.6%) were the most dominant. Actinobacteria comprised only 10.2%, whereas the other phyla were present at <1% of the bacterial community. Moreover, within Proteobacteria, Gammaproteobacteria (57.3%) was the most represented class followed by Alphaproteobacteria (28.3%) and Deltaproteobacteria (13.5%). At a finer scale, Flavobacteriaceae (35.0%) and Alteromonadaceae (15.2%) were the most abundant families, contributing to half of the total bacterial diversity. In contrast, families Rhodobacteraceae (order Rhodobacterales, class Alphaproteobacteria) and Vibrionaceae (order Vibrionales, class Gammaproteobacteria) dominated the water sample with 34.6 and 32.1%, respectively. We further identified a core microbiome that consisted of 49 OTUs (**Table 1**, Table S1), and that included the 10 most abundant bacterial taxa, accounting for >80% of all OTU sequence counts. These bacteria represented abundant and rare members of the *Exaiptasia* H2 microbiome, with mean abundances that range from 2 to 7,309 counts. *Kordia* sp. (Flavobacteriaceae) was the most dominant OTU (34%) across samples.

Distinct Bacterial Communities in *Exaiptasia* H2 and CC7

We compared the taxonomic composition of the microbial assemblages of symbiotic *Exaiptasia* H2 and CC7 strains infected with the *Symbiodinium* strain SSB01 that was previously isolated from H2, thus allowing for a comparison of the two *Exaiptasia* strains without potential symbiont effects (Xiang et al., 2013; Röthig et al., 2016a). We examined the diversity and relative abundance of bacterial OTUs and found significant differences (*t*-test, $p < 0.05$ post Benjamini–Hochberg correction). More than 17 phyla were represented in CC7 (Röthig et al., 2016a), depicting a more taxonomically diverse microbiome than the 10 phyla detected in H2. Average abundances of Proteobacteria (69.7%), Actinobacteria (25.9%), and Firmicutes (1.5%) were notably higher in CC7 than H2, with 52.6, 10.2, and 0.1%, respectively. In contrast, Bacteroidetes (1.63%) showed more than a 20-fold decrease in CC7 compared to H2. A detailed analysis of the Proteobacteria assembly in CC7 showed higher and lower abundances in Betaproteobacteria (8.9%) and Alphaproteobacteria (7.2%), respectively. Furthermore, Pseudomonadaceae (19.7%) was the most abundant family in CC7, yet present at only very low numbers in H2 (<0.1%). On the OTU level, the bacterial diversity of CC7 and H2 was also different (**Table 2**). The average number of OTUs (118), and the inverse Simpson (11.78) and Simpson's evenness (0.10) indices were higher in CC7 than H2 (96.00, 5.87, and 0.06, respectively). Similarly, the Chao1 estimator of species richness was lower in H2 (114.76) than in CC7 (124.20). Comparisons amongst core microbiomes revealed 13 OTUs in common (**Table 3**), of which only four were observed to also be significantly more abundant in CC7 than H2 (*t*-test, $p < 0.05$ post Benjamini–Hochberg correction). The occurrence of *Propionibacterium acnes* (Actinobacteria) and *Staphylococcus epidermis* (Firmicutes) bacteria in both H2 and CC7 core microbiomes called for precaution when interpreting results, as these taxa are known for being common contaminants (e.g., human skin-associated organisms, contamination of DNA extraction kits, and other laboratory reagents) in sequenced-based microbiome studies (Salter et al., 2014). For this reason, and due to their low abundance, we removed these bacterial taxa from further taxonomy-based functional analysis.

Taxonomy-Based Functional Profiling of Bacterial Communities in *Exaiptasia*

We also characterized the functional potential of the bacterial communities of *Exaiptasia* by examining the enrichment and depletion of different metabolic processes based on phylogenetic inference using METAGENassist (Arndt et al., 2012). Putative functions were inferred from the taxonomic composition

FIGURE 1 | Taxonomic community composition of bacteria associated with *Exaiptasia* H2. The inner ring represents different phyla, and as the plot progresses outwards, there is an increasing taxonomic resolution for each ring (i.e., class, order, family, respectively). Only OTUs with relative abundance >0.1% are reported.

underlying the microbiome of H2 and CC7. A total of 84 OTUs were assigned, mapped and condensed into 73 distinct functional taxa. This analysis showed that the bacterial community associated with *Exaiptasia* H2 covered a range of nutrient cycling pathways, in particular sulfur ("sulfate reduction," "sulfide oxidation") and nitrogen ("nitrogen fixation," "nitrite reduction," "ammonia oxidation") cycling. Further, other metabolic processes involving the breakdown of complex organic molecules were observed ("xylan," "chitin and chlorophenol degraders," and "sugar fermenters").

TABLE 1 | *Exaiptasia* H2 49 core microbial OTUs.

OTU	Average abundance (%)	Taxonomy (bootstrap value)
OTU001	7,309 (36.95)	*Kordia* sp.
OTU002	1,780 (9.00)	Unclassified Rhodobacteraceae
OTU003	1,834 (9.27)	*Brevibacterium casei*
OTU004	1,812 (9.16)	Unclassified Alteromonadaceae
OTU005	745 (3.77)	*Photobacterium angustum* (60)
OTU006	1,230 (6.22)	*Plesiocystis* sp.
OTU007	15 (0.07)	*Nautella italica* (86)
OTU008	1,105 (5.59)	*Alteromonas* sp.
OTU009	546 (2.76)	Unclassified Oleiphilaceae
OTU010	471 (2.38)	*Thalassobius meditarraneus* (77)
OTU011	395 (2.00)	*Thalassobaculum litoreum*
OTU012	297 (1.50)	Unclassified Alteromonadales
OTU013	349 (1.77)	Unclassified Ectothiorhodospiraceae
OTU014	216 (1.09)	*BD7-3* sp.
OTU015	91 (0.46)	Unclassified Flavobacteriaceae
OTU018	230 (1.16)	*Oceanospirillum* sp.
OTU020	177 (0.90)	Unclassified Spirobacillales
OTU022	152 (0.77)	*Brachybacterium* sp.
OTU023	125 (0.63)	Unclassified Oceanospirillaceae
OTU024	99 (0.50)	Unclassified Hyphomonadaceae
OTU025	99 (0.50)	*Dietzia* sp.
OTU026	71 (0.36)	*BD2-13* sp.
OTU028	83 (0.42)	Unclassified Rhodospirillaceae
OTU029	12 (0.06)	*Roseivirga* sp.
OTU030	40 (0.20)	*Pseudoalteromonas porphyrae* (89)
OTU031	15 (0.08)	*Francisella* sp.
OTU032	61 (0.31)	Unclassified Alphaproteobacteria
OTU033	56 (0.28)	Unclassified Gammaproteobacteria
OTU034	60 (0.30)	Unclassified Proteobacteria
OTU035	54 (0.27)	Unclassified Saprospiraceae
OTU038	8 (0.04)	*Methylophaga* sp.
OTU040	39 (0.20)	unclassified Cryomorphaceae
OTU044	29 (0.15)	*Bacteriovorax* sp.
OTU045	30 (0.15)	*Candidatus Rhabdochlamydia* sp.
OTU046	12 (0.06)	Unclassified Rhizobiales
OTU048	21 (0.11)	*Orientia chuto* (94)
OTU049	21 (0.11)	*Rhodovulum* sp.
OTU052	16 (0.08)	*Roseovarius* sp.
OTU054	15 (0.07)	Unclassified Gammaproteobacteria
OTU055	13 (0.07)	*ZD0117* sp.
OTU057	3 (0.02)	*Cohaesibacter gelatinilyticus*
OTU061	5 (0.03)	Unclassified Flavobacteriaceae
OTU063	7 (0.04)	*Pelomonas puraquae*
OTU065	6 (0.03)	Unclassified Piscirickettsiaceae
OTU066	6 (0.03)	*Marinobacter zhejiangensis*
OTU067	6 (0.03)	*Brevibacterium aureum*
OTU068	6 (0.03)	Unclassified Alteromonadaceae (66)
OTU072	4 (0.02)	*Pseudoalteromonas ruthenica*
OTU092	2 (0.01)	unclassified Spirobacillales

Only OTUs present in all anemones were considered members of the core microbiome. Relative abundances are also shown in percent. Lowest taxonomic level from Greengenes classification is shown. Bootstrap value is shown if < 100.

TABLE 2 | Summary of alpha diversity indices (mean ± SD) of the microbial communities associated with *Exaiptasia* H2 and CC7 strains.

	H2[*]	CC7[§]
Coverage	1.00 (0.00)	1.00 (0.00)
Observed OTUs	96.00 (12.19)	118.20 (9.34)
Chao1	114.76 (17.25)	124.20 (11.61)
Shannon	2.35 (0.21)	
Simpson	0.19 (0.05)	
Inverse simpson	5.87 (1.95)	11.78 (1.12)
Shannon's evenness	0.52 (0.04)	
Simpson's evenness	0.06 (0.01)	0.10 (0.01)

[*]*This study, n = 5, subsampled to 20,816 sequences.*
[§]*Röthig et al. (2016a), n = 5, subsampled to 11,000 sequences.*

TABLE 3 | Differences in the average relative abundance in percent (±SD) of shared bacterial taxa (OTUs) between *Exaiptasia* H2 and CC7 core microbiomes.

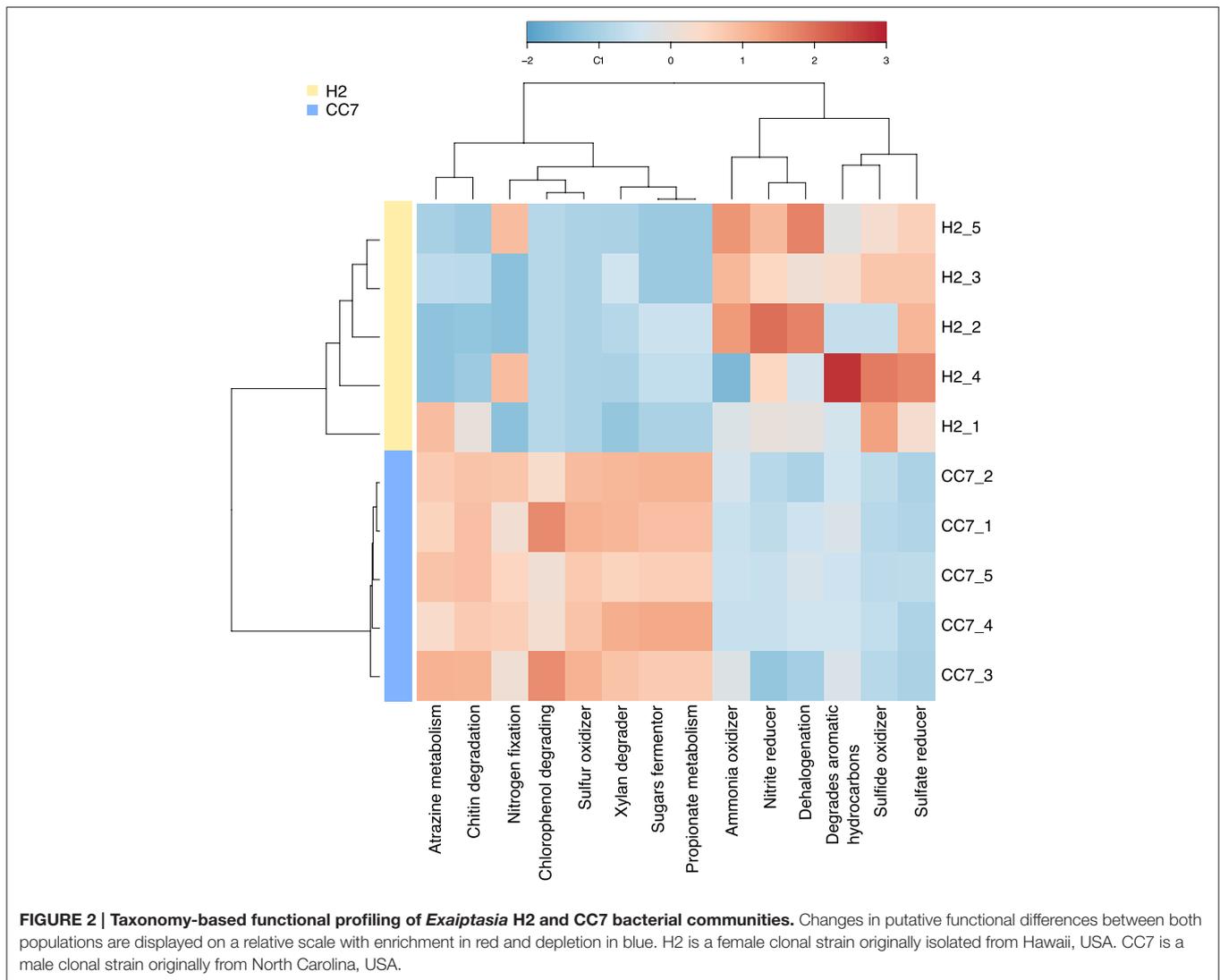| Taxa	H2	CC7	*p*-value
Alteromonas sp.	5.31 (4.78)	11.57 (4.08)	0.106
Bacteriovorax sp.	0.14 (0.13)	0.24 (0.11)	0.228
Brachybacterium sp.	0.73 (0.40)	12.11 (1.90)	0.064
Brevibacterium aureum	0.03 (0.03)	1.38 (0.46)	*0.002*
Candidatus Rhabdochlamydia sp.	0.15 (0.17)	0.82 (0.89)	0.186
Dietzia sp.	0.48 (0.29)	8.13 (1.24)	*0.002*
Francisella sp.	0.07 (0.10)	2.76 (1.20)	*0.004*
Nautella italica	0.07 (0.02)	0.63 (0.74)	0.195
Oceanospirillum sp.	1.10 (0.67)	1.06 (0.93)	0.939
Pelomonas puraquae	0.03 (0.03)	7.07 (1.00)	*0.002*
Photobacterium angustum	3.58 (6.75)	30.18 (26.54)	0.106
Plesiocystis sp.	5.91 (6.30)	19.09 (17.19)	0.186
Thalassobius meditarraneus	2.26 (2.02)	1.79 (1.10)	0.707

Significant p-values after Benjamini–Hochberg correction are shown in italic.

Overall, the putative functional profile of H2 samples appeared to be more variable than CC7 (**Figure 2**). In particular two samples (H2_1 and H2_4) were different from the remaining samples, yet they still clustered together (Figure S4). Processes such as "sulfate reducer," "sulfide oxidizer," and "nitrite reducer" were consistently enriched in H2, whereas up regulation of processes like "chitin degradation," "atrazine metabolism," "sulfur oxidizer," "xylan degrader," "sugars fermentor," and "propionate metabolism" was observed for CC7. Other processes, "degrades aromatic hydrocarbons," "ammonia oxidizer," and "dehalogenation" were inconsistently enriched in H2, reflecting a greater variation across individuals (**Figure 2**).

DISCUSSION

Exaiptasia H2 and CC7 Strains Only Share a Small Common Microbiome

Interestingly, we found significant differences in the bacterial diversity of the two *Exaiptasia* strains that have been cultured in the laboratory at the same conditions for long-term. Several

FIGURE 2 | Taxonomy-based functional profiling of *Exaiptasia* H2 and CC7 bacterial communities. Changes in putative functional differences between both populations are displayed on a relative scale with enrichment in red and depletion in blue. H2 is a female clonal strain originally isolated from Hawaii, USA. CC7 is a male clonal strain originally from North Carolina, USA.

studies (e.g., Littman et al., 2009; Pantos et al., 2015; Roder et al., 2015) have suggested that geographic location and environmental settings can determine the holobiont microbiome, whereas others (e.g., Hentschel et al., 2002; Fraune and Bosch, 2007; Montalvo and Hill, 2011; Lawler et al., 2016) have shown that bacterial communities are species-specific associations conserved over time and space. Moreover, coevolution of bacteria and coral has been proposed (Thompson et al., 2015), as it was demonstrated for *Endozoicomonas* symbionts in corals with contrasting life-history traits and across global scales (Bayer et al., 2013; Neave et al., 2016, 2017). Detailed analysis of these studies revealed fine scale host-*Endozoicomonas* specific patterns suggesting phylosymbiosis between corals and this bacterial genus. On the other hand, other bacteria have been universally found in different coral lineages from widely separated geographical habitats (Ainsworth et al., 2015), supporting the idea that consistent, specific host-bacterial interactions occur.

The presence of distinct microbial communities in *Exaiptasia* H2 and CC7 strains aligns with differences in algal symbiont specificities observed for the populations these lab strains were originally obtained from, i.e., Hawaii and North Carolina, respectively (Thornhill et al., 2013; Grawunder et al., 2015) and lends further support to the distinction of these *Exaiptasia* lineages. It should be noted that the CC7 anemones (Röthig et al., 2016a) used for these comparisons were also symbiotic with *Symbiodinium minutum* (SSB01), which has been previously isolated from H2 anemones (Xiang et al., 2013).

Our results showed a comparably lower richness at the OTU level for *Exaiptasia* H2, with a larger core microbiome (50 OTUs) than CC7 (37 OTUs). Only a small fraction (∼15%) of the microbiomes was shared between both groups, and it did not include the dominant bacterial symbionts *Kordia* sp. or *Pseudomonas veronii*, present in H2 and CC7, respectively. *Kordia* is an algicidal bacterium (Sohn et al., 2004) capable of inhibiting the growth of diatoms, and even killing them by active

lysis (Paul and Pohnert, 2011). Its ubiquity and high abundance (37%) in the H2 core microbiome indicates that these bacteria might be important for the host; however, this has to be studied in more depth. *P. veronii*, on the other hand, was the most abundant taxon in CC7. This has been repeatedly found in corals from the Red Sea (Röthig et al., 2016b; Ziegler et al., 2016), and yet it was absent in H2. It is also possible that differences in dominant taxa in *Exaiptasia* are due to sex-specific factors, since H2 are females and CC7 males (Grawunder et al., 2015). For example, studies on mice (Yurkovetskiy et al., 2013) have shown that sex hormones drive differences in the microbiota of males and females during growth; and that sex-specific dietary requirements of crickets determine their microbial symbioses (Schmid et al., 2014). Changes in the bacterial communities before and after spawning have also been documented for different coral species (Ceh et al., 2012), suggesting that certain taxa might be correlated with reproduction.

Noteworthy is that 2 out of the 13 OTUs (**Table 3**) that were defined as shared core microbes between both *Exaiptasia* strains are involved in sulfur cycling processes. Both *Alteromonas* sp. and *Oceanospirillum* sp. bacteria can degrade dimethylsufoniopropionate (DMSP), a compound that has been found within animals that harbor symbiotic algae, such as corals, marine bivalves, and polychaetes (Raina et al., 2009; Morrow et al., 2012). It has also been shown that these bacteria can be vertically transmitted in corals (Sharp et al., 2012), implying a strong role in symbioses. Further, bacteria of the genus *Photobacterium* are remarkably resistant to ultraviolet radiation (e.g., UVB; Matallana-Surget et al., 2009) and have been associated with coral bleaching (Thompson et al., 2005; Moreira et al., 2014). Also linked to bleaching is *Nautella italica*, a bacterial pathogen that can invade, colonize, and induce bleaching in marine macroalgae (Fernandes et al., 2011). Knowledge of the ecology and functional importance of these bacteria is still lacking, and is crucial to better understand their role in shaping the core microbiome of *Exaiptasia*.

Functional Differences Associated with the Bacterial Community of Different Hosts

A clear separation between H2 and CC7 was observed, depicting differences in the putative functional profiles of their bacterial communities. Consistent enrichment of carbon cycling related functions (e.g., "chitin degradation," "xylan degrader," and "sugars fermentor") in CC7 indicate potential differences in carbon utilization pathways. It should be noted that CC7 harbored the heterologous *Symbiodinium* SSB01 strain, which has previously been isolated from *Exaiptasia* H2. It can therefore not be excluded that nutrient interactions within the holobiont might have changed to allow the microbiome to take advantage of different carbon pathways, as has been observed in corals under stress (Bourne et al., 2016).

Changes in sulfur (DMSP degradation) and nitrogen cycling processes are also dependent on *Symbiodinium* (Raina et al., 2009; Bourne et al., 2016). It assimilates and converts most of the sulfur pool in the holobiont to DMSP, a more stable and soluble molecule that is later used as a source of reduced sulfur and

carbon for marine microorganisms (Raina et al., 2009), and likely determines their abundance and diversity in the host (Bourne et al., 2016). Nitrogen availability, as well as other nutrients, in the holobiont is also controlled by the algal symbionts (Rädecker et al., 2015; Bourne et al., 2016), and thus, increased nitrogen availability may promote the activity of nitrogen-fixating bacteria (i.e., diazotrophs). Interestingly, a higher diversity of diazotrophs was observed in CC7; at least 18 OTUs within the group *Rhizobiales* were part of the bacterial community whereas only 8 were present in H2.

Although, we see functional differences in the bacterial community between *Exaiptasia* H2 and CC7, caution should be applied. It has to be stressed that the functional classification of OTUs is based on comparison to well-characterized bacterial groups and the assumption that these functions are conserved in the related groups present in the microbiome of *Exaiptasia*. Further, functional groups might not be retained purely due to their metabolic pathways but because of host-specific preferences, even under the same environmental conditions. Different bacteria can provide similar functions; for example, nitrogen-fixating bacteria in the H2 core microbiome (e.g., unclassified Rhizobiales and *Cohaesibacter gelatinilyticus*) are different from the ones found in CC7 (e.g., unclassified Phyllobacteriaceae and *Methylobacterium hispanicum*).

CONCLUSIONS

Exaiptasia is a cnidarian-dinoflagellate symbiosis model system on the rise, yet until recently, little was known about its bacterial associations. This study provides a baseline on the bacterial diversity of the widely used laboratory strains *Exaiptasia* H2 and compared it to the bacterial microbiome of *Exaiptasia* CC7. Our data suggest the presence of a host-specific microbiome. We believe that knowledge on the specific microbiota of these two laboratory-cultured strains, which are commonly used for diverse experiments worldwide, is a valuable contribution to further our understanding of the *Exaiptasia* holobiont system. We also provided a basic level of functional understanding of the microbiome by assigning functional profiles based on taxonomic data. Our taxonomically inferred functional data showed a clear difference in the metabolic processes of H2 and CC7, which implies that there is still more research needed to better understand how hosts actively select their microbial symbionts and how the different bacterial taxa contribute to the overall functioning of the holobiont.

DATA ACCESSIBILITY

Sequence data have been deposited on NCBI under BioProject Accession no. PRJNA3060672 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA360672).

ETHICS STATEMENT

Study was exempt due to the use of invertebrate animals.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2017.00115/full#supplementary-material

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