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Diversity and Abundance of Symbiodiniaceae and Bacteria in Corals *Sarcophyton trocheliophorum* and *Euphyllia ancora* Under Thermal Stimulation

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ABSTRACT

Coral Symbiodiniaceae and bacteria maintain a symbiotic relationship that is essential for coral survival. The symbiotic communities component in the polyps host could affect coral resistance and the ability to recover from stress. In this study, we increased the cultured range temperature (26 ± 1 ℃) of *Sarcophyton trocheliophorum* and *Euphyllia ancora* at 32 ℃ for 12 hours, and then amplified the *ITS2* sequence of *Symbiodinium* and the bacterial 16S rRNA sequence in the sample, respectively to compare Symbiodiniaceae and bacteria diversity and abundance with normal culture temperature. The results showed that there was no change in the dominant species of *Symbiodinium* in these two corals in the heat treatment group, but the diversity of *Symbiodinium* in the two corals was significantly different. On the other hand, after thermal treatment, the endophytic bacteria of *S. trocheliophorum* represented more aerobic bacteria *Delfitia*, while the *E. ancora* was infected with more pathogenic endosymbiotic bacteria. This difference observation can be attributed to the different tolerance of corals.

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Introduction

Coral reefs are one of the most important communities in the world (Knowlton, 2001). In recent decades, due to global warming, the frequency and extent of coral bleaching around the world have been increased, and the coverage of coral reefs has been decreased significantly (Brown, 1997).

Global warming raises the response of the cnidarian host and associated coral symbionts against stress, resulting in changes in coral tissue in biological symbiosis and destruction, and finally coral bleaching (Hoegh-Guldberg *et al*., 1999). Coral symbionts include *Symbiodinium*, bacteria, fungi, archaea, and viruses (Rohwer *et al*., 2002). The interaction between symbiotic organisms and hosts in coral reefs plays an important role in host health, and most importantly in symbiotic algae and bacteria. (Bourne *et al*., 2016). Relationships between bacteria and corals affect the behavior of coral symbiotic Symbiodiniaceae (Teplitski *et al*., 2009).

Bleaching is a response of corals to environmental changes. The loss of Symbiodiniaceae can be used as a bioassay of coral bleaching (Jones, 1997). Symbiodiniaceae are important and photosynthetic symbionts in corals (Baker *et al*., 2003). The coral ecosystem

is threatened by the rising of SST (Sea-surface temperature) and the stress produced by this change usually leads to the release of Symbiodiniaceae, which causes bleaching and death of corals (Gil-Agudelo *et al.*, 2017). When the environmental conditions change, Symbiodiniaceae also alter their composition in the host, to better adapt to life in the coral (Lin *et al*., 2015; Hume *et al*., 2016). The ability of Symbiodiniaceae to adapt to the environmental conditions is determined by the stable changes in host and symbiont populations (Parkinson *et al*., 2015). Berkelman *et al*. (2006) suggested that the tolerance of corals to temperature alterations was related to the type of symbiotic algae. The Symbiodiniaceae diversity, morphology, and ultrastructure have been studied for a long period (Freudenthal *et al*., 1962; Trench *et al*., 1987), but due to the Symbiodiniaceae variety, the morphological classification problems have not been completely solved (Wakefield *et al*., 2000). Regarding the traditional morphological classification, it is more efficient and convenient to use the DNA sequence to classify the species of Symbiodiniaceae (Rowan *et al*., 1991). Concerning molecular methods, it was found that Symbiodiniaceae diversity is very high (Santos *et al*., 2010) and it has a wildly geographical distribution (Lajeunesse, 2000; Lajeunesse, 2010). At present, Symbiodiniaceae can be divided into 9 major clade groups of A-I (Pochon *et al*., 2010; Carlos *et al*., 1999). These clades can be further subdivided into species that may include several hundreds of sub-species and biotypes (Ziegler *et al*., 2017). Rowan and Powers (1992) used the 18S rDNA sequence combined with RFLP for Symbiodiniaceae diversity studies. Later researchers focused on the ITS barcoding to distinguish species (Lajeunesse, 2001). The internal transcribed spacer 2 (ITS2) region is still the most commonly used DNA marker for the study of the diversity of Symbiodiniaceae (Stat *et al*., 2006; Sampayo *et al*., 2009). Different clades of Symbiodiniaceae have been studied by DGGE combined with the *ITS2* gene (Apprill *et al*., 2007; Stat *et al*., 2012), but there are still, different positions on the gel migration lines, which cannot be completely separated (Lajeunesse, 2002).

Next-generation sequencing (NGS) technology has been applied in biological and environmental studies and other disciplines since 2005 (Shokralla *et al*., 2012). Recently, the NGS method for Symbiodiniaceae diversity research has been gradually accepted by researchers (Boulotte *et al*., 2016; Ziegler *et al*., 2017). Thomas *et al*. (2014) using 454 amplicon pyrosequencing technology on *Acropora* symbiotic Symbiodiniaceae diversity exploration and provided the molecular basis of NGS technology of the sensitive molecular techniques for coral reef combating global warming. Arif *et al*. (2014) analyzed the diversity of ITS2 sequences by comparing the next-generation sequencing technology and the traditional DGGE method to assess the diversity of Symbiodiniaceae. Ziegler *et al*. (2017) used a 454 pyrosequencing platform and MiSeq Illumina platform to analyze ITS2 sequences of symbiotic *Symbiodinium*, and studied the molecular diversity of symbiotic *Symbiodinium* from corals around the Arabia peninsula. These results also indicate that the diversity of Symbiodiniaceae is very high; it means that the traditional method at the molecular level has not better separated different Symbiodiniaceae, and the use of next-generation sequencing technologies in Symbiodiniaceae diversity research is a better method.

Endosymbiotic bacteria are the basic functional unit of the coral symbiosis system and have a direct impact on the health of corals (Moran *et al*., 2015). The bacteria associated with corals are mainly concentrated in their mucus and body, and play a very important role in maintaining coral health and resistance to diseases (Rosenberg *et al*., 2007; Ziegler *et al*., 2016). Bourne and Munn (2005) analyze the bacterial diversity of corals by cloning 16S rRNA and rpoB-DGGE. Bacterial diversity analysis by NGS has been widely used. Sunagawa *et al*. (2009) used high-throughput sequencing methods to sequence 16S rRNA genes to analyze the diversity of endophytic bacteria in Montastraea faveolata corals. Bayer *et al*. (2013) also used the method of high-throughput analysis on the red sea coral symbiotic bacteria, *Stylophora pistillata*, and found that Endozoicomonas bacteria are abundant in the coral endoderm tissues.

Kushmaro *et al*. (1996) found that when the seawater temperature increased, the toxicity of bacteria increased. Many studied were performed on coral pathogens consist of *Serratia* (Enterobacteriaceae) and *Vibrio* (Vibrionaceae). These studies found that *Vibrio* causes bleaching and death of coral (Kushmaro *et al*., 1997; Ben-Haim *et al*., 2003). Under thermal stress, Different Symbiodiniaceae are affected by *Vibrio* in different ways (Gil-Agudelo *et al*., 2017). *Vibrio shilonii* produces exotoxins and inhibits Symbiodiniaceae photosynthesis, and finally, the Symbiodiniaceae are dissolved (Banin *et al*., 2001) and coral is bleached. The response of different Symbiodiniaceae to *Vibrio shilonii* toxin is different (Gil-Agudelo *et al*., 2017). *Serratia* causes the coral white pox disease, the final impact resulting in coral death (Patterson *et al*., 2002).

Symbiotic bacteria are not only important to the adaptability and viability of coral but also have obvious responses to environmental changes (Bourne *et al*., 2016). Teplitski *et al*. (2009) suggested that coral and symbionts cannot be separated so, must be studied as a whole. The interaction mechanisms among symbiont members play an important role in maintaining coral health and stabilizing microbial communities' structure. The balance of algae and bacteria is very important to the stability and health of corals. When this balance is broken by the change of the external environment, the coral algae-bacteria symbiotic system is broken, causing coral bleaching or infection to death. We investigated the changes in the diversity and total abundance of symbiotic algae and bacteria in the corals during the short-term period after thermal stimulation. There is a lack of information about the effect of short-term period changes. The global climate is warming up, and we hope to provide a reference for the protection and restoration of coral reef ecosystems.

Materials and Methods

Coral sample collection and processing

All coral samples were collected from the Ganzhe islands in the eastern part of Wanning City, Hainan Province, China. Each of the five individuals of the Alcyonacea coral- *S. trocheliophorum* and the Scleractinia coral-*E. ancora* were collected and all corals were cut into small pieces (Fig. 1).

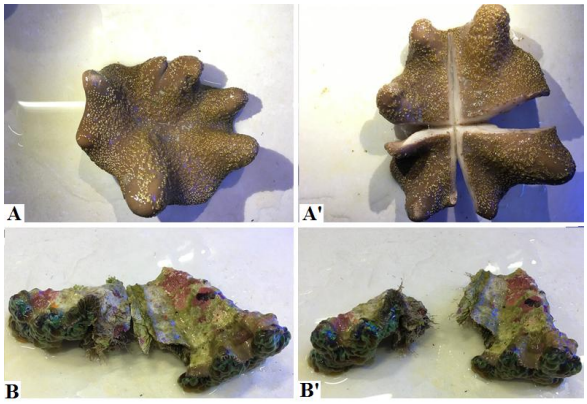

Fig. 1. *S. trocheliophorum* and *E. ancora* collection: A and A') *S. trocheliophorum* were collected and all corals were cut into small pieces; B and B') *E. ancora* was collected and all corals were cut into small pieces.

The samples were held in Hainan Tropical Ocean University Coral Museum for two weeks in tank water: volume: 6l temperature: 26℃, salinity: 35‰, light: using lights on a 12 h light-dark cycle (light duration: 7 am to 7 pm; night-time: 7 pm to 7 am). All small pieces of the two corals are completely independent individuals.

Experimental setup

Each individual was placed in tank 1 (temperature: 26 °C, salinity: 35‰, volume: 1×1×1 m) and control group tank 2 (temperature: 26 °C, salinity: 35‰, volume: 1×1×1 m). When the coral tentacles were fully extended, each individual was cut into the small tissues and was placed in a beaker, washed with sterile water 3 times, remove the remaining water was removed carefully after cleaning. The individual tissues of *S. trocheliophorum* were then transferred to a 1.5 ml centrifuge tube to mix the total DNA, experimental group markers S-1, and Control group markers S-c. Similarly, the individual tissues of *E. ancora* were then transferred to a 1.5 ml centrifuge tube to extract the total DNA: experimental group markers E-1 and control group markers E-c.

In this study, the water temperature was quickly increased to reach 32 °C and then maintained at this target temperature for 12h (Other environmental conditions remained unchanged). During the experiment, an automatic aquarium

heater was used to set and control the temperature. Finally, we extracted the total DNA of *S. trocheliophorum* (S-2) and *E. ancora* (E-2), just like the extracted DNA method before thermal stimulation.

DNA extraction, PCR amplification, and Illumina MiSeq Sequencing

DNA from each individual was extracted with the Column Marine Animals DNA Kit (Guangzhou DONGSHENG, China) according to the manufacturer's instructions. DNA was eluted in the Tris-EDTA buffer solution and stored at -20℃ until use. PCR amplification of the ITS2 sequencing (~320 bp) for Illumina MiSeq platform was performed using primers ITS intfor2 (LaJeunesse *et al.*, 2000) and ITS2-reverse (Coleman *et al*., 1994). The primer sequences were as:
Miseq-ITSintfor2: 5'-TACACGACGCTCTTCCGATCTACTGGAATTGCAGAACTCCGTG-3' and Miseq-ITS2-reverse:
5'-GAGTTCCTTGGCACCCGAGAATTCCAACTAGGGGATCCATATGCTTAAGTTCAGCGGGT-3'.
The bacterial variable regions 3 and 4 of the 16S rRNA gene was amplified using the primer pair:
341F (5-CCCTACACGACGCTCTTCCGATCTGCCTACGGGNGGCWGCAG-3) and
805R 5'-GACTGGAGTTCCTTGGCACCCGAGAATTCCAGACTACHVGGGTATCTAATCC-3' with MiSeq 16S (underlined) sequences.
Amplification was performed according to the following protocol: The First PCRs were run with 10μM primer 1μL, 2×Taq master Mix (Sangon Biotech Co., Ltd, Shanghai, China) 15μl, 20 ng DNA and DNase free water to make a total volume of 30μL：denaturation step of 94°C for 3 min; 5 cycles of denaturation at 94°C for 30s ， annealing at 45 °C for 20s and extension at 65 °C for 30 s, 20 cycles of denaturation at 94 °C for 20s, annealing at 55 °C for 20 s and extension at 72 °C for 30s, and a final extension at 72 °C for 5 min. The second 30 μL reaction mixture contained 20 ng of DNA, 15 μL of 2×Taq master Mix (Sangon Biotech Co., Ltd, Shanghai, China), 1 μL of each Bar-PCR primers (10 uM), and DNase free water to make a total volume of 30μL which was used for PCR under the following conditions: denaturation at 95°C for 3min, 5 cycles of denaturation at 94°C for 20s, annealing at 55°C for 20s and extension at 72°C for 30s, and a final extension at 72°C for 5 min. Pooled samples were cleaned with Agencourt AMPure XP magnetic bead system (Beckman Coulter, Brea, CA, USA). The samples were then quantified by Qubit3.0 DNA （Life Technologies, CA, USA) and pooled in equimolar ratios. Finally，the high-throughput was sequenced using Illumina MiSeq (Sangon Biotech Co., Ltd. Shanghai, China).

Analysis of sequence data

The Miseq sequencing dataset contains the barcode sequence, the primers, and linker sequences that are added at the time of sequencing. First, cutadapt was used to remove the primer sequence, and then according to PE overlap between the overlap, the use of PEAR pairs of reads merge into a sequence, merge sequence overlap area allows the maximum mismatch ratio is 0.1. Then according to the barcode tag sequence to identify and distinguish the sample to get the sample data, the Prinseq was used to remove the sample in the sample tail mass of 20 or less base. Set the 10bp port if the average quality value in the window was less than 20, starting from the window to remove the back end of the base. The cut contained the N part of the sequence and removed the short sequence in the data, with a length threshold of 200bp. Then the low complexity of the sequence of filtering, and finally get the sample valid data. Finally, we used Usearch and uchime to remove chimeric with nonspecific amplification sequences to get Filtered Reads. All the sample sequences were clustered according to the distance between the sequences. Finally, the sequences were divided into different Operational taxonomic Units (OTU) using a 97% similarity cut-off.
The OTUs obtained for each sample were analyzed for alpha diversity using MOTHUR 1.30.1. Diversity index analysis was estimated using statistical indicators (Shannon, Simpson, Coverage, ACE, and Chaol) that can reflect the abundance and diversity of biological communities. The community richness index

(Chao and Ace) were utilized to estimate the total number of species in ecology. The high values represented a large number of species. The community diversity index (Shannon and Simpson) was used to estimate the biodiversity index in the sample. The high value of the Shannon index can be referred to as the high diversity of the community. The coverage index reflects whether the results of sequencing represent the actual situation of the sample.

MiSeq ITS2 Sequence-Analysis

For the OTU-based analysis, according to the ITS2 database of GenBank in NCBI, the dataset was blasted by alignment analysis. The best alignment result of OTU sequences was screened, and the results were filtered. The default setting (similarity >90% and the sequence of coverage >90%) was used for subsequent classification, and other sequences were classified as unclassified (Arif *et al*., 2014; Ziegler *et al*., 2017). Finally, the larger abundance and representative OTUs were selected to construct a phylogenetic tree using the Neighbour-joining method.

The top 100 of the abundance of OTUs were applied for network analysis using QIIME, and draw a node with a significant contact (weight ≥ 100). The area size represented the size of the overall abundance.

Bacterial Microbiome-Analysis

To obtain the species classification information corresponding to each OTU, the species classification of OTU was carried out on the RDP database. We used the RDP classifier method based on Bergey's taxonomy to compute the probability values assigned to this rank for each sequence at different levels. It is generally believed that the probability value (RDP classification threshold) is greater than 0.8 when the classification results are credible. According to the previous analyses, the statistical results of the taxonomy species were used to show the histogram of the community structure by R. Samples, and community distribution information was also clustered and re-arranged. The results were plotted using the gplots package of R and displayed in the heatmap. Finally, according to the results of species classification, Fisher exact test was used to identify species with significant differences ($P \leq 0.05$) among species.

Results

Diversity of *Symbiodinium*

A total of 234547 raw numbers were obtained by ITS2 sequencing. Then the samples were identified and distinguished according to the barcode tag sequence (Table 1).

Finally, 233,991 Filtered Reads (S-1：44091；S-2：55356；E-1：38039；E-2：96505) were obtained after filtering and removing the chimera and nonspecific amplification sequences. All the sample sequences were clustered according to the distance between the sequences, and the sequences were divided into different OTUs according to the similarity between the sequences. Each sample obtained by the OTUs was analyzed following the diversity index (Table 2).

Table 1. *Symbiodinium ITS2* gene sequencing data statistics.

Sample	Barcode	Raw No.	Mean length	Clean No.	Mean length	Chimeras No.	Filtered No.
S-1	GGGCAG	44192	338.63	44116	290.94	25	44091
S-2	GCAAAG	55414	338.68	55357	291.15	1	55356
E-1	CCACCC	38116	336.82	38079	289.02	40	38039
E-2	AAATGC	96825	336.87	96698	289.02	193	96505

Table 2. *Symbiodinium* Alpha diversity index statistics.

Sample	Seq No.	OTU No.	Shannon index	Simpson	Chao1 index	ACE index	Coverage
S-1	44091	311	0.33	0.91	2269.06	5992.21	0.99
S-2	55356	310	0.32	0.91	4105	12889.91	1.00
E-1	38039	507	2.07	0.20	2219.69	4727.40	0.99
E-2	96505	1125	1.31	0.59	5186.76	11956.77	0.99

From the Chao index and the Ace index, it can be seen that after the heat treatment, the total number of *Symbiodinium* of the two corals was increased. The Shannon index of the *E. ancora* was reduced and it shows the diversity of *Symbiodinium* was reduced after heat treatment, but the Shannon index of *S. trocheliophorum* was almost unchanged. The same result can be obtained from the Simpson index analysis. According to the Shannon index and the Simpson index, it can be seen that the diversity of *E. ancora* is higher than that of *S. trocheliophorum*.

The OTUs of each sample were analyzed by blastn alignment using the ITS2 database of GenBank in NCBI. Finally, we selected the higher abundance of OTUs using Neighbor-joining to build the phylogenetic tree (Fig. 2). There were two Clades (C＆D) of *Symbiodinium* in *S. trocheliophorum*. Clade C was the dominant population. There were also two Clad of *Symbiodinium* in the *E. ancora*, but Clade D was the dominant population. We select the abundance of the top 100 bits of the OTU information and the node with significant contact (weight ≥ 100). Finally, the network analysis was performed and the results are shown in figure 3.

Diversity of bacteria

A total of 127630 raw numbers were obtained based on the sequencing of the bacterial 16S rDNA gene. The samples were identified and distinguished according to the barcode tag sequence (Table 3). Finally, a total of 115,692 Filtered Reads (S-1：27953；S-2：24543；E-1：37542；E-2：25654) were obtained by filtering and removing chimeras with nonspecific amplification sequences.

All the sample sequences were clustered according to the distance between the sequences. According to the similarity between sequences, the sequences were then divided into different OUT. The OTUs of each sample were examined by a diversity index (Table 4). Regarding the Chao index and the Ace index, it can be seen that the total number of symbiotic bacteria has decreased in both corals after heat treatment. However, Shannon and the Simpson indexes were different. After the heat treatment, the diversity of the symbiotic bacteria in *S. trocheliophorum* was significantly decreased, while this diversity was increased in the *E. ancora* (Fig. 4).

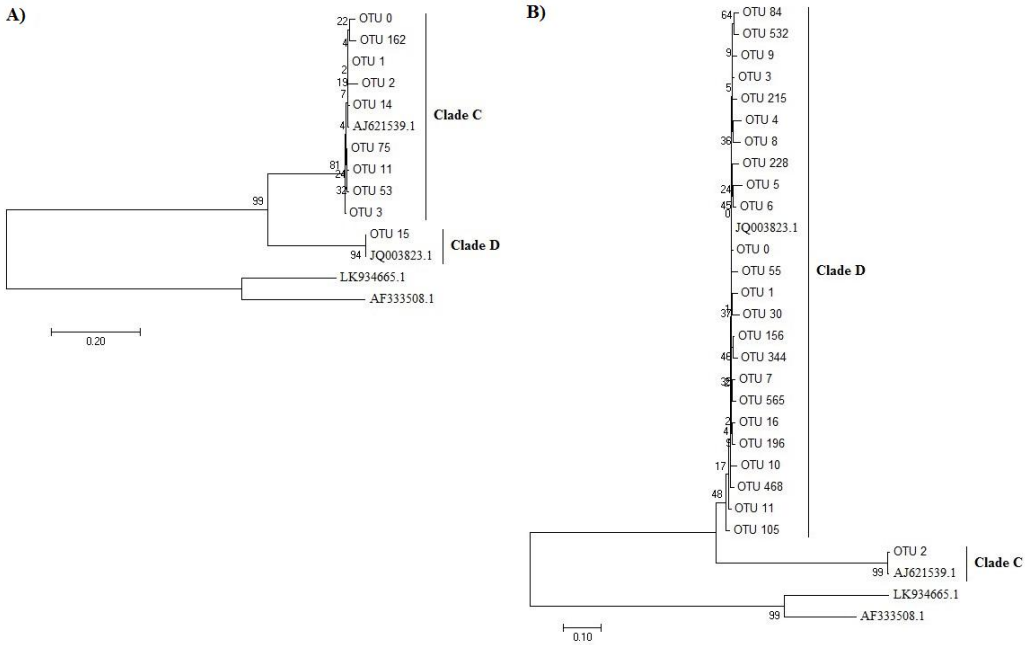

Fig. 2. Neighbor-joining tree for *Symbiodinium* based on *ITS2* gene Sequences of Different OTUs. (a) *S. trocheliophorum* (b) *E. ancora*.

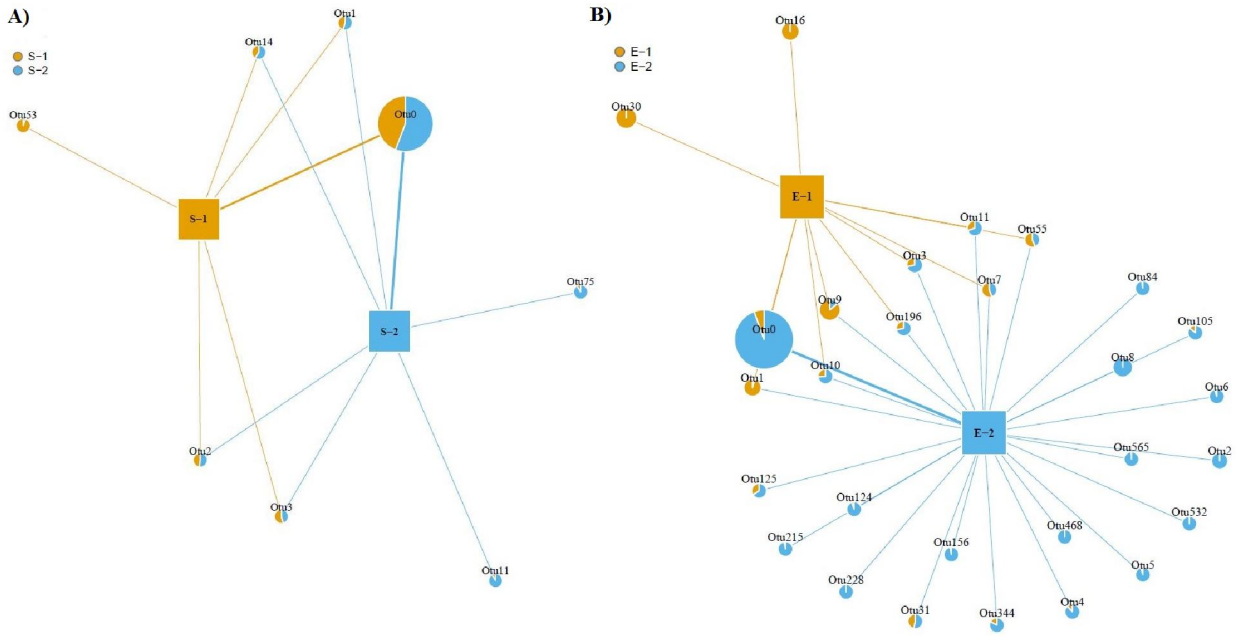

Fig. 3. The rectangles represent the samples, and the different samples are represented by different colors. OTU is marked as a pie chart, and the area size represents the size of the abundance. The different color patches in the pie chart represent the abundance of different samples on the OTU classification.

Table 3. Bacterial *16S rDNA* gene sequencing data statistics.

Sample	Barcode	Raw No.	Mean length	Clean No.	Mean length	Chimeras No.	Filtered No.
S-1	GCCGCT	33242	444.58	28300	425.22	258	27953
S-2	GAAACC	25545	468.13	24590	427.90	37	24543
E-1	CGCCAT	42082	453.30	37720	425.34	122	37542
E-2	GAGGTT	26761	461.50	25798	421.60	90	25654

Table 4. Bacterial Alpha diversity index statistics.

Sample	Seq No.	OUT No.	Shannon index	Simpson	Chao1 index	ACE index	Coverage
S-1	27953	2376	4.53	0.04	27598.23	51402.77	0.93
S-2	24543	2030	2.01	0.44	19660.40	47185.90	0.93
E-1	37542	2973	3.7	0.10	33036.98	74868.52	0.93
E-2	25654	2186	4.8	0.03	16508.96	30326.38	0.93

In *S. trocheliophorum* coral, *Spongiibacter*, *Achromobacter*, *Solimonas*, *Stenotrophomonas*, *Oceanococcus,* and *Citrobacter* were dominant populations before temperature rise, but the bacterial diversity in the coral decreased significantly after thermal stimulation (Figure 5), and *Delftia* and *Serratia* abundances rose sharply and became dominant populations (Fig. 5). The amount of *Delftia* and *Serratia* were significantly (P<0.01) increased after thermal stimulation (Fig. 6A). Before thermal stimulation, the dominant genera of *E. ancora* corals were *Oceanococcus*, *Solimonas*, *Marivita*, *Citrobacter,* and *Spongiibacter* (Figure 5), but the bacterial diversity was increased after heat stimulation, and the dominant population changed to *Alcanivorax*, *Loktanella*, *Neptuniibacter*, *Leisingera,* and *Achromobacter* (Figure 6). On the other hand, the abundance of *Oceanococcus*, *Spongiibacter*, *Marivita,* and *Solimonas* decreased significantly (P<0.01). So that, *Oceanococcus* and *Solimona* two kinds of bacteria after heat stimulation almost were disappeared completely, while *Alcanivorax*, *Neptuniibacter*, *Achromobacter*, *Loktanella*, *Pseudomonas,* and *Leisingera* were significantly (P<0.01) increased (Fig. 6B).

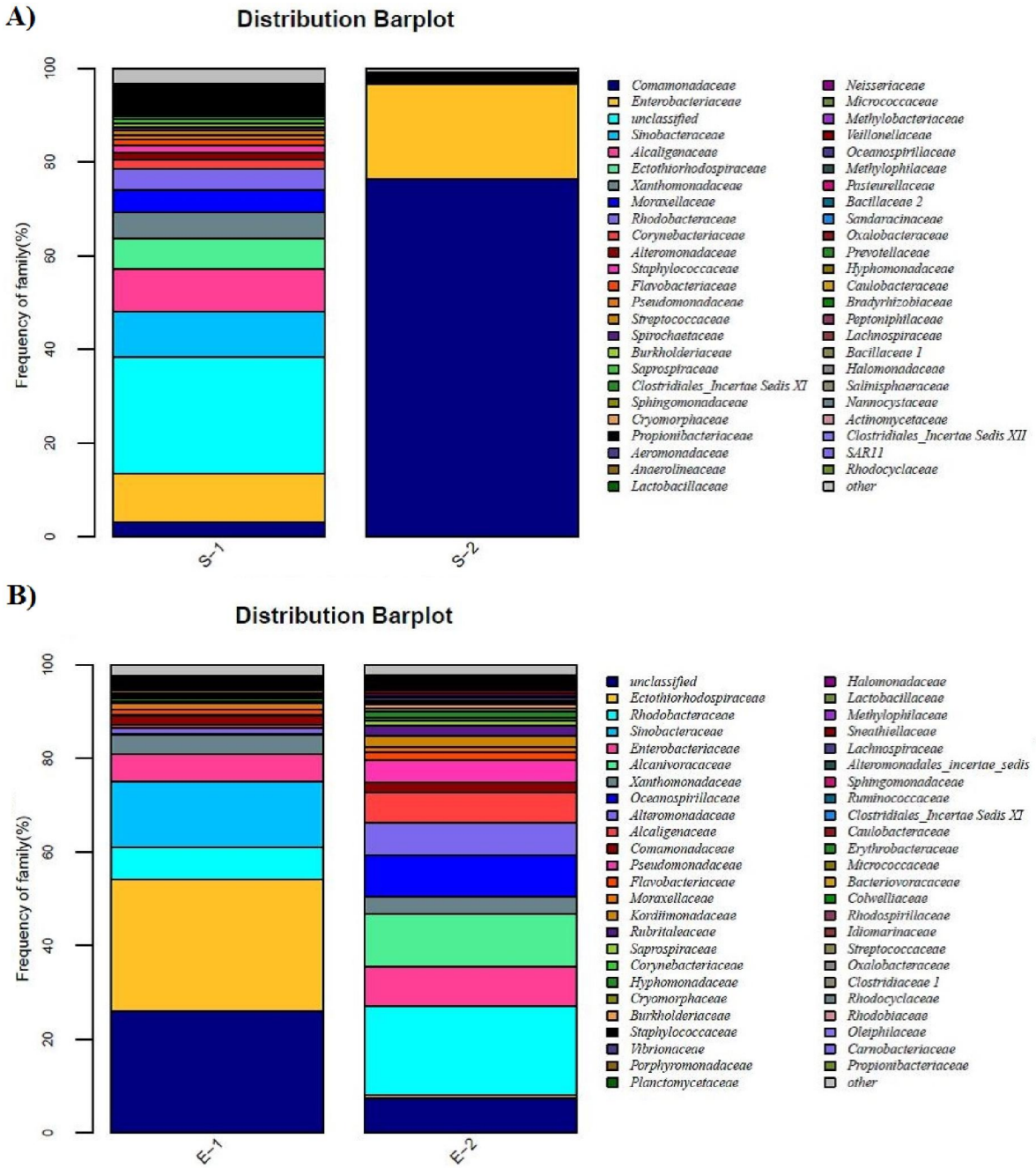

Fig. 4. Histogram of the changes in the internal symbiotic bacterial communities of the two corals before and after heat treatment. Different colors represent different genus, cannot be identified with unclassified representatives, low abundance with other representatives.

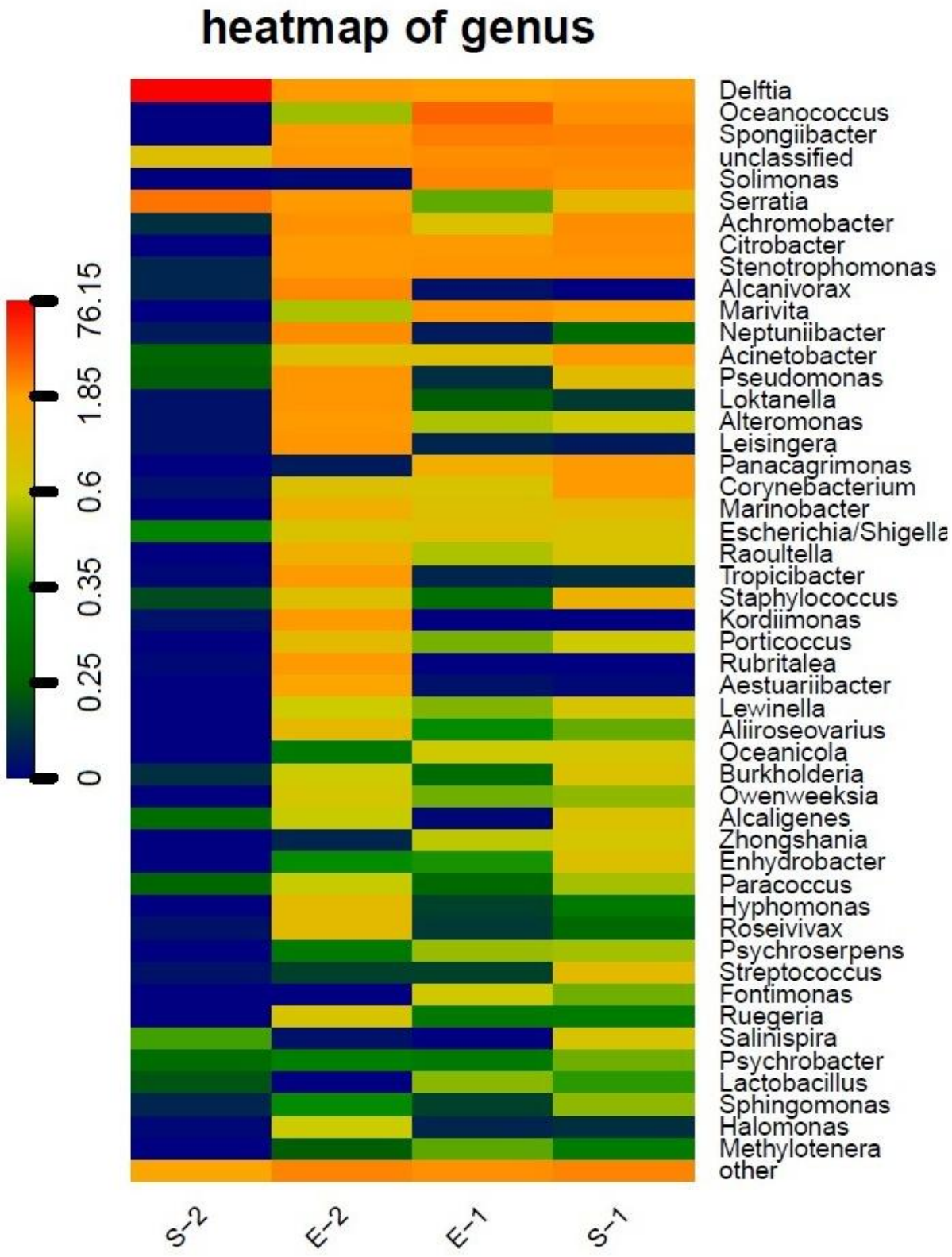

Fig. 5. Bacterial genera abundance heat map. The abundance of species is plotted with a species abundance matrix. Each column represents a sample. The rows represent the community structure. The color block represents the relative abundance of the species. The more red the color, the higher the relative abundance, and the more blue the color, the lower the relative abundance.

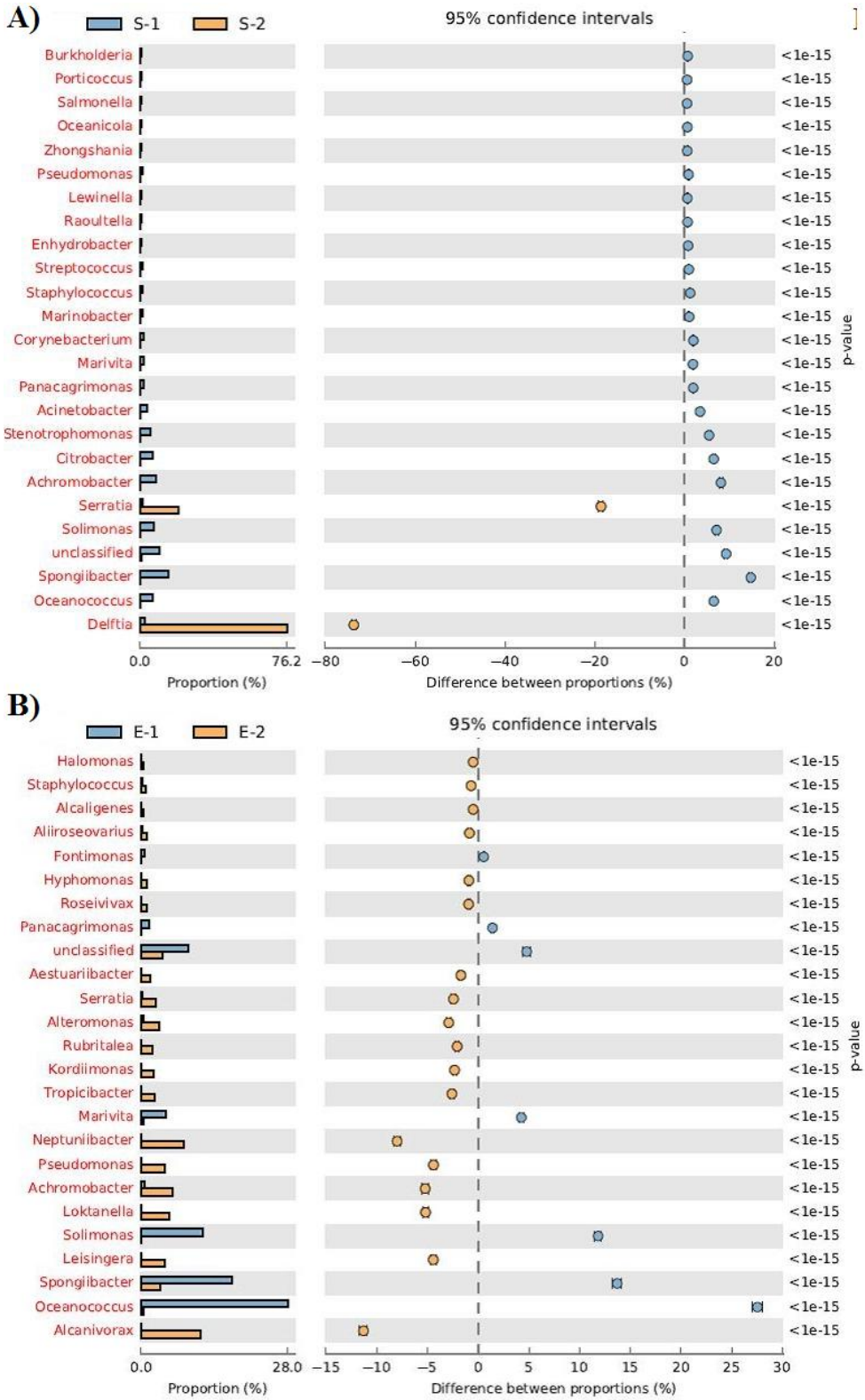

Fig. 6. Analysis of bacterial abundance before and after heat treatment. (a) *S. trocheliophorum* (b) *E. ancora*. The left side shows the abundance ratio of the different species in the two sample, the middle shows 95% confidence interval, Species classification abundance of the proportion of the difference, the right value of the P-value, P-value <0.05, representing significant differences, The name of bacteria is marked with red. (Only 25 of the lowest P values are listed).

Discussion

We used the next-generation sequencing techniques to study the effects of thermal stimulation on the symbiosis of *Symbiodinium* and bacteria of *S. trocheliophorum* and *E. ancora* corals in the South China Sea.

Changes in Symbiodiniaceae after heat stimulation

We analyze the diversity of OTUs and phylogenetic tree analysis, different OTUs represent different species of Symbiodiniaceae or subspecies. It was reported that the *Symbiodinium* of *Euphyllia* in the South China Sea was Clade C (Dong *et al*., 2009; Chen *et al*., 2005). But, the results of our phylogenetic tree showed that the dominant sample of *E. ancora* was Clade D and Clade C showed low diversity. In this study, the dominant species of *Symbiodinium* in *S. trocheliophorum* was Clade C, and the dominant species in *E. ancora* was Clade D. The dominant species in these two corals after heat stimulation did not change, which was consistent with the Stat study (Stat *et al*., 2012). However, we found that the total number of *Symbiodinium* after heat stimulation was increased. Strychar *et al*. (2004) documented that coral in the external thermal environment impact leads to the escape of *Symbiodinium* and eventually to coral death. Our results suggested that when the coral was subjected to thermal stimulation, the *Symbiodinium* responds to the environment and adjusts, therefore the number of *Symbiodinium* slightly rises and then declines. We also found that there were no significant changes in *Symbiodinium* diversity after the thermal stimulation of *S. trocheliophorum*, but the diversity of *Symbiodinium* in *E. ancora* has been declined. This may be related to the temperature tolerance of *Sarcophyton* (Marshall *et al*., 2000). However, *Euphyllia* is less resistant to high temperatures (Fang, 2015).

Abundance and diversity changes in Bacteria

After the thermal stimulation, the two corals were in a sub-health state and the total number of symbiotic bacteria have been declined. Because different bacteria have different heat resistance, many bacteria with low-temperature tolerance die. Bayer *et al*. (2013) analyzed the structure of the symbiotic bacteria in several corals of the Red Sea area by analyzing the V5-V6 region of the 16S rDNA gene and found that *Endozoicomonas* is a dominant bacterium in coral symbiotic bacteria. Generally, different corals have different symbiosis (Bayer *et al*., 2013). Sunagawa *et al*. (2009) evidenced *Alteromonadaceae*, *Enterobacteriaceae,* and *Vibrionaceae* are more abundant in morbid and sub-health corals. Similarly, in the soft *S. trocheliophorum*, we found that the diversity of symbiotic bacteria became lower after heat treatment and *Delftia* (Family *Comamonadaceae*) and *Serratia* (Family *Enterobacteriaceae*) became dominant populations. *Delftia* comparatively represents low abundances in healthy corals (Ainsworth *et al*., 2015), which is similar to our study. We found that the total abundance of *Delftia* increased after the heat stimulation because it is the aerobic bacteria and photosynthesis increased and released more oxygen. *Serratia* can lead to irregular white patches on the surface of coral tissue, called white pox disease, and the disease is more likely to occur when seawater temperatures rise (Sutherland and Ritchie, 2004). Ritchie (2006) found that coral mucus secretes certain antibiotics to resist *Serratia.* In contrast, we found that *Serratia* became a dominant bacterium after heat stimulation.

Chiu *et al*. (2012) studied the bacterial distribution of *Euphyllia glabrescens* by CARD-FISH showed that Alphaproteobacteria was frequently distributed between mucus and epidermis, while Gammaproteobacteria was detected in the gastrointestinal tract and was rarely observed in mucus and epidermis. We found that Gammaproteobacteria (Oceanococcus, Solimonas, Citrobacter, Stenotrophomonas) and Alphaproteobacteria (Marivita) were the dominant populations before the heat stimulation which was consistent with the results of Chiu.

Strychar *et al*. (2005) results have shown that *Sarcophyton* is bleached at ambient temperatures >32°C. Additionally, *Euphyllia* has a low tolerance to temperature, and when the temperature rose to 30℃, the coral was bleached and eventually died (Fang, 2015). In the study of coral bleaching, we showed that the more pathogenic bacteria were *Vibrio*, but the *Vibrio* aitration was not significant for the short period

of time after heat stimulation in both. Then most of these pathogens can be extracted and isolated from bleached or dead corals.

Each of the constituent units in the symbiotic body has an important contribution to the stability of the corals, and the interaction of each unit can keep the corals healthy life. We hope to provide basic data on the ecological and environmental adaptation of symbiotic algae and bacteria in coral reef systems through next-generation sequencing techniques by further research and restoration of coral reefs.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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