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Influence of temperature changes on symbiotic Symbiodiniaceae and bacterial communities' structure: an experimental study on soft coral *Sarcophyton trocheliophorum* (Anthozoa: Alcyoniidae)

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Abstract

It is well concluded that microbial composition and diversity of coral species can be affected under temperature alterations. However, the interaction of environmental accumulation of corals and temperature stress on symbiotic Symbiodiniaceae and bacterial communities are rarely studied. In this study, two groups of soft coral *Sarcophyton trocheliophorum* were cultured under constant (26 °C) and inconstant (22 °C to 26 °C) temperature conditions for 30 days as control treatments. After that, water was cooled rapidly to decrease to 20 °C in 24 h. The results of diversity analysis showed that symbiotic Symbiodiniaceae and bacterial communities had a significant difference between the two accumulated groups. The principal coordinate analyses confirmed that symbiotic Symbiodiniaceae and bacterial communities of both control treatments were clustered into two groups. Our results evidenced that rapid cooling stress could not change symbiotic Symbiodiniaceae and bacterial communities' composition. On the other hand, cooling stress could alter only bacterial communities in constant group. In conclusion, our study represents a clear relationship between environmental accumulation and the impact of short-term cooling stress in which microbial composition structure can be affected by early adaptation conditions.

Introduction

Coral bleaching has become a research hotspot for marine science since 1960s. This phenomenon was initially discovered as a consequence of large-scale loss of Symbiodiniaceae in corals under environmental disturbances (Yonge and Nicholls 1931). Goreau (1964) reported that shallow-water corals were whitened and had died in Jamaica due to the abundant inflow of freshwater and further proposed the term 'coral bleaching'. Nowadays, there are different opinions on the causes of coral bleaching, such as temperature rising (Aronson *et al.* 2000, Hoegh-Guldberg 1999), photoinhibition (Bhagooli & Hlidaka 2004), and chemical pollutants (Gervino *et al.* 2003, Philip *et al.* 2004). Afterwards influence of low temperature and bacterial infection were considered. The large-scale deaths of corals have been reported in Leizhou Peninsula during Holocene due to extensive cooling (Yu *et al.* 2004). In an experimental study, Kushmaro *et al.* (1996) documented that infected *Oculina patagouica* by *Vibrio* AK-1 bacteria could exhibit bleaching. Further studies showed *Vibrio shilonii* AK-1 releases quartile sensing signal molecules in the process of coral infection (Li *et al.* 2016). Additionally, Meyer *et al.* (2016) confirmed that quartile sensing signal molecules could be involved in the infection process of coral leukosis.

Regarding current studies, there are several causes for coral bleaching. However, a consensus has been achieved that coral bleaching is mainly caused by losing in vivo pigments of symbiotic Symbiodiniaceae. Symbiodiniaceae lives in the vacuole of the entoderm cells of the host and provides more than 95% of the products of photosynthesis (e.g., amino acids, sugar, carbohydrate, and small molecular peptide) for hosts (Loh *et al.* 2001, Lu *et al.* 2021a,b). Photosynthetic products can supply energy and essential compounds for corals; instead, Symbiodiniaceae can get elemental nutrients (e.g., amine and phosphate) from the metabolic products by corals (Saxby 2000). Weglry *et al.* (2007) found that symbiotic microorganisms (e.g., bacteria, fungi, and archaea) play key roles in coral metabolism, such as energy supply, transformation of substances, and disease immunity. Additionally, Anthozoans can provide shelter and raw materials.

The probiotics have an indispensable biological role for corals. During coral bleaching, the nitrogen-fixing bacteria in the mucus of corals can replace with Symbiodiniaceae and provide nutrients for coral. For example, the symbiotic *Cyanobacteria* can synthesize nutrients through

the bleaching stage in *Oculinary patagonica* (Teplitski & Ritchie 2009). Moreover, probiotics can also facilitate the ecological balance of flora in the host or in surrounding environments (Merrifield *et al.* 2010). The symbiotic microorganisms (Symbiodiniaceae and bacteria) of corals are groups of complicated dynamic combinations.

Regional disparity (McKew *et al.* 2012), eutrophication (Thurber *et al.* 2009), and diseases (Rosenberg *et al.* 2007) can change the symbiotic microflora of corals. Terraneo *et al.* (2019) used the Internal Transcribed Spacer (ITS) sequence of Symbiodiniaceae to study on *Porites* corals in the Red Sea. It was calculated that the communities' structure of Symbiodiniaceae has a relationship with latitude. Environmental condition can be an important factor to restrict their distribution. Pootakham *et al.* (2019) also found that coral symbiotic bacteria are sensitive to temperature, and temperature alterations cause a change in the structure of the bacterial community.

In Similan Archipelago (Thailand), large-amplitude internal waves bring daily average temperature differences of 5–7 °C in seawater. Wall *et al.* (2015) reported that such temperature differences induce the strong adaptation of corals. Studies on the dynamic changes in the holobiont of corals can provide a new approach to better understand coral's biology and their microbial communities' relationships with environmental changes. We hypothesize that accumulation condition can cause non-identical responses in coral host to determine symbiotic Symbiodiniaceae and bacterial composition under different environmental stress. The aim of this study was to point out the effect of rapid cooling of water temperature on symbiotic Symbiodiniaceae and bacterial communities' structure of cultured *Sarcophyton trocheliophorum* under constant (26 °C) and variable (22 °C to 26 °C) temperature conditions.

Materials and methods

Sampling

Following Yu *et al.* (2020), a specimen of *S. trocheliophorum* was collected (in December 2020) from a fringing reef at a depth of 6 m (salinity: 34 ppt, temperature: 27 °C, water temperature: 25 °C) around the Xiaozhou Island (18°12'30.43"N; 109°23'27.62"E) in South China Sea (Figure S1). Taxonomic status of specimen has been confirmed using 28S and mtMutS markers sequences (Benayahu *et al.* 2018, Quattrini *et al.* 2019) and morphology of sclerites (Benayahu *et al.* 2018). The 17 branches of the specimen were split and cultured in a glacial aquarium (ca. 100 L) filled with natural seawater (salinity: 33 ± 1 ppt; temperature: 26±1 °C). The aquarium was illuminated with white and blue cool LCD fluorescent bulbs (Philips T5HO Activiva Active 54 W) at a light intensity of 518 μmol photons m^{-2} s^{-1} in a 12 h/12 h light–dark cycle for 4 months to recover full colony and acclimatise to laboratory condition following Tang *et al.* (2018).

Temperature acclimation and simulation of cooling event

Twelve well-grown full colonies have been chosen and divided into two groups (constant and inconstant temperatures) in which each group including control and rapid cooling treatments (each treatment consists of three replicates). Temperature of constant group (Cg) was set as acclimation condition (26 °C) following Tang *et al.* (2018). To simulate the effect of temperature changes, its range was set from 22 °C to 26 °C for inconstant group (Ig), which was automatically coordinated every 12 h. Both groups (including control and rapid cooling treatments) were acclimated to 30 days simultaneously. At the end of 30 days, water temperature was swiftly decreased to 20 °C in rapid cooling treatments. This condition was maintained for 24 h (control treatments continued in the previous temperature condition).

DNA extraction, PCR amplification, and Illumina MiSeq sequencing

Whole DNA from each individual was extracted using the Column Marine Organism DNA Kit (Guangzhou DONGSHENG, China). DNA was stored in Tris-EDTA buffer solution and kept at −20°C for further studies.

PCR amplification of the ITS2 sequence (approximately 320 bp) for Illumina MiSeq platform was performed using primers ITS intfor2 and ITS2 reverse (Coleman *et al.* 2010, LaJeunesse & Trench 2000). The primer sequences were as follows: Miseq-ITSintfor2 (5′-GA ATTGCAGAACTCCGTG-3′) and Miseq-ITS2-reverse (5′-GG GATCCATATGCTTAAGTTCAGCGGGT-3′).

The bacterial variable regions 3 and 4 of the 16S rRNA gene were amplified using the primer pair: 341F (5′-CCTACGGGNG GCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAA TCC-3′) (Liang *et al.* 2017).

Amplification was performed according to the following protocol. First, PCRs were run with 10 μM primer, 1 μL 2×Taq master Mix (Sangon Biotech Co., Ltd., Shanghai, China), and 15 μL of 20 ng DNA. DNase-free water was added to obtain a total volume of 30 μL. The mixture was exposed to the following condition: denaturation at 94 °C for 3 min; five cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 20 s, and extension at 65 °C for 30 s; 20 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 5 min. A second 30 μL of reaction mixture containing 20 ng DNA, 15 μL 2×Taq master Mix (Sangon Biotech Co., Ltd., Shanghai, China), 1 μL of each Bar-PCR primer (10 uM), and DNase-free water was prepared to make a total volume of 30 μL and was used for PCR under the following condition: denaturation at 95 °C for 3 min, five cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s and extension at 72 °C for 30 s, 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. A PE2×300 library was constructed according to the standard operating procedures of the platform. Finally, sequencing was conducted on the IlluminaMiseqPE300 platform (Majorbio).

Analysis of sequence data

The Miseq data 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. According to PE overlap between the overlap, PEAR pairs of reads were then used to merge the sequence. The merge sequence overlap area was allowed a maximum mismatch ratio of 0.1. According to the barcode tag sequence used 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. The port was set at 10 bp when the average quality value in the window was less than 20, starting from the window to the back end of the base. The cut contained the N part of the sequence and removed the short sequence in the data according to the length threshold of 200 bp. Low-complex sequence filtering was conducted, and the sample valid data were finally obtained. Usearch and uchime were used to remove chimaeric with non-specific amplification sequences to get filtered

reads. All 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.

Nucleotide sequence accession numbers

Raw reads of Symbiodiniaceae (accession numbers: SRP279038) and bacteria (accession numbers: SRP279036) were submitted to the NCBI SRA.

MiSeq ITS2 sequence analysis

A comparison database corresponding to the Symbiodiniaceae subgroup ITS was downloaded from GeoSymbio (http://sites.google.com/site/geosymbio/downloads), and the ITS2 database was uploaded onto the website of CD-HIT Suite. CD-HIT was set at 100%, and other parameters were set to default. Repetitions were deleted, and annotations were combined. The remaining results were used to construct a non-redundancy ITS database. The best alignment results of OTU sequences were screened, and the results were filtered. The default satisfied a similarity >90%, and a sequence of coverage >90% was used for subsequent classification (Arif *et al.* 2014, Ziegler *et al.* 2017). The diversity of single sample was gained from the alpha diversity index based on sample clustering results with Mothur software. OTU abundance and diversity were reflected by Chao 1 and Simpson indices, respectively. To measure the change in the diversity of Symbiodiniaceae from one condition to another, the principal coordinate analysis (PCoA) at the OTU levels (OTU68, OTU49, OTU44, and other identified taxa) has been analyzed.

Bacterial microbiome analysis

Non-repetitive sequences of 16S were extracted from optimal sequences for OTU clustering (excluding single sequence) according to 97% similarity. Chimaeras in the clustering process were eliminated to obtain the representative sequence of OTU. A taxonomic analysis of OTU representative sequences of 97% similar level was conducted using the RDP classifier Bayesian algorithm to acquire the specific classification information of each OTU. Community composition of different samples was analyzed on different classification levels (Inc. domain, kingdom, phylum, class, order, family, genus, and species). The diversity of a single sample was gained from the alpha diversity index analysis of sample clustering results with Mothur software. Coverage, abundance, and diversity of microflora were reflected by coverage, Chao 1, and Simpson indices, respectively. The PCoA has been performed to consider the beta diversity analysis at the genus and OTU levels.

Results

Diversity of Symbiodiniaceae

Based on OTU classification, an abundance of the symbiotic Symbiodiniaceae is summarized in Table 1 and Figure 1. OTUs were identified in three major levels, including OTU68, OTU49, and OTU44. The OTU68 presented the highest abundance in all treatments. The highest and lowest values of OTU68 were recorded in Cg (control: 98.8%, cooling: 96.9%) and Ig (control: 62.07% and cooling 63.52%), respectively. In contrast, the highest and lowest values of OTU49 and OTU44 were revealed in Ig and Cg treatments consecutively.

Table 1. Percentage of Symbiodiniaceae OTUs abundance (Cg: constant group, Ig: inconstant group).

	Treatment			
OTU	Cg (control)	Cg (cooling)	Ig (control)	Ig (cooling)
OTU68	98.8	96.9	62.07	63.52
OTU49	0.51	1.38	35.22	33.42
OTU44	0.12	0.06	2.44	2.81
Others	0.57	1.66	0.27	0.25

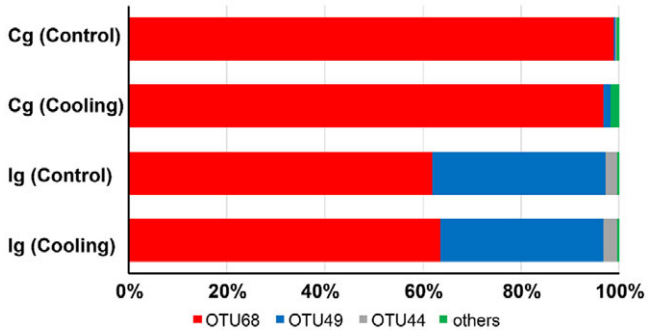

Figure 1. The OTU-based Symbiodiniaceae genera profiles (Cg: constant group, Ig: inconstant group).

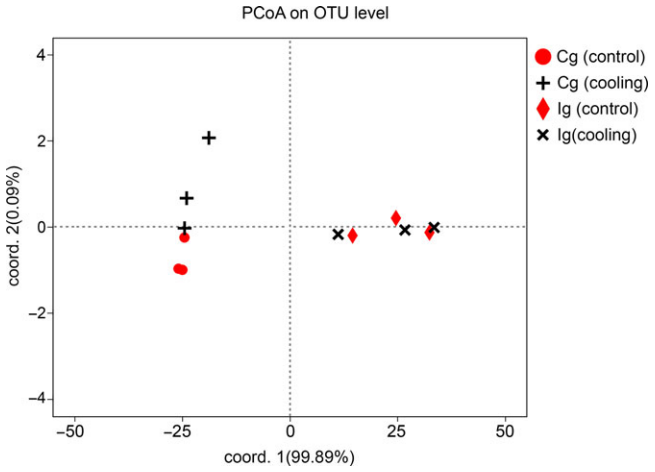

Figure 2. Relationships between Symbiodiniaceae diversity and groups/treatments using principal coordinate analysis (PCoA) based on the OTU level.

Based on OTU levels, PCoA shows that the first and second coordinates held 99.89% and 0.09% of the variance, respectively. PCoA documented that Cg and Ig were completely divided into two clusters following first coordinate, while there was no indicative differentiation between treatments within each group (Figure 2).

Chao and Shannon indices represent significant differences among treatments (Table 2; Figure 3). Overall, the highest values of both Chao and Shannon indices were observed in Cg. Although Shannon index could not show a difference between control (0.979 ± 0.012) and rapid cooling (0.930 ± 0.050) treatments in Cg, the highest value of Chao index was obtained in rapid cooling treatment of Cg (17.3 ± 0.577). There was no significant difference between control and rapid cooling treatments in Ig ($p>0.05$).

Table 2. Mean values (± SD) of Chao index and Shannon index for Symbiodiniaceae communities under different treatments. (Cg: constant group, Ig: inconstant group).

Treatment	Chao index	Simpson index
Cg (Control)	13.1^{b} (±1.644)	0.979^{cd} (±0.012)
Cg(cooling)	17.3^{abc} (±0.577)	0.930^{ab} (±0.050)
Ig (Control)	11.0^{c} (±0.000)	0.544^{bd} (±0.049)
Ig(cooling)	11.2^{a} (±0.288)	0.542^{ac} (±0.048)

Same letter in each column shows significant difference ($p<0.05$).

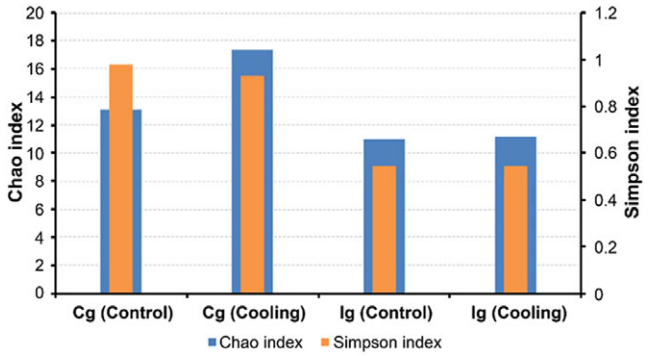

Figure 3. Mean values (± SD) of Chao index and Shannon index for Symbiodiniaceae communities under different treatments. (Cg: constant group, Ig: inconstant group).

Diversity of bacteria

Four groups of sample databases were obtained from high-throughput sequencing. All samples were screened, and repeated results were eliminated. Table 3 represents the bacterial communities' structure in different taxonomic levels. Overall, the highest and lowest bacterial contribution were observed in both control and rapid cooling of Cg, respectively. The symbiotic bacterial structure was composed of seven most dominant phyla, including Tenericutes, Protebacteria, Bacteroidetes, Firmicutes, Actinobacteria, Spirochaetae, and Cyanobacteria (Table 4). Percentage of bacterial communities of phyla is shown in Figure 4. Our finding demonstrated that generally there was no significant difference in phyla composition between control and rapid cooling in Ig, while in Cg, Tenericutes (36.3%) and Protebacteria (53.21%) were dominant phyla in control and rapid cooling treatments, respectively. The highest percentage of cyanobacteria (phototrophic bacteria) was presented in control treatment of Cg samples. Increasing the percentage of Bacteroidetes after rapid cooling in Cg was considerable (7.72% *vs.* 38.06%). Similar result was recorded in genus level in which both treatments of Ig mostly revealed parallel composition. In the other part, genera composition showed dissimilarity within Cg treatments (Figure 5). Control and rapid cooling treatments contained the highest percentage of *Spiroplasma* (36.24 *vs.* 0.14%) and *Marinifilum* (24.15 *vs.*0.00%), respectively. Additionally, the high value of *Enterococcus* (11.47 *vs.* 0.00%) was distinguished in the control treatment (Table S1). *Vibrio* which was known as a pathogen, was recorded in coral treatments, except in the control treatment of Cg although.

In genus level, PCoA represents that the first and second coordinates consist of 40.37% and 22.33% of variation and totally the two coordinates involve with 62.7% of variety (Figure 6). Our results showed that four treatments were grouped into three separated clusters consisting of Cg control, Cg rapid cooling, and another containing both treatments of Ig together. In OTUs level, the first and second PCoA coordinates contribute 32.93% and 22.81% of the variance, respectively (overall, 55.74% of total difference). Same as genus level, Cg control and Cg rapid cooling treatments were divided into two clusters, and Ig control and Cg rapid cooling treatments were placed in a single one (Figure 7).

Chao and Simpson indices evidenced that there was no significant difference in abundance communities composition of bacteria between the control and rapid cooling treatments within each group, as well as between both control treatments (Table 5). The highest and lowest values of Chao index were observed in both rapid cooling treatments in Cg (506.5 ± 73.41) and Ig (328.4 ± 33.39), respectively. Meanwhile, Simpson index presented contrary results, which the highest and lowest values belonged to cooling treatments in Ig (0.32 ± 0.09) and Cg (0.09 ± 0.02), respectively (Figure 8).

Discussion

This is the first experimental study to understand the effect of different conditions of thermal adaptation on the communities' structure of symbiotic Symbiodiniaceae and bacteria of coral in association with rapid cooling stress.

Diversity changes of Symbiodiniaceae

Diversity of symbiotic Symbiodiniaceae in host was analyzed with ITS2 marker. This method has been widely accepted and used by many researchers to compare the traditional Symbiodiniaceae classification method (Arif *et al.* 2014, Quigley *et al.* 2014, Thomas *et al.* 2014, Ziegler *et al.* 2017).

With regard to our results, Symbiodiniaceae communities were identified with OTU68, OTU49, and OTU44, out of which OTU68 had the highest abundance in all treatments. According to taxonomical relationship, Symbiodiniaceae has been classified into nine genera which formally named A-I clades (Chen *et al.* 2019). Four clades of Symbiodiniaceae, including clade A, B, C, and D have been reported as common symbiotic relationships with corals (Pochon *et al.* 2004, Ziegler *et al.* 2017). In our study, OTU68 belongs to clade C (*Cladocopium*), whereas OTU49 and OTU44 fit in clade G (*Gerakladium*) (see Pochon & Gates 2010) (Figure S2). Clade G has been recorded in stony corals with low abundance from South Chain Sea for the first time by Chen *et al.* (2019). It is the first time that we could report the existence of clade G in soft coral with high abundance from South Chain Sea. Zhou and Huang (2011) proved the dominant species of Symbiodiniaceae consisted of Clade C in stony corals surrounding the Hainan Island (South China Sea). Cooper *et al.* (2011) also reported that Clade C showed high distribution of Symbiodiniaceae in *Acropora millepora* from the Great Barrier Reef (Australia). While different latitudes represented variable communities' composition of Symbiodiniaceae in corals, the most abundance of OTUs were involved in clade C (Chen *et al.* 2019). Our study also confirmed that clade C was the dominant community.

Previous studies have documented a significant relationship between temperature extremes and dominance of clade D1 (Oliver & Palumbi 2009, Oliver & Palumbi 2011). Lajeunesse

Table 3. Number of bacteria associated with different groups in different taxonomic levels (Cg: constant group, Ig: inconstant group).

Treatment	Phylum	Class	Order	Family	Genus	Species	OTU
Cg (control)	30	59	136	244	488	696	1034
Cg (cooling)	17	36	89	173	341	451	646
Ig (control)	22	45	105	205	380	499	719
Ig (cooling)	22	40	93	192	352	463	636

Table 4. Percentage of bacterial abundance on phylum level (Cg: constant group, Ig: inconstant group).

	Treatment			
phylum	Cg (control)	Cg (cooling)	Ig (control)	Ig (cooling)
Tenericutes	36.3	0.14	54.76	55.86
Protebacteria	15.24	53.21	25.68	27.59
Bacteroidetes	7.72	38.06	6.18	3.63
Firmicutes	23.53	7.07	5.43	4.99
Actinobacteria	3.82	0.45	3.87	4.65
Spirochaetae	2.81	0.04	2.02	1.93
Cyanobacteria	6.03	0.06	0.4	0.41
Others	4.55	0.97	1.66	0.94

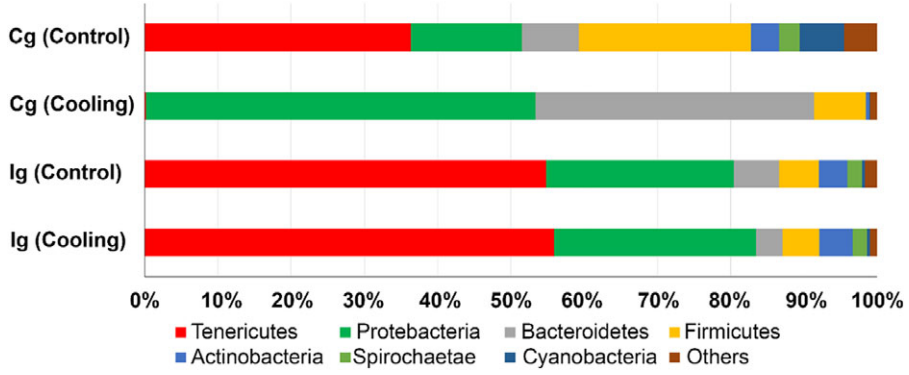

Figure 4. The OTU-based bacteria phyla profiles (Cg: constant group, Ig: inconstant group).

et al. (2010) concluded that local environmental conditions have most likely impacted the relative dominance of coral symbionts. For example, high temperature and high turbidity may cause increasing frequency of *Symbiodinium trenchi* (clade D1) in the Andaman Sea (northeastern Indian Ocean). They found that with increasing the rate of turbidity among studied localities, abundance and frequency of *S. trenchi* have increased. It was suggested that water transparency and sediment quality (especially nutrient levels) could affect on the distribution of D1 in coral hosts (Cooper *et al.* 2011). Overall it seems communities' structure of Symbiodiniaceae can be determined by environmental conditions (Chen *et al.* 2019).

Our findings evidence that inconstant thermal conditions can significantly increase the abundance of clade G. Though clade G has been identified as the lowest abundance in stony corals (Chen *et al.* 2019, LaJeunesse *et al.* 2018), high percentage of this clade in Ig was considerable. Generally there is a lack of information about the relationship between environmental factors and distribution of clade G (Chen *et al.* 2019). Ziegler *et al.* (2017) showed that the members of this clade have more potential to adapt with high temperature and salinity. In this study, although OTU68 (clade C) was reported as dominant Symbiodiniaceae in both groups, inconstant thermal conditions (22 °C–26 °C) has distinctly increased the abundance of OTU49, which belongs to clade G (35.22%–33.42% *vs.*0.5%–1.38%). Additionally, increasing of OTU44 (clade G) abundance was considerable (0.12%–0.01% *vs.* 2.44%–2.81%). Our results contradict previous observations in the Arabian Sea by Ziegler *et al.* (2017). It can be conservatively concluded that clade G might exhibit adaptive potential in relationship with temperature alterations and high/low temperature stress. Additionally, we document that clade C is sensitive to long-term temperature alterations while it cannot be affected by rapid cooling stress.

According to low values of Simpson index, the most important effect of inconstant culture condition (22 °C–26 °C) was the increased diversity of Symbiodiniaceae (Table 5). This showed that temperature changes provide more opportunities for large number of symbiotics to adapt with host corals. Meanwhile low values of

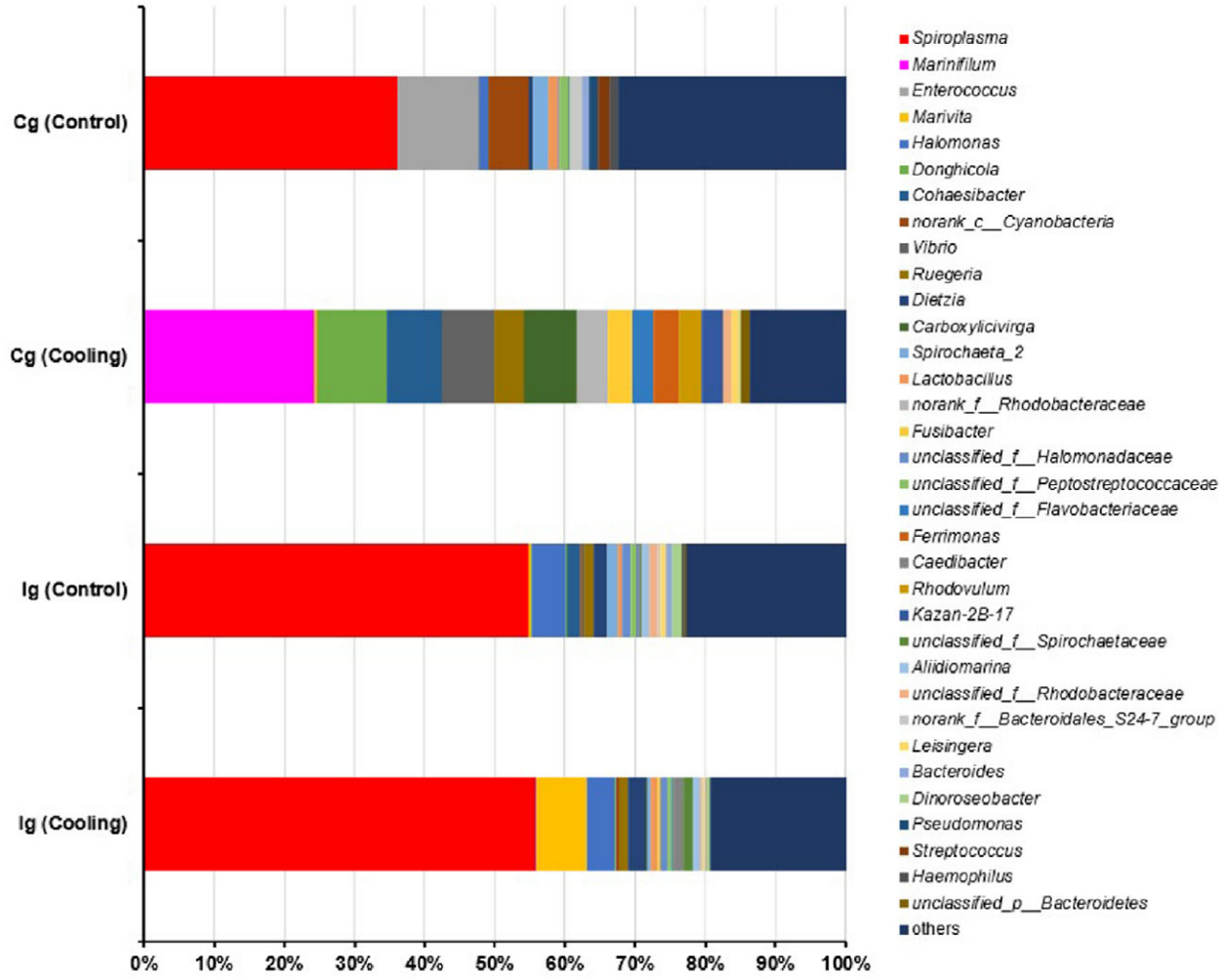

Figure 5. The OTU-based bacteria genera profiles (Cg: constant group, Ig: inconstant group).

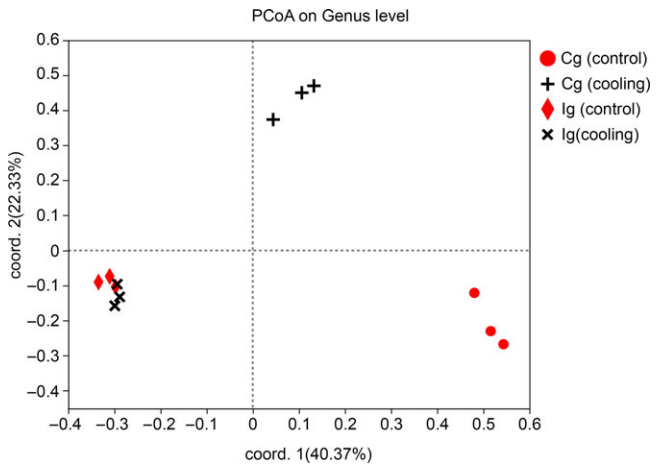

Figure 6. Relationships between bacterial diversity and groups/treatments using principal coordinate analysis (PCoA) based on the genus level.

Chao index in this group suggested that symbiotics could not reach too high abundance than constant culture conditions. This finding can be attributed to the effect of temperature changes and/or culture duration (30 days in this experiment). An extended culture duration is suggested to get further information.

PCoA indicated that both groups (constant and inconstant) completely represented different patterns so that, they were divided into two separated clusters, while there was no significant differentiation between structure and abundance of OTUs after cooling within each group. This observation suggested that symbiotic Symbiodiniaceae could change under different long-term condition but short-term rapid cooling could not change the structure of communities. In addition, long-term adaptation could play an important role to maintain diversity and abundance after a sudden decrease in water temperature.

Diversity changes of bacteria

Coral bacterial communities can perform an important practical function in the hosts, such as antibacterial activities (Kelman *et al.* 1998, Ritchie 2006), nutrient metabolism (Naumann *et al.* 2009, Wild *et al.* 2004), and nitrogen fixation (Grover *et al.* 2014, Lawler *et al.* 2016). They also can be associated with coral diseases (Egan and Gardiner 2016, Mouchka *et al.* 2010). It seems beneficial bacterial communities may support coral's adaptive potential for rapid changes (Yu *et al.* 2020, Ziegler *et al.* 2017). Symbiotic bacteria in corals perform a key duty in the nutrition

Table 5. Mean values (± SD) of Chao index and Shannon index for bacterial communities under different treatments. (Cg: constant group, Ig: inconstant group).

Treatment	Chao index	Simpson index
Cg (control)	489.1 (±221.58)	0.28 (±0.334)
Cg (cooling)	506.5[a] (±73.41)	0.09[a] (±0.027)
Ig (control)	435.3 (±109.35)	0.25 (±0.203)
Ig (cooling)	328.4[a] (±33.39)	0.32[a] (±0.097)

Same letter in each column shows significant difference ($p<0.05$).

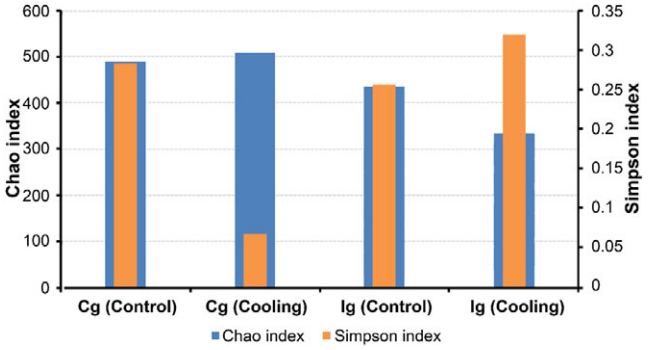

Figure 8. Mean values (± SD) of Chao index and Shannon index for bacterial communities under different treatments. (Cg: constant group, Ig: inconstant group).

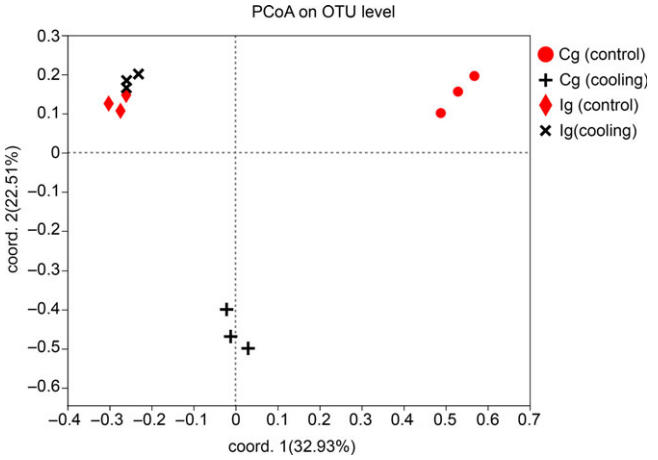

Figure 7. Relationships between bacterial diversity and groups/treatments using principal coordinate analysis (PCoA) based on the OTUs level.

resource (Lesser *et al.* 2004), energy supplying (Mao-Jones *et al.* 2010), and healthy growth of coral ecosystems (Mahmoud & Kalendar 2016). Temperature alterations represented a significant relationship with bacterial composition (Hutchins & Fu 2017). It was evidenced that change and/or replacement of bacterial communities in corals can be a positive response to adapt with temperature stress (Glasl *et al.* 2016, Reshef *et al.* 2006).

Environmental factors can modify relationships between symbiotic bacterial community and its coral hosts. Neulinger *et al.* (2008) showed that there was no indicative dissimilarity between the bacterial communities of deep-sea stony coral *Lophelia* from Gulf of Mexico and Norway. In contrast, McKew *et al.* (2012) documented that bacterial compositions of *Porites* and *Acropora* from two distinct geographical localities in Caribbean Sea (Mexico) and Indo-Pacific (Indonesia) were significantly different. In addition, samples of soft coral *Scleronephthya gracillimum* from different geographical localities showed distinguished differentiation in bacterial communities (Seonock *et al.* 2017). It is obvious that differences in environmental factors can change the communities of bacteria at different geographical areas. We suggest that differences in colouration of corals in different habitats can be attributed to communities of bacteria with different types and abundance of pigments.

Many studies aim to understand whether diseases are spread in corals by invasive pathogens or by increased symbiotic bacteria (Duvallet *et al.* 2017, Olesen & Alm 2016). Measuring bacterial diversity and abundance alone cannot supply sufficient information to make a decision for this question (Maher *et al.* 2019). To better understand the relationship between bacterial communities and coral disease, the first step is to investigate the role of environmental factors on abundance and diversity of symbiotic bacteria. Generally, increasing seawater temperatures have been linked with alterations in the bacterial composition which can cause infections and disease in corals (Seonock *et al.* 2017) but there is a lack of information about low-temperature stress.

Previous studies have been documented that Proteobacteria is typically dominated phylum in deepsea, tropical, and cold water stony corals (Kellogg *et al.* 2009, Robertson *et al.* 2016). Additionally, in some samples, Spirochaetes (van de Water *et al.* 2016) and Tenericutes (Holm & Heidelberg 2016) were the most abundant phyla. In this study, sequencing of a partial of the 16S rRNA marker provided us a dataset to identify differences in bacterial communities between different thermal culture conditions. As observed, rapid cooling could change the bacterial composition and diversity in Cg. According to our results, Tenericutes and Firmicutes have been replaced with Proteobacteria and Bacteroidetes by cooling stress, while there was non-significant change in bacterial community's structure after cooling in Ig, statistically. Furthermore, bacterial families, including Rhodobacteraceae, Vibrionaceae, Flavobacteriaceae, Burkholderiaceae, and Campylobacteraceae were formerly reported in high abundance in diseased corals (Daniels *et al.* 2015, Weiler *et al.* 2018). Within this group, pathogenicity of Rhodobacteraceae is considerable (Roder *et al.* 2014, Sunagaw *et al.* 2009). In our data, three families of Rhodobacteraceae, Vibrionaceae, and Flavobacteriaceae were reported. Interestingly abundance and diversity of these three families increased after rapid cooling in constant temperature conditions, meanwhile their increase was not noticeable in Ig.

In this study, rapid cooling treatments completely represented different consequences in different groups. While rapid cooling could change the bacterial composition in Cg, inconstant condition maintained bacterial abundance against cooling stress. Same observations were recorded using PCoA in genus and OTU levels. Both treatments of Cg were strongly divided into two clusters, and treatments of Ig were placed in single one. This evidenced that long-term temperature changes can provide suitable adaptation situations for bacterial communities to prevent the effect of short-term changes. Weiler *et al.* (2018) proved the bacterial compositions of seawater were dissimilar with communities of host corals. We cautiously suggest that environmental

alterations may cause primary symbiotic communities structure; and in this condition, bacteria in surrounding seawater cannot play an important role in new communities' composition.

Conclusion

In this study, we noted the soft coral *S. trocheliophorum* symbionts association during long-term temperature adaptation and its response to rapid cooling changes. Because examined individuals have been originated from single specimen, consequently the results can be only referred to host response to temperature changes. Our results show that microbial communities' composition is directly related to water temperature conditions. Symbiodiniaceae composition was strongly adapted with long-term temperature acclimation of hosts while there was no significant difference in their diversity and abundance after rapid cooling. On the other hand, temperature compatibility has different effects on the bacterial communities. Our findings prove that bacterial composition of *S. trocheliophorum* that cultured under constant temperature conditions can be significantly altered with rapid cooling stress while long-term acclimation under inconstant temperature conditions increased coral adaptation ability to sustain its microbial communities' structure. Overall, it can be hypothesised that long-term adjustment following inconstant thermal state in nature provides adaptive conditions which may protect Symbiodiniaceae and bacterial communities of corals against rapid temperature decreasing during seasonal typhoons and internal waves. Although rapid cooling stress has been suggested as a key factor in increasing the incidence of disease in corals via increasing abundance and diversity of pathogenic bacteria, long-term acclimation under temperature range can greatly reduce the risk of infection after rapid cooling stress rather than constant thermal conditions.

In conclusion, temperature changes in the experimental states can provide better symbiotic adaptation between microbial communities and corals. This adaptation can play a protective role for Symbiodiniaceae and bacterial compositions against cold stress and can protect corals from disease. Additional experimental studies on different thermal conditions are needed to get more information about the relationship between thermal adaptations and environmental stress on coral microbial communities' structure.

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