REPORT

Different responses of scleractinian coral *Acropora pruinosa* from Weizhou Island during extreme high temperature events

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Abstract Ecological surveys observe coral "winners" and "losers" in global coral bleaching events. However, the key contributors to holobiont tolerance and interactions between symbionts remain unclear. Herein, we compared bleaching and unbleaching *Acropora pruinosa* corals from Weizhou Island, during an extreme high-temperature event in the northern South China Sea in 2020. We found the dominant Symbiodiniaceae subclade in the bleaching and unbleaching corals to be C1; however, the density of Symbiodiniaceae in the latter was significantly higher than that in the former. Additionally, the symbiotic bacteria α diversity in the unbleaching coral was significantly higher than that in the bleaching coral, with a reorganized bacterial community structure. Core microbiome analyses revealed 55 bacterial core operational taxonomic units (OTUs), of which 10 were significantly differentially enriched between the two coral groups. The significantly enriched bacterial core OTUs in the unbleaching coral were primarily nitrogen cycling related, while those enriched in the bleaching coral were associated with antimicrobial activity. RNA-Seq analyses revealed that significantly upregulated genes in the bleaching coral were primarily associated with diseases and autophagy, while those in the unbleaching coral were associated with immune defense and maintenance of the symbiotic relationship between

corals and symbionts. We propose that the differences in tolerance of *A. pruinosa* result from the cooperation between coral host, Symbiodiniaceae, and symbiotic bacteria. In extreme high-temperature events, unbleaching corals may maintain stable symbiotic relationships by increasing the diversity of symbiotic bacteria, regulating the structure of the symbiotic bacteria community, improving the interaction between coral host and symbiont and enhancing host immunity, thus avoiding coral bleaching. This study illuminates the relationship between the coral symbiont and tolerance differences of coral holobionts, providing new insights for further exploration into the adaptability of scleractinian corals in the context of global warming.

Introduction

Some corals are "winners" while others are "losers" in major stress events (Loya et al. 2001). In the coral bleaching event, obvious differences exist in the bleaching time of different corals, with some corals not bleaching from beginning to end (Palmer 2018). This interesting ecological phenomenon suggests potential for coral "winners" to adapt to future climate change. However, with the increasing frequency of coral bleaching, it remains unclear whether reefs can recover. Further, the specific adaptation mechanism underlying annual coral bleaching requires further investigation (Andréa et al. 2014).

To date, numerous studies have investigated tolerance differences among different coral species or within geographic regions. However, much remains unknown

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regarding why the same coral can survive or die during extreme high-temperature events, and which key factors lead to these tolerance differences. The holobionts of scleractinian coral are composed of the coral host and multiple symbionts, including Symbiodiniaceae and bacteria (Brener-Raffalli et al. 2018). Alterations in commensal bacterial diversity, community structure reorganization, and dominant bacteria promote increased tolerance of coral symbionts (Liang et al. 2017; Qin et al. 2020a; Yu et al. 2020a, b, 2021b; Chen et al. 2021). Moreover, Symbiodiniaceae types are associated with the environmental adaptability of scleractinian corals in the South China Sea (Chen et al. 2019; Qin et al. 2019). Meanwhile, higher heat tolerance is related to coral host tissue thickness, trophic status (Xu et al. 2021), non-coding RNA, and host transcriptional response (Qin et al. 2020b; Yu et al. 2020a, b, 2021a). The differential susceptibilities of coral taxa during and after bleaching events are only partially explained by a single symbiont (Palmer et al. 2010). These different evolution rates of various components of the coral holobiont must be considered when studying the response of corals to environmental perturbations, including climate change (van Oppen and Medina 2020), which will provide more comprehensive, scientific and generalizable results (Palmer 2018).

Weizhou Island, in the northern margin of the South China Sea, was viewed as a potential refuge for scleractinian corals in response to climate change (Yu et al. 2019). As such, it provides a study system to reveal the coral host and symbionts interact and are affected by environmental change, as well as potential regulatory factors affecting coral survival in abnormal high-temperature events. However, in the summer of 2020, an abnormal high-temperature event occurred in the northern South China Sea with disastrous effects on the local coral reefs. Although coral bleaching was extensive, there were considerable differences in colony survival, especially *Acropora pruinosa*. In order to reveal potential molecular mechanisms underlying differences in thermal tolerance of scleractinian coral "winners" in abnormal high-temperature events, we collected bleaching and unbleaching *A. pruinosa* coral samples from this unusual event. *A. pruinosa*, widely distributed in tropical and subtropical regions of the Indian Ocean and the Pacific Ocean, is one of the most vulnerable genera to climate change (Palacio-Castro et al. 2021). A large number of indoor and outdoor studies related to coral thermal tolerance have also been conducted in previous study (Yu et al. 2020a, b, 2021b), this further implies that it is an ideal research object of this study. Using next-generation sequencing technology, we compared the different responses among coral host, Symbiodiniaceae, and symbiotic bacteria. The primary research questions were as follows: (1) Which component is the main contributor to coral "winners" adapting to extreme high temperature? and (2) Are there correlations among the components of coral "winners"? Addressing these queries will provide insights into the adaptive mechanism of coral "winners" to extreme high temperature, thus improving our understanding of the potential adaptability of corals at relatively high latitudes to future climate change.

Materials and methods

Environmental characteristics data

To showcase the abnormal high-temperature event in 2020 and bleaching levels of Weizhou Island (Fig. 1A) we used a set of heat stress gauges developed by the National Oceanic and Atmospheric Administration (NOAA) Coral Reef Watch (CRW) (https://coralreefwatch.noaa.gov/). We also used NOAA satellite data from Hainan Island, the location nearest Weizhou Island, to analyze abnormally high seawater temperature and pressure level of coral reefs in Weizhou Island from 1985 to 2021 (Fig. 1B). Data on Sea Surface Temperature (SST) (°C) (Fig. 1C), degree heating week (DHW) (Fig. 1D), and bleaching alert area around Weizhou Island (Fig. 1E) were collected. Collectively, it was confirmed that an abnormal high-temperature event occurred in Weizhou Island in the summer of 2020.

Coral sample collection

Bleaching and unbleaching samples of *A. pruinosa* from Weizhou Island (N21° 8.27′, E109° 12.6′) were collected by a SCUBA diver using a hammer and chisel in August 2020 (Fig. 1F, 1G). All corals were sampled from the same depth (approximately 3–5 m in depth). Morphologically distinct *A. pruinosa* colonies were collected to minimize the chances of sampling the same ramet more than once (Chen et al. 2020). However, the selected coral samples were within 10 m of each other to ensure consistency. Branching samples (approximately 5 cm in length) were collected from each coral. Strict sterile techniques were used in the sample collection of this study, such as wearing personal protective equipment, using sterile tools and containers. The entire sampling process was completed in the shortest possible time. To prevent contamination, the coral was rinsed with 0.2 μm filter-sterilized seawater to remove loosely attached microbes from the coral tissue. Next, the coral was subsampled into 5 mL cryogenic vials. All samples were frozen in liquid nitrogen and stored at −80 °C.

Fig. 1 Study area, environmental characteristic data, and representative images of coral fragments from each treatment. **A** Study area in the South China Sea (SCS). **B** Bleaching alert levels. **C** Sea surface temperature. **D** Degree heating weeks. **E** Bleaching alert area. **F** Unbleaching coral. **G** Bleaching coral
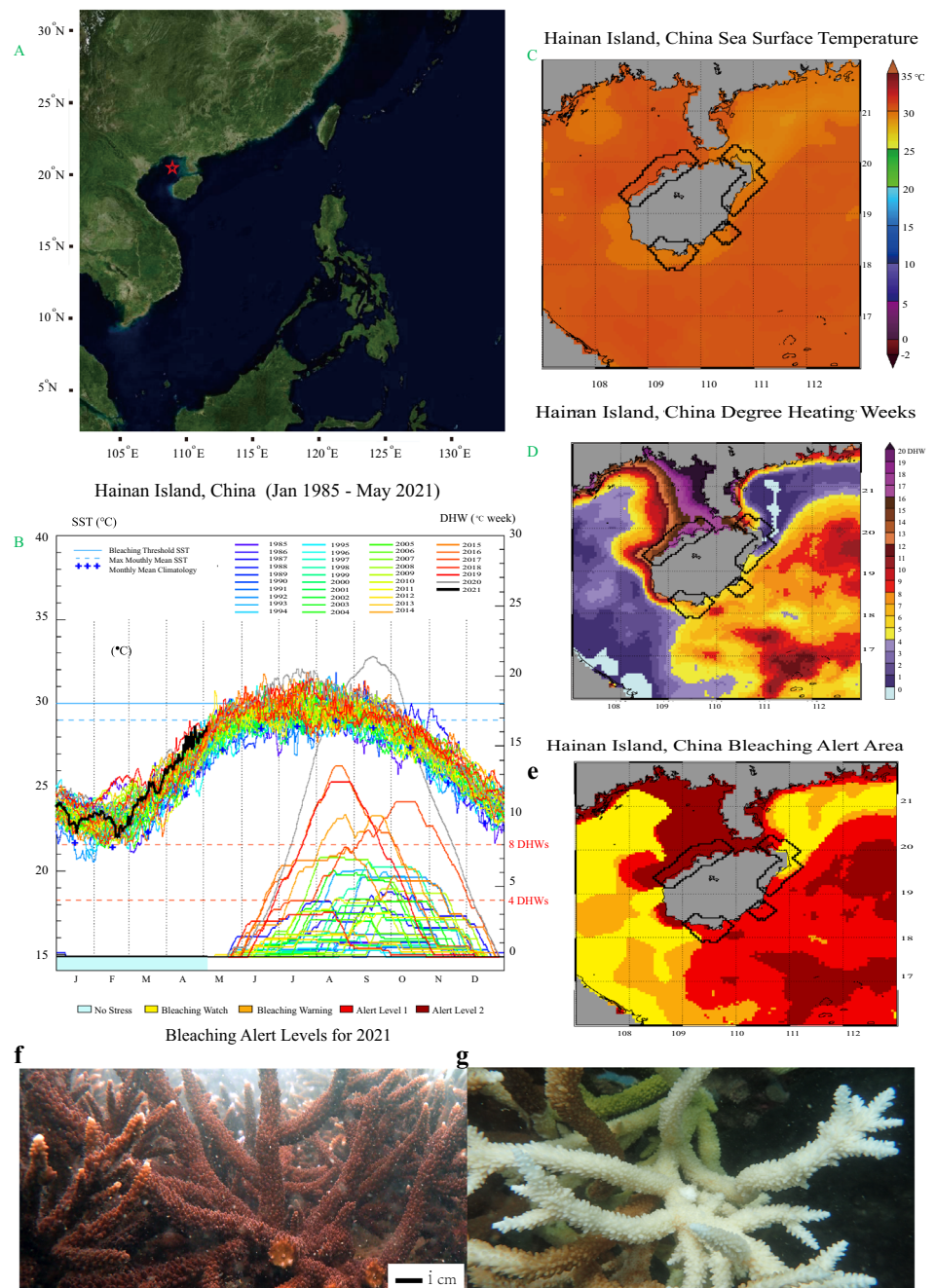
DNA extraction and metagenome sequencing

The frozen fragments were crushed quickly at low temperatures, and the total genomic DNA was extracted from the whole coral fragments of 14 coral colonies using the TIANamp Marine Animals DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's protocols. After DNA quality inspection, PCR amplification was performed using previously reported primers (338F and 806R of bacterial 16S rRNA V3V4) and cycling conditions (Liang et al. 2017). Purified amplicons were

sequenced using the Illumina MiSeq platform (Illumina, San Diego, USA). Raw data submitted to NCBI Sequence Read Archive (SRA) (PRJNA732278).

The demultiplexed raw sequencing reads of the 16S rRNA gene were quality-filtered using the software Trimmomatic. Next, FLASH was used to merge quality-filtered reads. First, reads shorter than 50 bp, with ambiguous characters, or truncated 300 bp reads over a 50 bp sliding window with an average quality score < 20 at any site, were discarded. Second, the maximum number of mismatches was set to 0.2 and the overlapping sequences were

assembled with a length > 10 bp. Reads that could not be assembled were discarded. Samples were distinguished based on barcodes and primers and the sequence direction was adjusted.

UPARSE software (version 7.1) was used to identify and remove the chimeric sequences of operational taxonomic units (OTUs), the similarity threshold was 97% (Edgar 2010). The taxonomic analyses were based on high-quality reads. RDP Classifier software was used to analyze the taxonomy of each OTU representative sequence, the confidence threshold was set as 70%, the SILVA 16S rRNA database (SSU128) was used (Edgar 2010). Coverage, ACE estimator, and α-diversity were determined using MOTHUR software (Schloss et al. 2011). Microbial community β-diversity, non-metric multidimensional scaling (NMDS) was calculated based on the Bray–Curtis distance matrix using rarefied OTU abundance tables and visualized (Zaneveld et al. 2017). Analyses of similarities (ANOSIM) and partial least-squares discriminant analyses (PLS-DA) were used to determine microbial community differences based on the Bray–Curtis distances with 999 permutations (Ziegler et al. 2017a). Statistically different biomarkers between different groups were searched using the LEfSe method with a linear discriminant analyses score > 3 (Segata et al. 2011). The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to determine the predictive metagenomes of the symbiotic bacterial populations in each coral sample using 16S rRNA sequences (Langille et al. 2013). Considering that the core bacterial microbiome is associated with the environmental responses and ecological function of coral holobionts (Chen et al. 2021), we assessed changes in core microbial abundance to elucidate the potential molecular mechanism underlying different responses of scleractinian coral A. pruinosa from Weizhou Island during extreme high-temperature events (Chen et al. 2021; Yu et al. 2021b). QIIME2 was used for the core microbiome bacteria determination (Bolyen et al. 2019). According to previous studies, the members present in more than 80% of the sample were considered to be microbiome bacteria (Hernandez-Agreda et al. 2017; Chen et al. 2021).

Symbiodiniaceae clade type determination

After rapidly crushing the frozen fragments at low temperatures, genomic DNA was extracted from the 10 whole coral samples using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). After quality and purity evaluation, the internal transcribed spacer 2 (ITS2) sequences were amplified from the extracted DNA using the primers ITSintfor2 and ITS2-reverse, as previously described (Chen et al. 2019). Purified amplicons were sequenced using the Illumina MiSeq platform (Illumina, San Diego, USA). Raw data submitted to SRA (PRJNA732290).

The Symbiodiniaceae diversity and community composition were analyzed based on the ITS2 sequence (Ziegler et al. 2017b; Chen et al. 2019). To ensure high-quality reads, a read tail mass value < 20 was filtered using the Trimmomatic software (Bolger et al. 2014). The consolidated PEAR data were used to obtain the full-length ITS2 rDNA fragments (Zhang et al. 2014). MOTHUR was used to trim read quality and detect chimeras. Reverse and forward primer sequences were trimmed using CUTADPAT with sequence identity cut-off set to 100% (Chen et al. 2019). Due to the limitations of the *ITS2* gene as a multicopy marker, sequence-based OTU analyses and ITS2 analyses were used to reveal symbiotic Symbiodiniaceae diversity and community composition in different *A. pruinosa* samples. Using the previously described parameters and pipeline settings, all sequences were aligned with the ITS2 database using BLASTn (Ziegler et al. 2017b; Chen et al. 2019). ITS2 sequences, present at a minimum cut-off > 5% in at least one sample and filtered, were subsampled using MOTHUR (https://www.mothur.org) (1000 reads per sample) (Ziegler et al. 2017b). With sequence similarity set to 97%, sequences with a retention length > 90% were clustered into an OTU (Arif et al. 2014; Thomas et al. 2014; Ziegler et al. 2017b). After removing non-Symbiodiniaceae OTUs, the most abundant OTU sequence was considered as the representative sequence (Arif et al. 2014). The statistical assessment of Symbiodiniaceae diversity and community composition was based on the selected sequences.

RNA-seq analyses

Total RNA was extracted from 10 frozen coral samples using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Qualified RNA samples were used to prepare RNA-seq transcriptome libraries. Following the default stranded RNA protocol of the HiSeq 4000 instrument (Illumina), high-quality RNA was prepared and sequenced with equal quantities, generating 150 nt-long paired-end reads. Raw data submitted to SRA (PRJNA732840).

SeqPrep and Sickle were used to trim the raw paired-end reads and verify their quality. The Trinity program default parameters were used to assemble all cleaned reads de novo (Manfred et al. 2011), which were then mapped to the assembled unigenes (Tanwar et al. 2017). Six databases (Pfam, NR, COG, Swiss-Prot, KEGG, and GO) were used for unigene annotations via BLAST search (Evalue = 1×10^{-5}) (Camacho et al. 2009). Sequences of corals and Symbiodiniaceae were distinguished based on previous studies (Tang et al. 2018; Yu et al. 2020a), and the BLASTx was referenced to the reported genomes of

Symbiodiniaceae *Symbiodinium microadriaticum* (Aranda et al. 2016), and corals *Orbicella faveolata* (Prada et al. 2016), *Stylophora pistillata* (Voolstra et al. 2017), and *Acropora digitifera* (Shinzato et al. 2011). These transcripts were used in subsequent analyses. Based on the expression matrix, the samples were analyzed using correlation analyses and principal component analyses (PCA). The transcripts per million (TPM) was used to measure transcript and gene expression level using RSEM software (Li and Dewey, 2011). DESeq2 software identified differentially expressed genes (DEGs) from bleaching and unbleaching coral samples (FDR < 0.05) (Love et al. 2014). GO enrichment analyses were performed using software Goatools (version 0.6.5) (Benjamini–Hochberg adjusted *p*-value < 0.05) (Klopfenstein et al. 2018). KEGG annotation and enrichment analyses were performed according to default parameters using KOBAS software (Wu et al. 2006). The over-representation analyses (ORA) using Fisher's exact test was used for KEGG and GO enrichment of DEGs analyses (Benjamini–Hochberg adjusted *p*-value < 0.05) (Backes et al. 2007).

Results

Changes in coral-associated bacterial communities

A total of 2,213 OTUs were assigned from 570,002 processed bacterial sequences after subsampling for equal sequencing depth (Table 1, Supplementary Table S1). The mean length of the bacterial sequences was 417 bp (Table 1). Good's coverage accounted for $\geq$ 99.88% of the diversity and near-saturation of the rarefaction curve suggested that the sequencing results represented the true condition of symbiotic bacteria in the coral samples (Table 1). The mean numbers of OTUs were 351 $\pm$ 56 and 373 $\pm$ 67 in bleaching and unbleaching groups, respectively. The Chao and Ace indices OTU richness did not differ significantly between the two groups, while the Simpson index suggested a significant difference in microbial α-diversity (Table 1).

The NMDS analyses results indicated significant differences between the bacterial community structures of the bleaching and unbleaching groups (ANOSIM, stress = 0.13, R = 0.1312, p = 0.04; Fig. 2A). Furthermore, PLS-DA revealed different microbiota associated with the bleaching and unbleaching corals (Fig. 2B).

The most dominant OTUs in both the bleaching and unbleaching coral groups were *Ralstonia* sp. (OTU2147) and *Rhodococcus* (OTU2162) (Fig. 3A, Fig. 3B). The LEfSe method (LDA > 3.0; p < 0.05; Fig. 3C) identified 20 bacterial OTUs associated with bleaching coral samples.

To further analyze the function of symbiotic bacteria in the coral holobiont, we identified 55 bacterial core OTUs associated with all coral samples (Supplementary Table S2, Fig. 4A, Fig. 4B). Core microbiomes accounted for 70.99% and 66.66% of the bacterial abundance in the bleaching and unbleaching coral groups, respectively. Among the core bacteria, *Ralstonia* sp. (OTU2147) and *Rhodococcus* sp. (OTU2162) predominated, comprising 50.85% and 23.63% of the core microbial community in the bleaching coral groups, and 39.39% and 20.94% in the unbleaching coral groups, respectively.

To characterize functional changes in the symbiotic microbiomes, PICRUSt was used to predict the functional composition profiles based on the 16S rRNA sequencing data (Supplementary Table S3, Table S4). We identified 41 s-level functional categories in KEGG. Furthermore, four mean relative abundances differed significantly between the bleaching and unbleaching coral groups. The pathways enriched in the unbleaching coral group highlighted metabolism of cofactors and vitamins and energy metabolism pathway. In contrast, the excretory system and immune system diseases pathway were enriched in the bleaching coral group.

Changes in Symbiodiniaceae community structure

Within the Symbiodiniaceae community, *Cladocopium* was the dominant genera in all coral samples (Fig. 5). At the subclade level, clade C1 was the dominant Symbiodiniaceae (mean relative abundance, 83.93%). In addition, clades C1ca, C1p, C72, Cspc, and C1g were present in low abundance. Hence, the community structure of symbiotic Symbiodiniaceae in the different groups is stable at the

Table 1 A 16S rRNA gene sequencing data

Estimators	bleaching-Mean	bleaching-Sd	unbleaching-Mean	unbleaching-Sd	*P* value
No. of sequences	37,421	5074	44,007	3221	
Sobs	350.57	55.503	373.43	67.236	
Ace	358.29	57.432	381.05	69.728	
Chao	363.39	58.545	383.33	71.233	
Simpson	0.16409	0.032283	0.10971	0.040097	*
Coverage	0.99937	0.00026512	0.99935	0.00022025	

Fig. 2 Composition and diversity differences in bleaching and unbleaching corals at operational taxonomic unit (OTU) level. **A** NMDS. **B** Partial least-squares discriminant analyses (PLS-DA) score plots
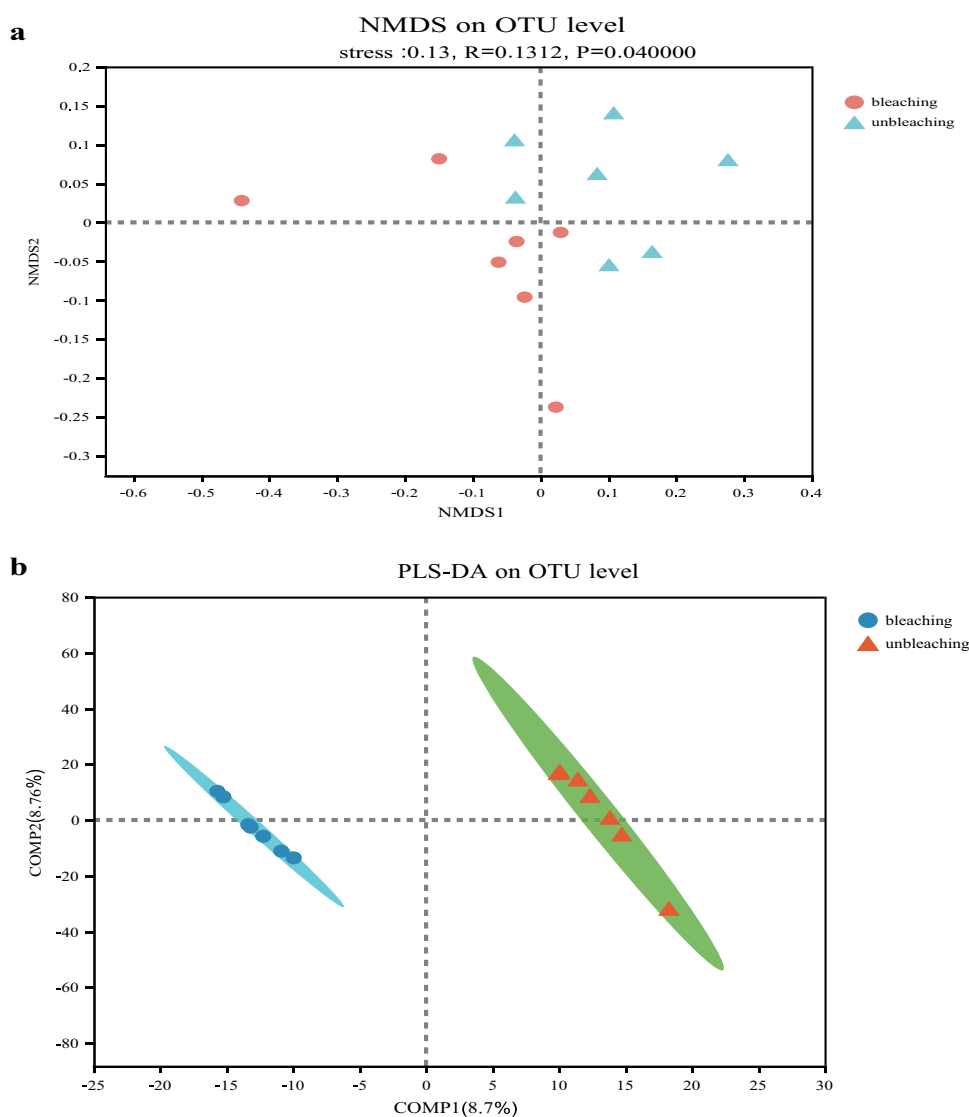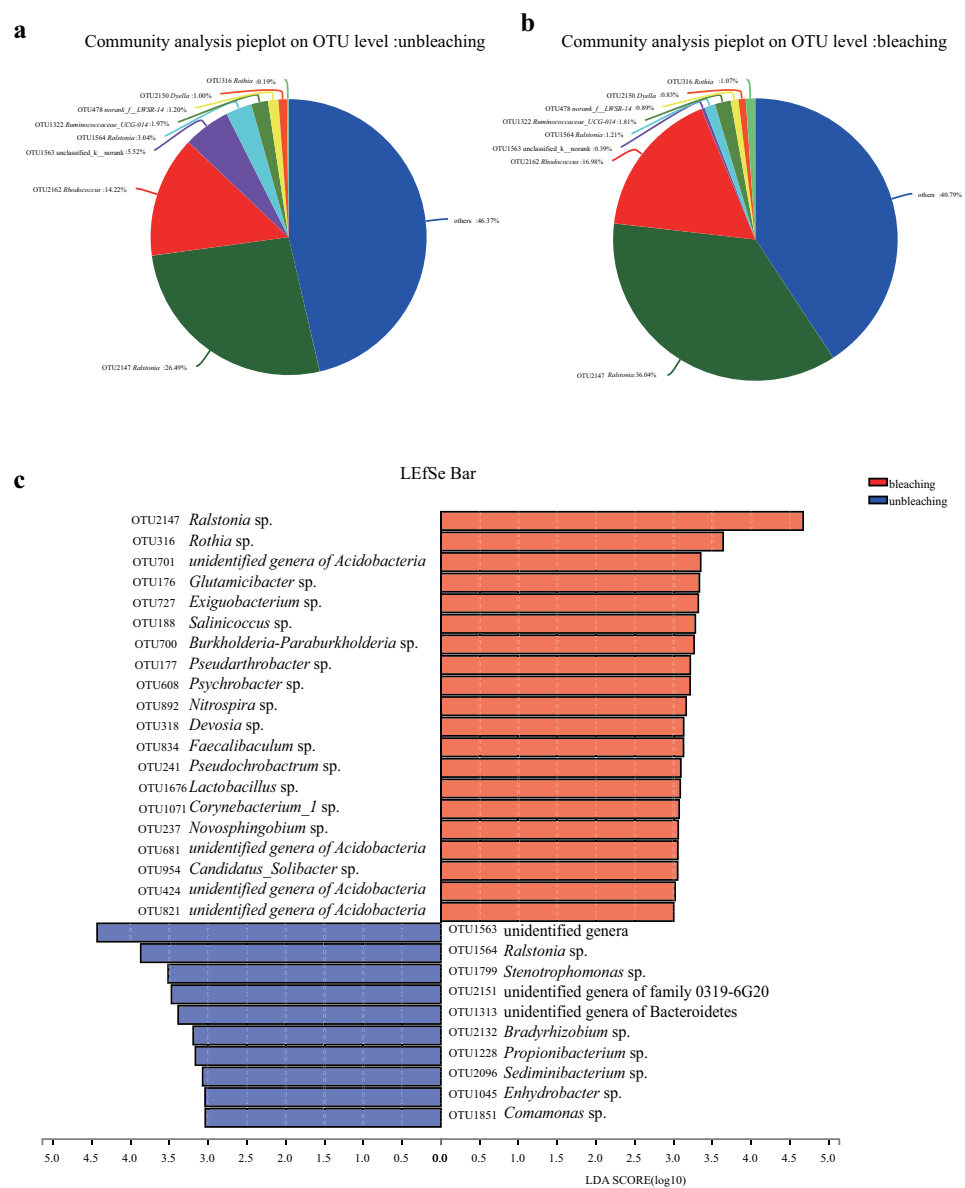

compositional and relative abundance levels, although small differences exist across samples.

Analyses of gene expression response of the bleaching and unbleaching coral

Ten high-quality metatranscriptome libraries of *A. pruinosa* were established using Illumina technology. We obtained 524,239,848 raw reads (Supplementary Table S5). Functional information of genes as showed in Supplementary Table S6. After removing ambiguous reads, adapters, and low-quality sequences, we assembled 519,905,138 high-quality clean reads into 104,482 unigenes, with an N50 length of 2017 nt and an average length of 948 nt (Supplementary Table S7). The mapped rate to the coral host of each sample was 29.76–66.30%, while that to Symbiodiniaceae was 16.56–55.20% (Supplementary Table S8, Table S9). RSEM was used to analyze the expression levels of 104,482 host and 40,982 Symbiodiniaceae unigenes (Supplementary Table S10, Table S11). PCA plots of unigene expression levels demonstrated that unbleaching and bleaching coral groups were clearly distinguished, accounting for 43.98% and 8.53% of the total variance, respectively (Fig. 6A). The variation between samples in different groups was greater than that between samples in the same group (Fig. 6B). From the DEGs with adjusted *p*-values < 0.001 (FC ≥ 20, Bonferroni), we discovered 3326 (1441 upregulated and 1885 downregulated) differentially expressed coral genes (Fig. 6C) and 109 (41 upregulated and 68 downregulated) differentially expressed Symbiodiniaceae genes (Fig. 6D) in the bleaching group compared with the unbleaching group (Supplementary Table S12, Table S13). All DEGs were subjected to GO and KEGG enrichment analyses (Benjamini-Hochberg) (Supplementary Table S14–Table S21) (adjusted *p*-value < 0.05). Significantly upregulated coral genes in the

Fig. 3 Microbial community composition based on significantly different operational taxonomic units (OTUs). Microbial composition of **A** Unbleaching group and **B** Bleaching group at the genus level. Others denote those OTUs with abundances < 0.01%. **C** Enriched bacterial OTUs of both groups. Linear Discriminant Analyses score > 3

a Community analysis pieplot on OTU level :unbleaching

b Community analysis pieplot on OTU level :bleaching

c LEfSe Bar

bleaching group were assigned to 8 KEGG pathways (Fig. 7A), while significantly downregulated coral genes in the bleaching group were assigned to 3 and 27 significantly enriched GO and KEGG pathways, respectively (corrected p-value < 0.05; Fig. 7B, Fig. 7C). However, significantly differentially expressed Symbiodiniaceae genes were not associated with enriched GO or KEGG pathways.
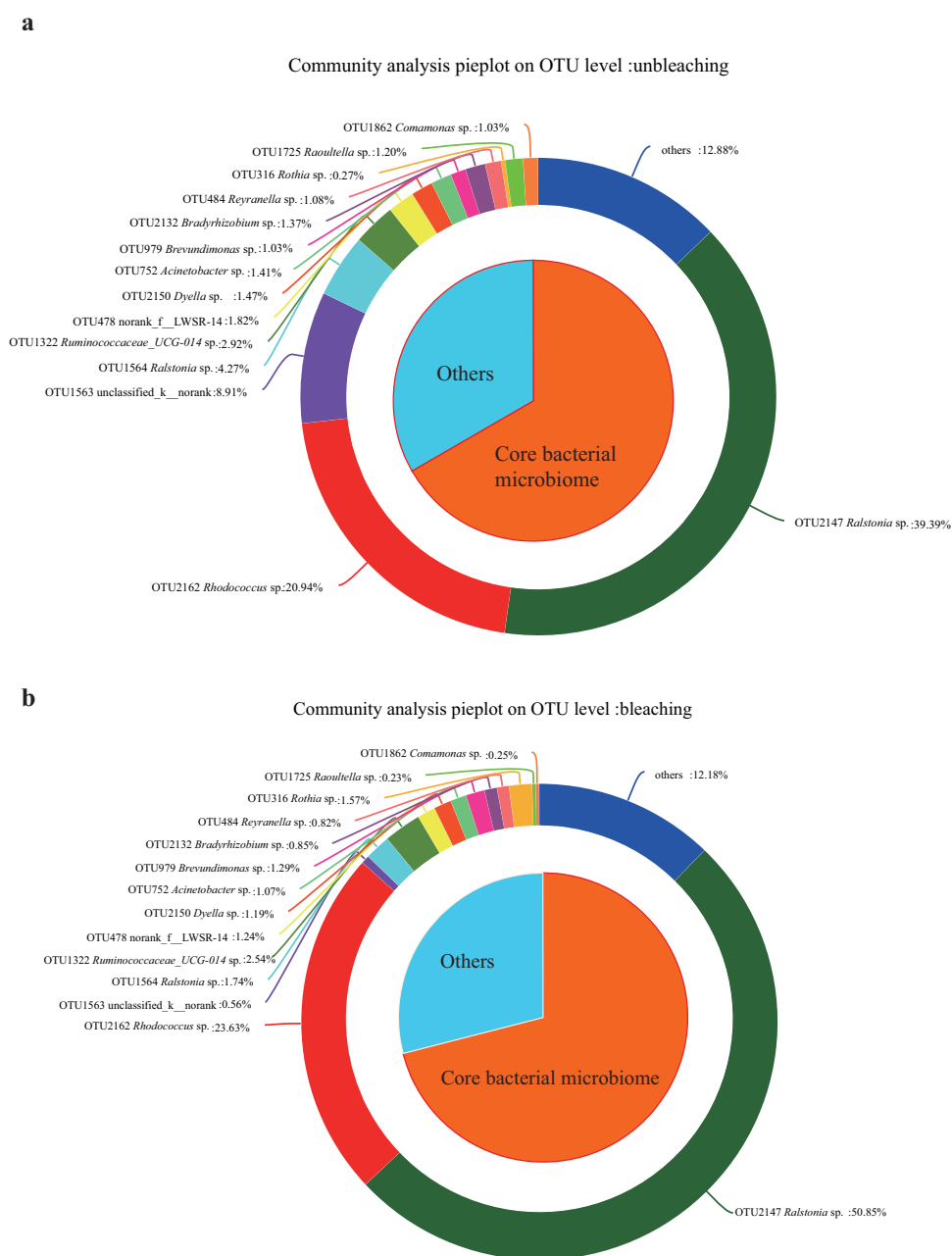
Discussion

Herein, we collected coral samples of bleaching and unbleaching *A. pruinosa* from Weizhou Island during a large-scale coral bleaching event in 2020 and studied the

underlying mechanisms that led to these different fates from the perspective of whole coral holobionts.

Community structure reorganization of symbiotic bacteria may be related to tolerance differences

Symbiotic bacteria are an important part of the scleractinian coral holobiont and participate in many important biological processes (Ziegler et al. 2017b). Here, we compared the α-diversity, community structure, and bacterial core OTUs associated with coral samples. NMDS and PLS-DA analyses revealed different microbiota associated with bleaching and unbleaching coral samples, with recombined community structures and different relative abundances of symbiotic bacteria between the two coral

Fig. 4 Relative abundance and composition of the core bacterial microbiomes. The inner pie chart shows the proportional core microbiome in coral bacterial community composition; the outer pie chart illustrates the core bacterial microbiome composition in coral holobionts. Others denote operational taxonomic units (OTUs) with abundances < 0.01%.
A Unbleaching group.
B Bleaching group

a Community analysis pieplot on OTU level :unbleaching

OTU1862 *Comamonas* sp. :1.03%
OTU1725 *Raoultella* sp. :1.20%
OTU316 *Rothia* sp. :0.27%
OTU484 *Reyranella* sp. :1.08%
OTU2132 *Bradyrhizobium* sp. :1.37%
OTU979 *Brevundimonas* sp. :1.03%
OTU752 *Acinetobacter* sp. :1.41%
OTU2150 *Dyella* sp. :1.47%
OTU478 norank_f__LWSR-14 :1.82%
OTU1322 *Ruminococcaceae_UCG-014* sp. :2.92%
OTU1564 *Ralstonia* sp. :4.27%
OTU1563 unclassified_k__norank :8.91%
others :12.88%
OTU2147 *Ralstonia* sp. :39.39%
OTU2162 *Rhodococcus* sp. :20.94%
Others
Core bacterial microbiome

b Community analysis pieplot on OTU level :bleaching

OTU1862 *Comamonas* sp. :0.25%
OTU1725 *Raoultella* sp. :0.23%
OTU316 *Rothia* sp. :1.57%
OTU484 *Reyranella* sp. :0.82%
OTU2132 *Bradyrhizobium* sp. :0.85%
OTU979 *Brevundimonas* sp. :1.29%
OTU752 *Acinetobacter* sp. :1.07%
OTU2150 *Dyella* sp. :1.19%
OTU478 norank_f__LWSR-14 :1.24%
OTU1322 *Ruminococcaceae_UCG-014* sp. :2.54%
OTU1564 *Ralstonia* sp. :1.74%
OTU1563 unclassified_k__norank :0.56%
OTU2162 *Rhodococcus* sp. :23.63%
others :12.18%
OTU2147 *Ralstonia* sp. :50.85%
Others
Core bacterial microbiome

groups. Similarly, significant differences in symbiotic bacteria were found in coral samples with differential tolerance to environmental stress and disease (Ziegler et al. 2017a; Yu et al. 2020a, b; MacKnight et al. 2021). *Ralstonia* sp. (OTU2147) and *Rhodococcus* (OTU2162) were the dominant bacteria species in both groups; however, their relative abundance differed significantly between groups. LEfSe analyses identified 20 and 10 bacterial OTUs associated with bleaching and unbleaching corals, respectively, thus, further confirming the phenotype plasticity of coral symbiotic bacteria. Symbiotic bacteria have the ability to change more rapidly than coral hosts, which

may help corals with long life-spans adapt to climate change (Ziegler et al. 2017a).

Therefore, microbial adaptation is a potential mechanism for coral holobionts to counteract the impact of environmental changes. According to the coral probiotics' hypothesis, a dynamic relationship between coral symbiotic microorganisms and environmental conditions promotes the most favorable symbiotic bacteria for coral holobionts (Reshef et al. 2006). Furthermore, the α-diversity of the bleaching corals was significantly lower than that of the unbleaching corals. Previous studies have found that coral symbiotic bacterial α-diversity increased under

Fig. 5 Relative abundance of different Symbiodiniaceae subclades in coral samples. Others denote subclades with abundances < 0.05%. **A** Unbleaching group pie chart. **B** Bleaching group pie chart. **C** Bar plot of all coral samples
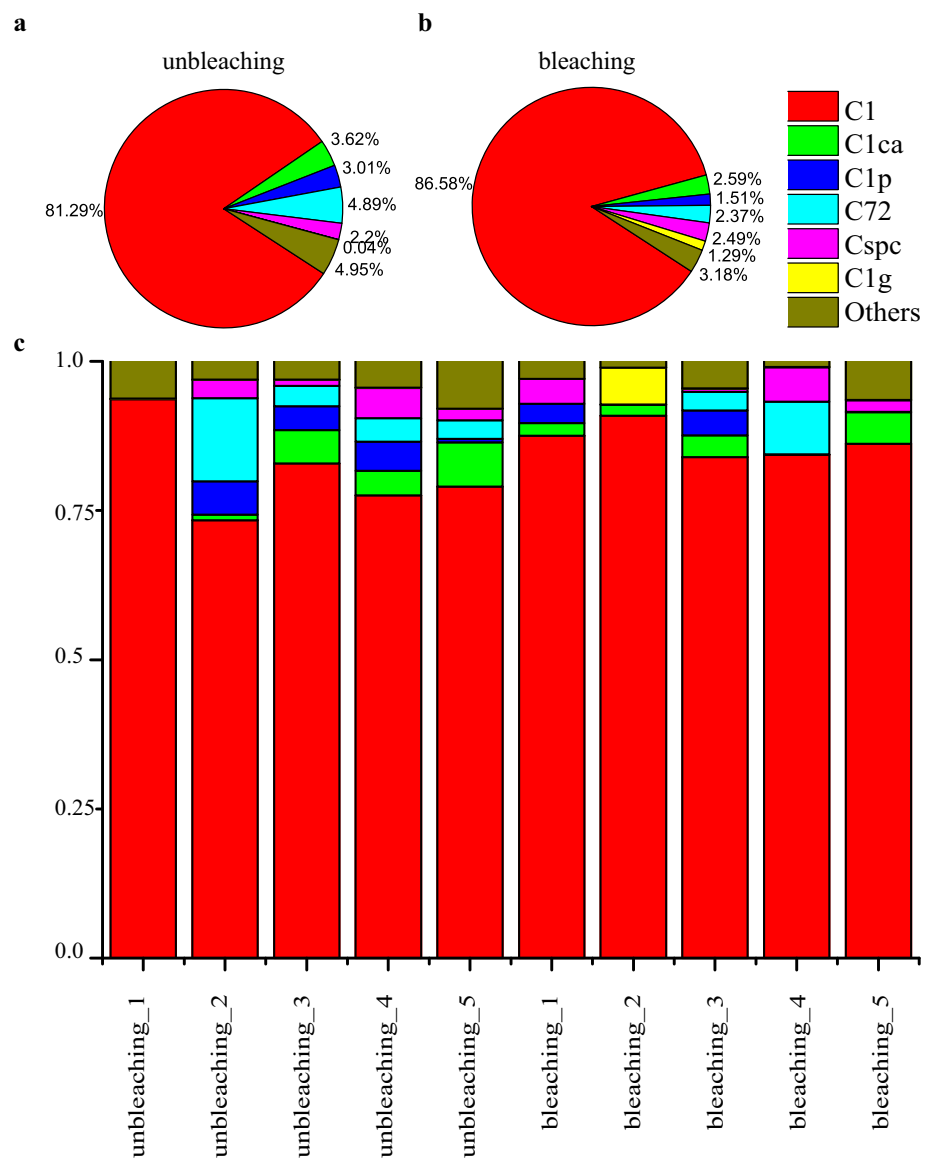

various adverse conditions (Jessen et al. 2013; Pratte et al. 2018; Oppen and Medina, 2020; Yu et al. 2020a, b). High abundance and diversity of symbiotic bacteria also allow coral hosts to transform their major symbiotic bacteria to facilitate the establishment of an improved symbiotic relationship (Pollock et al. 2019). Thus, higher diversity may help maintain the physiological function of the whole organism, and may partially account for the higher tolerance (Yu et al. 2020a, b).

To further explore the role of symbiotic bacteria in differential tolerance, we analyzed differences in the core bacterial OTUs, revealing the relative abundance of the core bacterial microbiome of *A. pruinosa* shifted from 70.99% (bleaching) to 66.66% (unbleaching), suggesting that the core microbiome is critical for the health of coral symbiosis. The dominant core OTUs of the two groups

were consistent, although their relative abundance differed significantly. Combining the LEfSe and core bacterial microbiome analyses, we found that the core bacterial OTUs *Rothia* sp. (OTU316), *Glutamicibacter* sp. (OTU176), *Pseudarthrobacter* sp. (OTU177), *Psychrobacter* sp. (OTU608), and an unidentified genus of *Acidobacteria* (OTU424) were significantly enriched in the bleaching group, whereas *Ralstonia* sp. (OTU1564), *Bradyrhizobium* sp. (OTU2132), *Propionibacterium* sp. (OTU1228), *Enhydrobacter* sp. (OTU1045), and an unidentified genus (OTU1563) were significantly enriched in the unbleaching group. The core bacterial OTUs significantly enriched in the unbleaching groups were mainly associated with nitrogen cycling function, while those in the bleaching groups were associated with antimicrobial activity. *Ralstonia* sp. (OTU1564), a keystone species in

Fig. 6 Comparison between unbleaching group and bleaching group. **A** PCA. **B** Heat map. Differentially expressed coral genes **C** and Symbiodiniaceae genes **D** (red = upregulated, grey = nosig, green = downregulated)
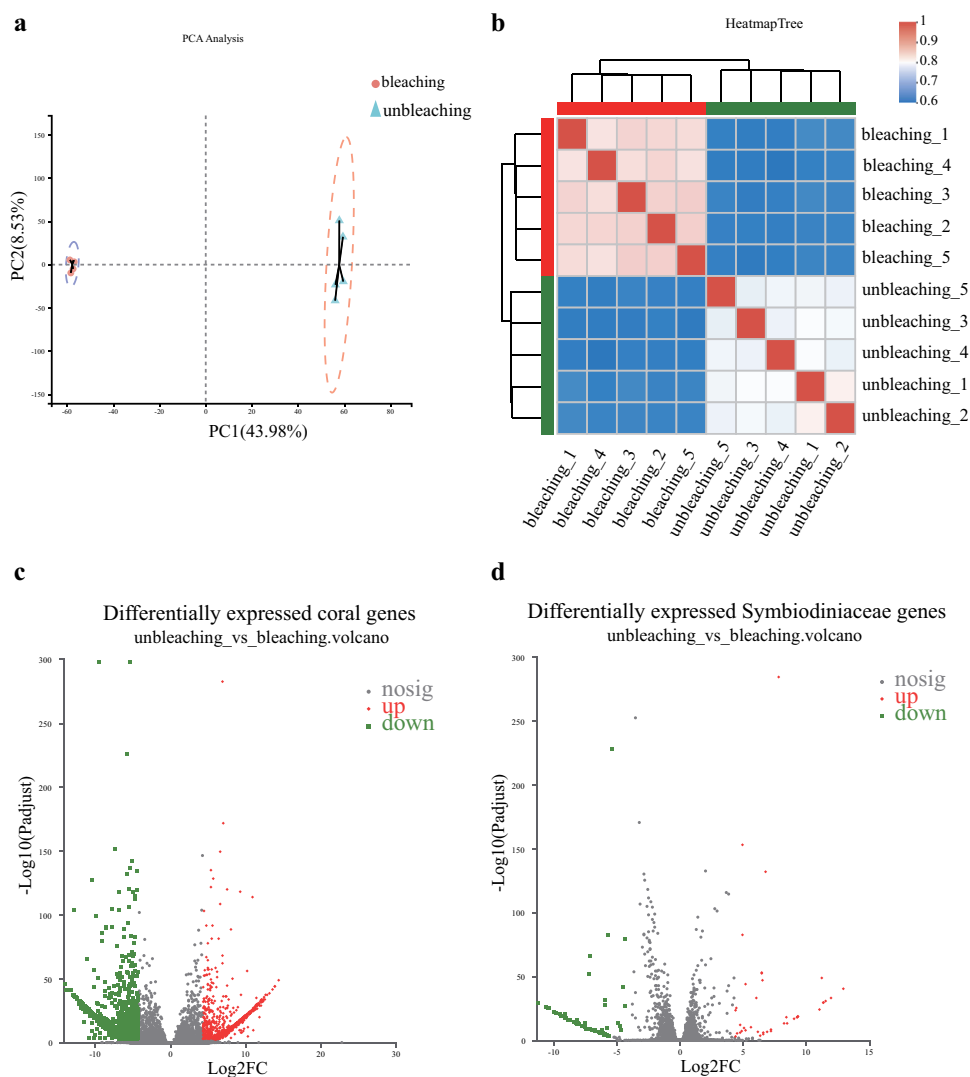
coral, is the core bacterial population in *Mussismilia hispida*, and can be vertically transferred through the mucus (Leite et al. 2017). *Ralstonia* is also reportedly related to Symbiodiniaceae, although it is a minor coral symbiotic genus (Yang et al. 2017). Additionally, *Ralstonia* has important roles in ion-coupled transport and amino-acid metabolism in corals (D Ainsworth et al. 2015). In the current study, the relative abundance of *Ralstonia* sp. (OTU1564) was low; hence, it likely persists at relatively low abundance as an important taxon in corals (Giovannoni and Stingl 2005).

Bradyrhizobium sp. (OTU2132) is a nitrogen-fixing bacterium that may participate in the nitrogen metabolism process of coral holobionts, and may be associated with symbiotic Symbiodiniaceae (Olson et al. 2009). It is also reportedly the dominant bacteria of coral *Turbinaria peltata* from the Weizhou Island (Chen et al. 2021).

Propionibacterium sp. (OTU1228), which reduces nitrate to nitrite, could facilitate nitrogen metabolism

(Weiler et al. 2018). *Propionibacterium,* a conserved member of the core microbiome across a number of tropical and mesophotic corals, has been found within the Symbiodiniaceae (Kellogg et al. 2017) and may have a role as a conserved symbiont (Kellogg et al. 2017) while representing a dominant bacteria of corals *Alcyonium grandiflorum*, *Anthothela* sp., and *Anthothela grandiflora* collected from Norfolk and Baltimore canyons in the mid-Atlantic (Lawler et al. 2016).

The sponge-associated *Rothia* and coral-associated *Glutamicibacter* from the southeast coast of India have documented antimicrobial activities (Rajasabapathy et al. 2020). Moreover, *Rothia* sp. (OTU316) was detected in diseased corals from collection sites HHWMP and PS in Hong Kong in the winter and summer of 2010 (Chiu et al. 2012). Members of this genus are core microorganisms associated with different aged organisms, such as *Coelastrea Aspera*, in the fluctuating tidal environment of Phuket Island, Thailand (Sweet et al. 2017). Meanwhile,

Fig. 7 Functional enrichment analyses of the differentially-expressed genes (DEGs). **A** KEGG enrichment analyses of the significantly upregulated coral genes in the bleaching group. **B** GO and **C** KEGG enrichment analyses of the significantly downregulated coral genes in the bleaching group. The significance of enrichment is indicated by the bar chart, and the abundance of DEGs is indicated by the line chart
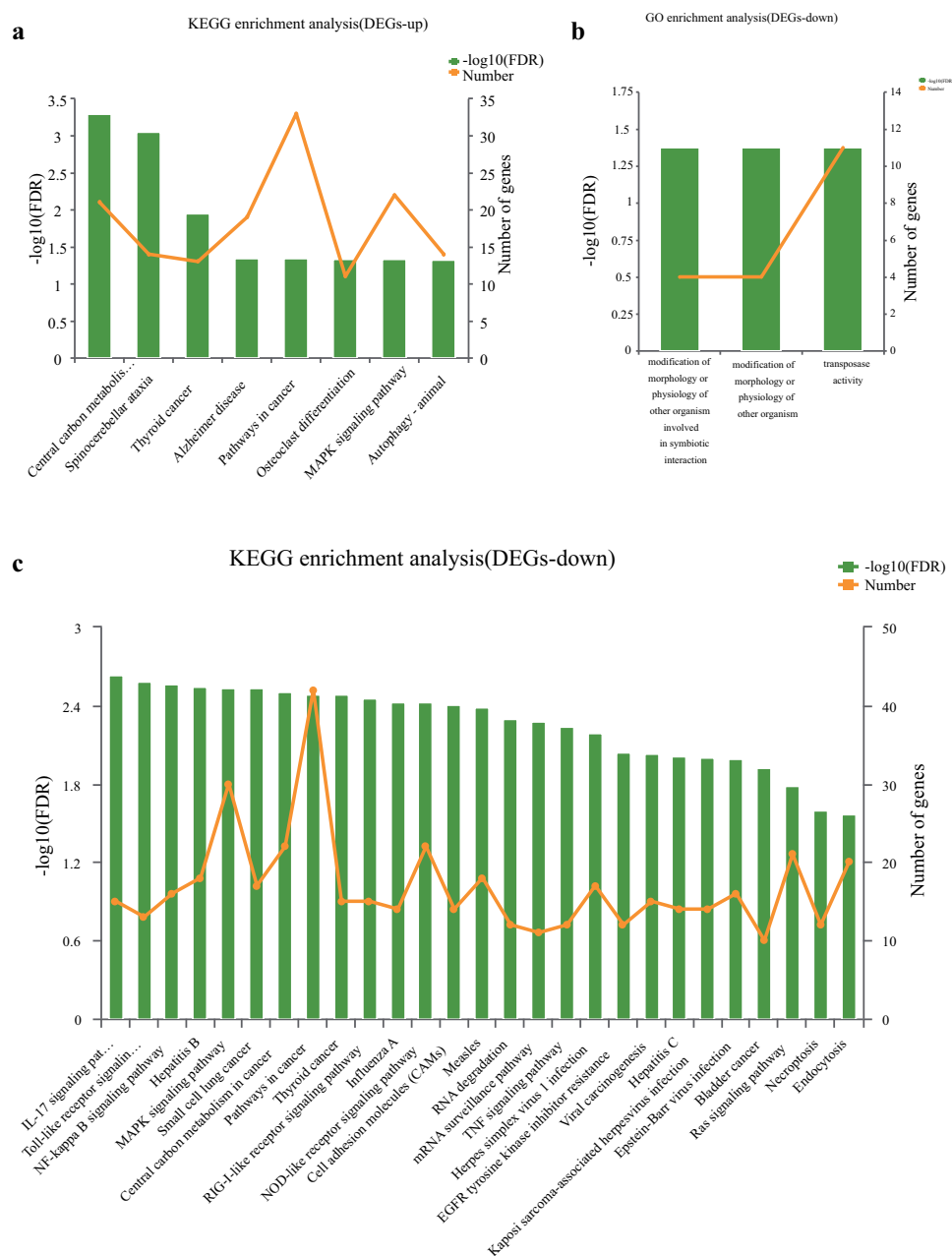
Glutamicibacter sp. (OTU176) is a melanin-producing bacterium with antioxidant activity that protects animal cells from photo-toxicity (Vijayan et al. 2017).

Previous studies have reported that *Pseudarthrobacter* sp. (OTU177) is an important indicator of PAH contamination in agricultural soils (Li et al. 2019). Further, *Pseudarthrobacter* sp., associated with the surface of three co-occurring Antarctic macroalgae, reportedly exhibits antibiotic activity against at least one indicator strain (Alvarado et al. 2018). Combined with the fact that Symbiodiniaceae density is low in summer (Sawall et al. 2014), we speculate that the high abundance of nitrogen cycle-related bacteria in healthy corals may replace Symbiodiniaceae to supplement the necessary nitrogen source for coral holobionts. However, further research is required to confirm this interesting phenomenon. Nevertheless, reorganization of the symbiotic bacterial community structure plays an important role in the environmental adaptation of coral holobionts.

A. pruinosa host and Symbiodiniaceae from a stable symbiotic combination under extreme high temperature stress

The recycling and assimilation of nutrients between Symbiodiniaceae and coral host is the functional basis for the

survival of coral holobionts in oligotrophic sea areas (Muscatine and Porter 1977). Indeed, significant differences in the density of Symbiodiniaceae have been found in coral symbionts with different tolerances (Qin et al. 2019). Moreover, the adaptive bleaching hypothesis suggests different combinations of symbiotic relationships between corals and Symbiodiniaceae, which can be reorganized to improve the tolerance of symbionts under environmental stress (Chen et al. 2019). Therefore, this study compared differences in the population and density of symbiotic Symbiodiniaceae to reveal the potential molecular mechanism leading to different degrees of scleractinian corals bleaching during extreme high-temperature events.

Although the density of Symbiodiniaceae in the two coral groups differed significantly, no recombination of coral host and Symbiodiniaceae was detected between the two groups. C1 was the dominant Symbiodiniaceae subclade of *A. pruinosa* in both groups, which agreed with results of previous studies on Symbiodiniaceae in *A. pruinosa* from Weizhou Island (Yu et al. 2020a, b). Moreover, in previous laboratory heat stress simulation experiment with *A. pruinosa*, changes in temperature and duration of heat stress did not lead to the recombination of coral host and Symbiodiniaceae (Yu et al. 2020b). Similarly, after the bleaching events in the Bahamas and Florida islands in 1997–1998, stable assemblages of symbiotic Symbiodiniaceae were found in many coral species (Thornhill et al. 2006). Weizhou Island is a relatively high latitude coral reef area (Yu et al. 2019). Previous studies showed that in the South China Sea, the relative abundance of C1 in scleractinian corals from high latitudes is higher than in low latitudes (Chen et al. 2019). Additionally, C1 is the dominant symbiotic Symbiodiniaceae in various scleractinian corals in the high latitude waters of Okinawa, and Jeju Island (Chen et al. 2019). We believe that coral hosts are generally symbiotic with a single Symbiodiniaceae, i.e., once a Symbiodiniaceae clonal lineage is established in the host, it will occupy an advantage (Thornhill et al. 2017). Therefore, we believe that the combination of *A. pruinosa* coral host and Symbiodiniaceae is the optimal result of natural selection and long-term evolution and is key to the coral's environmental adaptation on Weizhou Island. A stable coral host and Symbiodiniaceae combination do not imply that Symbiodiniaceae are uninvolved in the corals' response to temperature stress. Combined with the hypothesis of immune adaptive "homeostasis of self" of coral holobionts (Palmer 2018), coral holobionts can regulate the symbiotic relationship, expelling harmful or redundant Symbiodiniaceae (Palmer 2018), thus maintaining the stability of the symbiotic relationship under environmental stress.

Transcriptional plasticity of coral host contributes to higher tolerance of coral holobionts

We found that coral hosts displayed high transcriptional plasticity under environmental stress, which led to differential tolerance of coral holobionts. PCA plot and heat map analyses revealed high consistency between the five replicates within each group and differences between the groups, which provided greater statistical confidence for transcriptome comparison. Meanwhile, high-throughput RNA-Seq enabled the identification of a large number of DEGs involved in differential tolerance. Compared with the unbleaching group, we discovered 3326 (1441 upregulated and 1885 downregulated) and 109 (41 upregulated and 68 downregulated) differentially expressed coral and Symbiodiniaceae genes in the bleaching group. We employed a more rigorous analyses level to obtain fewer DEGs and help avoid false-positive results. Significantly upregulated coral genes in the bleaching group were assigned to 8 significantly enriched KEGG pathways, most of which were associated with diseases and autophagy, which agrees with the phenotypic characteristics observed during extreme high-temperature events. Extreme high-temperatures lead to the loss of Symbiodiniaceae, resulting in severe crisis in coral holobionts. We discovered 1885 significantly upregulated coral genes in the unbleaching group, among which three GO terms were overrepresented, including modification of morphology or physiology of another organism involved in symbiotic interaction (GO:0,051,817), modification of morphology or physiology of other organism (GO: 0,035,821), and transposase activity (GO: 0,004,803). Notably, the first two GO terms indicate involvement in the maintenance of the symbiotic relationship between corals and symbionts. Thermal stress can cause the breakdown of the coral symbiosis with loss of Symbiodiniaceae and/or their photosynthetic pigments within coral tissues (i.e., coral bleaching), which has severe consequences for the fitness and survival of coral hosts (van Oppen and Medina 2020). Therefore, we speculate that enhancing pathways related to maintaining the stability of the symbiotic relationship between corals and symbionts may prevent the collapse of this relationship in response to heat stress, thus avoiding coral bleaching during extreme high-temperature events.

Furthermore, among the 27 overrepresented KEGG pathways for significantly upregulated genes in the bleaching group, 11 were associated with immune defense. Toll-like receptors, NOD-like receptors, and tumor necrosis factor receptor (TNFR) are important receptors in corals (MacEwan 2002; Traylor-Knowles and Connelly 2017; Zhou et al. 2017) and are reportedly involved in heat stress (Barshis et al. 2013; Palumbi et al. 2014). The innate immune system is assumed to be the sole means by which

non-self cells are detected and either killed or contained in non-chordates (Beutler 2004). In scleractinian coral, the immune system is the purveyor of the homeostatic relationships among coral holobionts. Thus, immunity likely has an important effect on survival of scleractinian coral in response to climate change pressures (Palmer 2018). According to the damage threshold hypothesis of coral holobiont susceptibility, compared to those that employ resistance strategies, the holobionts with higher constituent immunity may better maintain homeostasis through perturbations (Palmer et al. 2010). Therefore, our results suggest that comparatively high constituent immunity can physiologically offset the damage of coral holobionts. Enhanced interactions between coral hosts and symbionts and comparatively, high constituent immunity may contribute to the higher tolerance of unbleaching coral.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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