Coral Reefs
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REPORT

Insights into the molecular mechanisms underlying the different heat tolerance of the scleractinian coral *Pavona decussata*

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Abstract The increasing threat of ocean warming has led to the more frequent endangerment of coral reefs, including the heat-tolerant *Pavona decussata*. To shed light on the molecular mechanisms involved in the response of coral to ocean warming, we investigated the gene expression profiles of *P. decussata* after natural thermal stress. Using PacBio Sequel II sequencing technology, we obtained relatively complete transcriptome data for *P. decussata* and then analyzed its gene expression quantitatively with Illumina RNA-seq technology. We acquired information on gene function, structure, and expression profile from coral host and zooxanthellae. Analysis of Illumina sequencing data revealed that unbleached coral host might rely on the active utilization of amino acids to maintain a stable living condition based on the tricarboxylic acid cycle under high temperature stress, and that zooxanthellae might benefit from ammonium produced by coral host. Moreover, the downregulation of unbleached coral host gene expression in innate immune pathways centered on the transcription factors that heat shock factor and nuclear factor (NF)-κB, as well as the tyrosine kinase pathway, might be crucial for maintaining the equilibrium of the zooxanthellae under thermal stress. Thus, the differences in these molecular mechanisms could determine, to some extent, whether coral host can maintain a symbiotic relationship with algae under heat stress. This study elucidated the molecular mechanisms underlying differences in thermal tolerance within *P. decussata* species and supported further theoretical basis in coral molecular biology and ecological conservation, which enhance our comprehension of coral responses to future climate change.

Keywords Thermal stress · Coral bleaching · *Pavona decussata* · Energy metabolism · Innate immunity

Introduction

Reef-building corals are the primary constructors of coral reef ecosystems, which are the most biodiverse, resource-rich, and highly productive ecosystems in the ocean (Reaka-Kudla 1997; Hughes et al. 2017). The contribution of corals to the ecosystem relies on a unique cnidarian-dinoflagellate symbiosis. The algal family Symbiodiniaceae exists within the gastrodermal cells of its coral host (Bourne et al. 2016; LaJeunesse et al. 2018), and both parties achieve a balanced mutualism through the exchange of nutrients (Rädecker et al. 2015). In stable symbiosis, photosynthetic carbon is produced by zooxanthellae through photosynthesis and then translated to the coral host to meet over 90% of its metabolic needs (Muscatine and Porter 1977; Rädecker et al. 2021), at this point, both the zooxanthellae and coral host are in a state of nitrogen limitation because of sufficient photosynthetic

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carbon (Cui et al. 2019; Thomas et al. 2020; Rädecker et al. 2021). However, bidirectional transporters on the epidermal cell membrane of the coral host absorb ammonium from the environment (Cui et al. 2023), and then transport it to gastrodermal cells and the zooxanthellae to alleviate nitrogen limitation (Thies et al. 2022). The endosymbiotic algae increase their nitrogen utilization efficiency for growth and division by absorbing ammonium (Rädecker et al. 2021), thus completing a nitrogen cycle between the symbiotic partners. This symbiotic relationship is not formed indiscriminately, but instead achieved through regulation of the innate immunity of the coral host (Mansfield and Gilmore 2019; Jacobovitz et al. 2021). Although not as complete as the immune system of vertebrates, the innate immunity of corals has a crucial role in ensuring their sustained survival. It distinguishes between harmful pathogens and beneficial symbiotic microorganisms through cellular pathways, such as immune cells (Snyder et al. 2021), pattern recognition receptors (Brennan and Gilmore 2018), complement system (Poole et al. 2016), transforming growth factor beta (TGFβ) (Detournay et al. 2012; Berthelier et al. 2017), and immunity transcription factors (TFs) (Desalvo et al. 2010; Mansfield et al. 2017). In addition, immune-related processes of coral host, including activation of the prophenoloxidase system and inflammation regulatory pathways, are associated with the loss of symbiosis (van de Water et al. 2015; Mansfield et al. 2017; Palmer 2018). Therefore, coral innate immunity holds considerable importance in controlling symbiotic tolerance.

Given the complexity of coral symbiotic relationships, they are highly sensitive to environmental factors, such as temperature. In recent years, climate-related environmental changes have been affecting marine ecosystems (Sully et al. 2019). Increasingly frequent heat stress events are making it difficult for corals to survive, and coral bleaching occurs when corals fail to adapt to the stress of rising sea temperatures (Hoegh-Guldberg 1999), resulting in a breakdown of the cnidarian-dinoflagellate symbiotic relationship. To explore the molecular mechanisms of symbiotic relationship breakdown between corals and their symbionts, "omics" are being increasingly used to study coral thermal bleaching events. For example, second-generation sequencing technology revealed transcriptional features of coral symbionts in response to heat stress. Research found that even if no visible bleaching occurred when corals were subjected to heat stress, the pressure from this increased temperature affected the tricarboxylic acid (TCA) cycle of coral host (Rädecker et al. 2021), suggesting that coral hunger might occur before the bleaching event. As corals gradually enter the bleaching phase, they strengthen protein catabolism (Savary et al. 2021) and promote amino acid utilization (Rädecker et al. 2021). At the same time, heightened oxidative stress leads to increased nitric oxide (NO) generation (Perez and Weis 2006), enhanced phenoloxidase (PO) activity (van de Water et al. 2015), and the positive action of the nuclear factor (NF)-κB (Mansfield et al. 2017), leading to enhanced innate immunity in coral host, which might have a counteracting effect on the maintenance of coral symbionts. Although high-throughput sequencing has expanded our understanding of the molecular mechanisms of coral bleaching, because of technological limitations of short-read sequencing in the second-generation sequencing approaches, results might contain sequence assembly errors or incomplete information (van Dijk et al. 2018). In contrast, full-length transcriptome sequencing has longer read lengths and can directly obtain complete sequences. Moreover, using long-read sequencing technology not only improves sequencing accuracy but also enables analysis and prediction of transcriptome structures (Guo et al. 2021; Liu et al. 2021).

During the summer of 2020, Weizhou Island, in the northern South China Sea, experienced an unusual heat event (Chen et al. 2022). Throughout this event, a considerable amount of coral (Yu et al. 2021b), including the thermotolerant *Pavona decussata*, underwent bleaching. However, there were significant individual differences in the extent of bleaching in *P. decussata*, indicating that the symbiotic relationship within the coral under natural heat stress might have different maintenance states. For *P. decussata* located at relatively high latitude, the Symbiodiniaceae composition seemed to be very stable. As previous report, the *Cladocopium* C1 were steadily dominated in both summer and winter, although temperature difference of up to 13.6 °C throughout the year in Weizhou Island (Yu et al. 2023). In addition, in Hong Kong at almost the same latitude, *P. decussata* experienced a similar thermal bleaching event, and both bleached and unblanched samples showed a stable Symbiodiniaceae composition, consistently with *Cladocopium* C1 as the dominant taxa (Ip et al. 2022). Overall, we hypothesized that *P. decussata* surviving in Weizhou Island also maintained a *Cladocopium* C1-dominated stable Symbiodiniaceae composition during the extreme high temperature event in 2020. And in order to get an in-depth molecular regulatory mechanism between the symbiotic partners, we therefore specifically used Pacbio and Illumina sequencing to investigate this heat-induced bleaching event in *P. decussata*. Two objectives were to: (1) effectively distinguish between coral host and symbiotic algal transcripts, annotating gene functions, and analyzing transcript structure; and (2) determine the potential molecular mechanisms by which coral holobionts responded to thermal stress by clarifying energy cycling between the symbiotic partners and changes in host innate immunity. This study will expand our understanding of how corals respond to climate anomalies and provide a theoretical basis for future conservation efforts associated with coral reefs.

Materials and methods

Specimen collection and RNA extraction

From May to August 2020, an anomalous high temperature event was observed with sea surface temperature (SST) anomalies exceeded a maximum of 2 °C in Weizhou Island (Feng et al. 2022). In this event, a maximum daily SST of 31.9 °C and a maximum monthly SST of 31.0 °C were recorded (Mo et al. 2022). Field investigations revealed widespread bleaching of corals (Yu et al. 2021b) and the co-occurrence of bleached and unbleached corals in the same colony. Six samples each of unbleached (HP) and bleached (BP) *P. decussata* were specially collected from six colonies (N21°8.27′, E109°12.6′) by SCUBA divers in August 2020 (Fig. 1), with the measured temperature at 31.1 °C. To ensure sample consistency, all samples were collected at the same depth (3 ~ 5 m), with a maximum distance of 10 m maintained between each sample site. After collection, all samples were rapidly stored using liquid nitrogen for subsequent analysis. Total RNA was extracted by grinding tissue from 12 frozen *P. decussata* samples in TRIzol reagent (Life Technologies, CA, USA) according to the manufacturer's protocol. RNA quality was assessed via both the Agilent™ 2100 Bioanalyzer (Agilent Technologies, CA, USA) and agarose gel electrophoresis. The RNA purity and concentration were determined with the Thermo Scientific™ NanoDrop™ One Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific, MA, USA).

Library construction and sequencing

SMRTbell library construction and sequencing

Six RNA samples of each group were extracted in equal amounts and mixed; the RNA sample was then enriched by Oligo (dT) magnetic beads and reverse transcribed into cDNA using a SMARTer™ PCR cDNA Synthesis Kit (Clontech, CA, USA), and the BluePippin™ Size Selection System for processing cDNA to prepare a SMRTbell library, following the isoform-sequencing protocol. Single-molecule real-time (SMRT) sequencing was provided by Gene Denovo Biotechnology Co. (Guangzhou, China) through the PacBio Sequel II platform (Pacific Biosciences, CA, USA). Raw Iso-seq data are available from National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (BioProject: PRJNA970555).

Fig. 1 Representative image of coral samples. In the same region, the individual of *P. decussata* exhibited two distinct states, with some appearing normal and others already undergoing bleaching

Illumina library construction and sequencing

After SMRTbell library construction, six biological replicates of each group were performed for Illumina cDNA library construction; the remaining RNA of two groups (> 1.5 μg per sample) was used for enriching by Oligo(dT) beads. Following RNA fragmentation, cDNA was synthesized with random primers. Using a QiaQuick PCR extraction kit (Qiagen, Venlo, The Netherlands), the second-strand cDNA fragments were purified, repaired, added poly(A) tails, and then ligated to Illumina sequencing adapters. Following the default stranded RNA protocol of the HiSeq™ 4000 instrument (Illumina, CA, USA), the ligation products were sequenced. The datasets generated from Illumina sequencing can be found in the same BioProject at NCBI SRA.

Data processing

Isoform-sequencing data processing

The raw reads of SMRT sequencing were analyzed according to the protocol of Pacific Biosciences (Gordon et al. 2015). The subreads BAM file was used to generate high-quality circular consensus sequences (CCSs), which retained CCSs with full passes greater than 1. To get full-length non-chimeric (FLNC) reads, sequences with both 5′ end adapters, 3′ end adapters and polyA tails were reserved, and the primers, barcodes, poly(A) tail trimming, and concatemers of the full passes were removed. Then, the FLNC reads were clustered hierarchically using Minimap2 v2.17 to generate unpolished consensus isoforms (consistency sequence). After polishing, high-quality isoforms (prediction accuracy ≥ 0.99) were removed for redundancy by CD-HIT v4.6.7 (Li and Godzik 2006). Finally, the full-length transcriptome of *P. decussata* was obtained, which contained structures from the 5′ UTR to the polyA tail. BLASTx v0.9.19.120 was used against the self-built protein sequence database to align the isoforms with an E-value threshold of 1e-5. Separately, the transcriptomes of the coral host and symbiotic algae were evaluated by BUSCO v3.0.2b (Simão et al. 2015) against the Eukaryota data set odb9.

Next-generation sequencing data processing

Quality control on the raw reads was performed using Fastp v0.18.0 (Chen et al. 2018), and low-quality data was filtered out to obtain clean reads. Isoforms assembled from Iso-seq were used as reference transcripts to align with the clean reads by RSEM v1.2.19 (Li and Dewey 2011). A calculation of gene abundance and normalization to RPKM were conducted. Based on the expression results of each sample, PCA analysis was performed to understand the repeatability between samples, followed by unigene differential expression analysis performed with DESeq2 v1.20.0 (Love et al. 2014). As differentially expressed genes (DEGs), genes with a false discovery rate (FDR) < 0.01 and $|\log_2$ fold change$| > 1$ were considered.

Basic annotation of isoforms, enrichment and correlation analyses of DEGs

Basic annotation of isoforms typically involved annotating protein function and their involvement in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) classifications. A BLASTx search of isoforms against Trembl and SwissProt protein databases (https://www.uniprot.org/) was conducted to identify their similarity to the genes from other species. GO annotation was analyzed by Blast2GO v6.0.1 (Conesa et al. 2005) using the protein annotation results of each isoform. WEGO (http://wego.genomics.org.cn/) was then used to perform GO functional classification statistics for all isoforms (Ye et al. 2006). Isoform pathway annotations were further obtained according to the KEGG database; in addition, DEGs were analyzed for GO functions and KEGG pathways using the methods described above. The further enrichment analyses using Fisher's exact test and $p < 0.05$ as a criterion for judgment. Correlations between gene expressions were determined by calculating Spearman rank correlation coefficient, with $p < 0.05$ being significant.

Structure analysis

The open reading frames (ORFs) were detected with ANGEL v3.0 (Shimizu et al. 2006), and the coding sequences (CDSs) and untranslated regions (UTRs) were obtained with isoform sequences. For the coral host, protein-coding sequences of isoforms were aligned by hmmscan to Animal TFdb (http://bioguo.org/AnimalTFDB/) to predict TFs, and those in the symbiotic algae were predicted using Plant TFdb (http://planttfdb.cbi.pku.edu.cn/). The transcriptome was analyzed using MIcroSAtellite (MISA, http://pgrc.ipk-gatersleben.de/misa/) for simple sequence repeats (SSR). Configuration parameters were as follows: definition (unit size, minimum repeats): 2–6 3–5 4–4 5–4 6–4; and interruption (maximum difference between two SSRs); 100. To assess the protein-coding potential of unannotated transcripts, CNCI v2.0 and CPC2 (http://cpc2.gao-lab.org/) were used with default parameters to identify potential long noncoding RNAs (lncRNAs) (Kong et al. 2007; Sun et al. 2013).

Quantitative real-time PCR (qRT-PCR) analyses

The BP and HP groups were both prepared for qRT-PCR assays. Five DEGs of coral host were randomly selected and specific primers were designed by Primer Premier v6.0 (Table S1). For each qRT-PCR reaction, 2.5 µL of cDNA, 0.4 µL of forward primer, 0.4 µL of reverse primer, 10 µL of AceQ Universal SYBR qPCR Master Mix, and 6.7 µL double distilled water were used in a total volume of 20 µL. All qRT-PCR reactions were performed on a LightCycler 96 System (Roche, Basel, Switzerland) with the following protocol: holding stage: 95 °C for 300 s; cycling stage: 95 °C for 10 s and 60 °C for 30 s and the cycling stage was run for 40 cycles. Finally, the temperature in the melt curve stage increased by 0.3 °C every 15 s from 60 to 95 °C. Relative expression levels were normalized to elongation factor 1α (EF1α) (Yu et al. 2021a) and calculated using the comparative C_T method.

Results

Full-length transcriptome sequencing and data processing

On the basis of the SMRT sequencing, we obtained 45,481,866,085 bp of raw data (~45 Gb) and 27,431,413 subreads, with an average subread length of 1666 bp and an N50 length of 1887 bp. With 640,853 CCS reads, the mean read length and pass number were 1860 bp and 40, separately. The CCS reads were clustered and corrected, resulting in 42,472 FLNC reads with an average length of 1822 bp and an N50 length of 2070 bp. Finally, redundant reads were removed by CD-HIT, resulting in 40,369 isoforms with a mean length of 1820 bp. Most isoforms were between 500 and 4000 bp, and the longest was 5441 bp. After comparison with previously published sequences, 35,221 isoforms from the coral host and 2811 from symbiotic algae were obtained for subsequent analysis (Fig. 2). According to BUSCO analysis, the coral host and zooxanthellae isoforms contained 82.3% (79.6% complete and 2.7% fragmented) and 5.2% (2.8% complete and 2.4% fragmented) of the Eukaryota data set odb9.

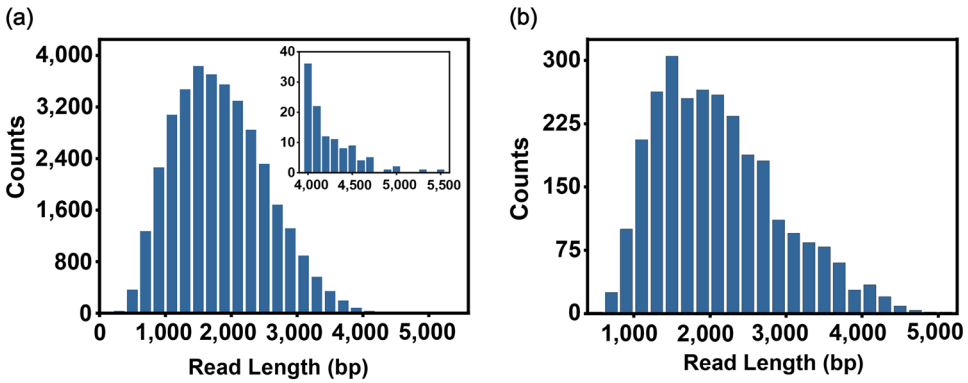

Fig. 2 Isoforms' length distributions of PacBio SMRT sequencing. **a** Isoforms of coral host. The inserted table showed the statistics after the length of 4000 bp. **b** Isoforms of symbiotic algae

Functional annotation

All isoforms from the coral host and symbiotic algae were functionally annotated by searching the Trembl, SwissProt, GO, and KEGG databases. In total, 33,825 isoforms (96.04%) from the coral host (Fig. 3a) and 2739 (97.44%) from the symbiotic algae (Fig. 3b) were identified to have at least one hit in the Trembl database, whereas 14,337 isoforms (40.71%) from the coral host and 536 isoforms (19.07%) from the symbiotic algae were annotated in all four databases.

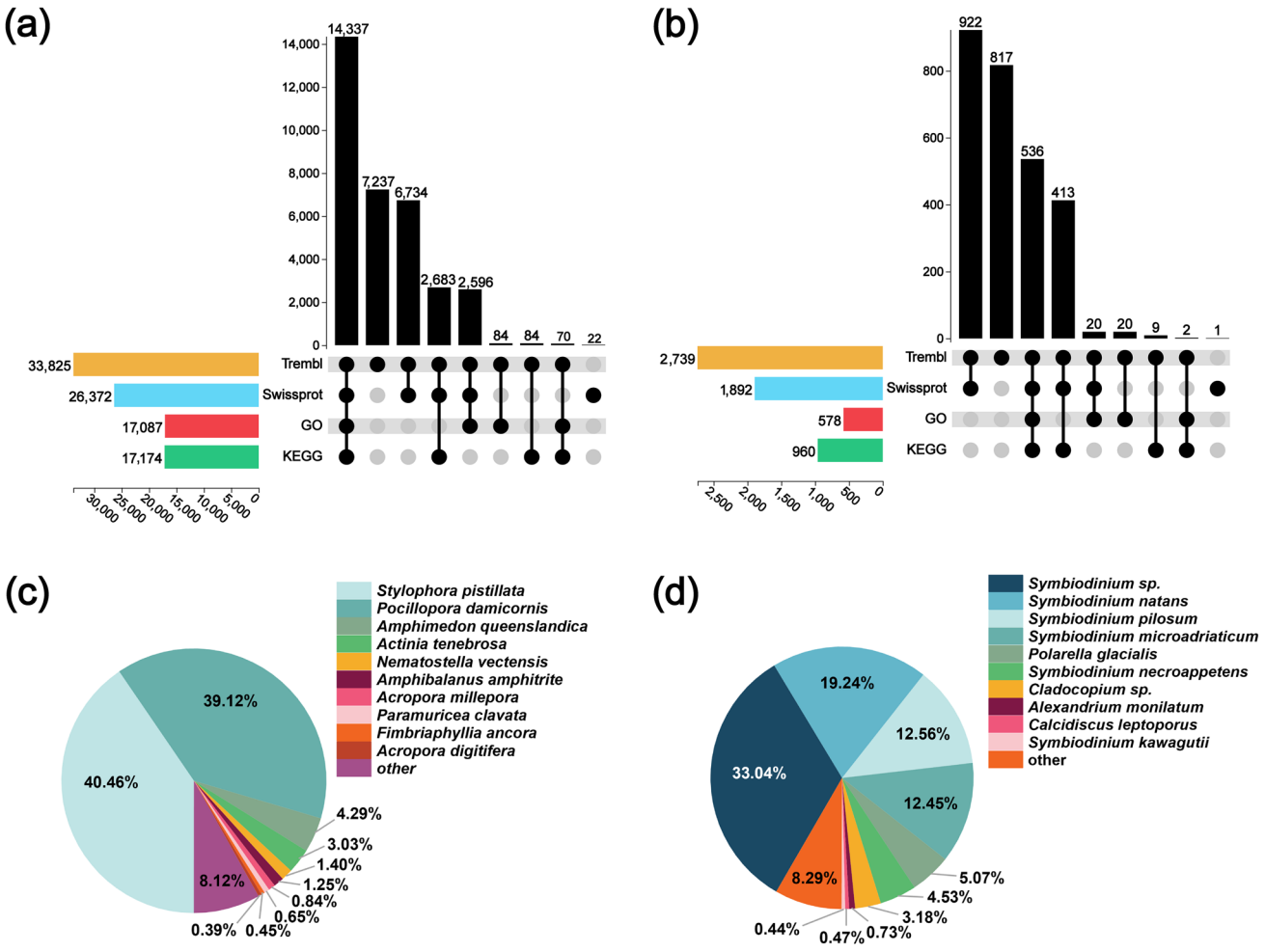

Fig. 3 The statistics of full-length isoforms annotated in all four databases, the coral host's results were shown on the left, and the symbiotic algae's results were on the right. **a**, **b** Annotation to public databases. **c**, **d** Trembl homologous species distribution diagrams

Homologous species were analyzed by comparing the isoform sequences to the Trembl database. The results showed that the homologous species for coral host in descending order were *Stylophora pistillata* (13,687, 40.46% of the total), *Pocillopora damicornis* (13,231, 39.12%), *Amphimedon queenslandica* (1451, 4.29%), *Actinia tenebrosa* (1026, 3.03%), *Nematostella vectensis* (472, 1.40%), and others species (Fig. 3c). For the symbiotic algae, the top five annotated species were *Symbiodinium* sp. (905, 33.04%), *S. natans* (527, 19.24%), *S. pilosum* (344, 12.56%), *S. microadriaticum* (341, 12.45%), and *Polarella glacialis* (139, 5.07%) (Fig. 3d).

GO analysis revealed that isoforms of both coral host and symbiotic algae were classified into three groups: biological process, cellular component, and molecular function (Fig. S1). In the biological process group, both coral host and symbiotic algal isoforms were enriched in cellular process, single-organism process, metabolic process, and biological regulation, among others. Of all the categories in the cellular component group, coral host isoforms were mainly annotated to cell, cell part, and organelle, and for symbiotic algae, the categories of cell and cell part were most prominent. Additionally, isoforms of both coral host and symbiotic algae were mainly annotated to the molecular function categories of binding and catalytic activity. The isoforms of coral host and zooxanthellae were annotated to complete and roughly consistent terms, indicating that the sequencing data showed relatively complete gene functions.

KEGG results demonstrated that 33,847 coral host isoforms and 2740 symbiotic algae isoforms were mapped to 389 and 277 KEGG pathways, respectively (Table S2). All KEGG pathways were then assigned to two levels of classification and presented as circular bar charts. The dominant isoforms of the coral host and symbiotic algae were enriched to human diseases, metabolism, and organismal systems (Fig. S2). The top five secondary classifications in the coral host (Fig. S2a) were signal transduction (7145 isoforms), global and overview maps (6932), endocrine system (3753), immune system (3516), and infectious disease: viral (3237). The global and overview maps (801), endocrine system (262), immune system (249), signal transduction (218), and carbohydrate metabolism (190) were the top five secondary classifications for symbiotic algae (Fig. S2b).

Structure analysis

In total, 45,251 CDS were predicted from coral host isoforms, and 3590 from symbiotic algae. The lengths of CDS ranged from 123 to 5340 bp, with 600 ~ 2000 bp being the most prevalent. The 5′-UTRs were observed in 26,662 isoforms, while 3′-UTRs were observed in 31,177 isoforms.

TFs are important regulatory genes involved in coral host and symbiotic algae life histories. In total, 2156 TFs and 44 TFs were respectively identified in the symbiotic partners (Fig. S3). The TFs of the coral host were distributed in 58 families, with the top five being bHLH (291), bZIP (188), zf-C2H2 (182), Homeobox (177), and HMG (160). The TFs of symbiotic algae were distributed in 10 families, with the top five being bHLH (9), HMG (9), MBD (3), NCU-G1 (3), and MYB (2).

SSR is a useful genetic marker with high polymorphism. In this study, 3400 SSRs in the coral host and 134 SSRs in symbiotic algae were identified. The most abundant SSR types in the coral host were trinucleotides (76.59%), followed by tetranucleotides (12.94%), hexanucleotides (4.71%), dinucleotides (3.12%), and pentanucleotides (2.65%) (Fig. S4a). For symbiotic algae, the most common type of SSR was trinucleotides (79.85%), followed by hexanucleotides (8.96%), dinucleotides (5.97%), and tetranucleotides (5.22%) (Fig. S4b).

RNA molecules with transcripts over 200 nt that are not protein-encoding are classified as lncRNAs. Given the lack of genomic information for *P. decussata*, lncRNA prediction was performed using CNCI and CPC2 software; lncRNAs predicted as "noncoding" by both software have been used as final results. A total of 633 lncRNAs were discerned from the coral host (Fig. S4c), with only eight lncRNAs found in the symbiotic algae (Fig. S4d).

Illumina sequencing and identification of DEGs

In total, 509,015,772 clean reads were produced based on 510,839,600 raw reads, which were then assembled into 33,760 unigenes. The sequencing profile is listed in Table S3. PCA using a linear model showed that coral host and symbiotic algae specimens were both divided into two groups (Fig. 4a, b). Compared with the BP group, it has been founded 10,279 (2282 downregulated and 7997 upregulated) differentially expressed coral genes (Fig. 4c) as well as 209 (165 downregulated and 44 upregulated) differentially expressed zooxanthellae genes in the HP group (Fig. 4d).

Enrichment analysis of DEGs in both coral host and symbiotic algae

After GO enrichment analysis, 1313 categories were enriched in coral host (Table S4), with 1028 categories belonging to biological processes. As for symbiotic algae, there were 32 enriched categories (Table S5), of which 18 were biological processes. In terms of KEGG analysis, 49 pathways were enriched in the coral host (Table S6), whereas only two pathways were enriched in the symbiotic algae. (Table S7).

To investigate the changes of *P. decussata* in energy metabolism and innate immunity, a selection of GO terms and KEGG pathways was made. The results of GO and

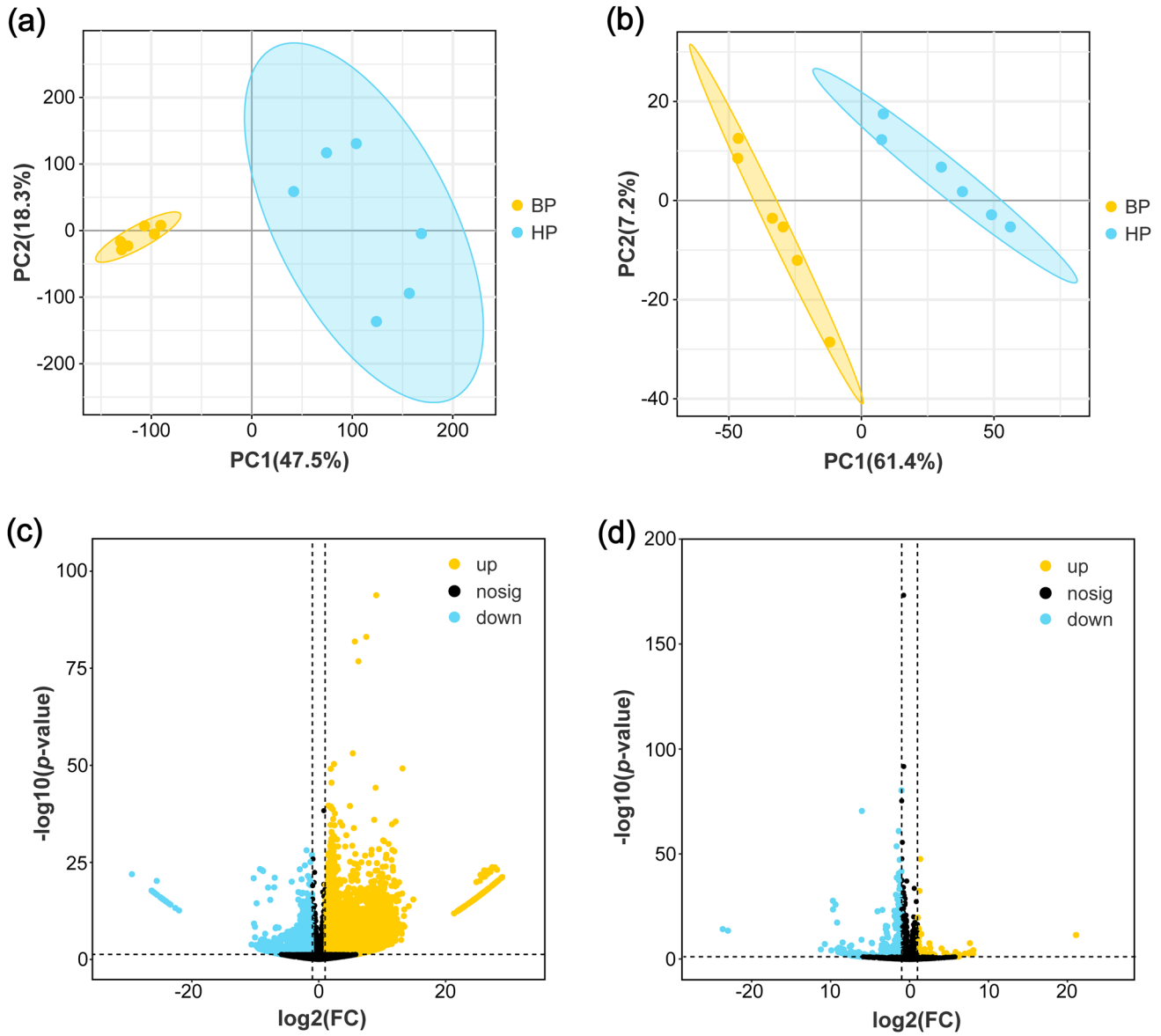

Fig. 4 Comparison between the HP group and BP group, coral host's results were shown on the left, and the symbiotic algae's results were on the right. **a**, **b** PCA plots. **c**, **d** Volcano plots showed the DEGs comparison

KEGG analyses of energy metabolism and innate immunity enriched from the coral host and zooxanthellae are shown in Fig. 5 and Table S8, S9. The enriched GO terms in the biological process category in the coral host were the energy homeostasis (GO:0097009), glutathione (GSH) metabolic process (GO:0006749), and regulation of NIK/NF-κB signaling (GO:1901222), among others (Fig. 5a). For zooxanthellae, terms were highlighted, such as secretion (GO:0046903) and carbohydrate derivative metabolic process (GO:1901135) (Fig. 5b). In terms of KEGG pathways, antigen processing and presentation were the most significantly enriched pathway in the coral host (Fig. 5c), while nitrogen metabolism prevailed in zooxanthellae (Fig. 5d).

Differential gene expression of coral holobiont under natural heat stress

DEGs of coral host were selected from the metabolic and innate immune pathways involved in Fig. 5, and their expression were visualized in Fig. 6. Several genes were involved in energy metabolism, which was mainly related to the coral host mitochondrial TCA cycle. Glutamate dehydrogenases (*GDH*s) and glutamate-cysteine ligase regulatory subunits (*GCLM*s), which regulate energy efflux from the TCA cycle, were upregulated in the HP group. Furthermore, relative to the BP group, host genes that adjust the influx of energy into the TCA

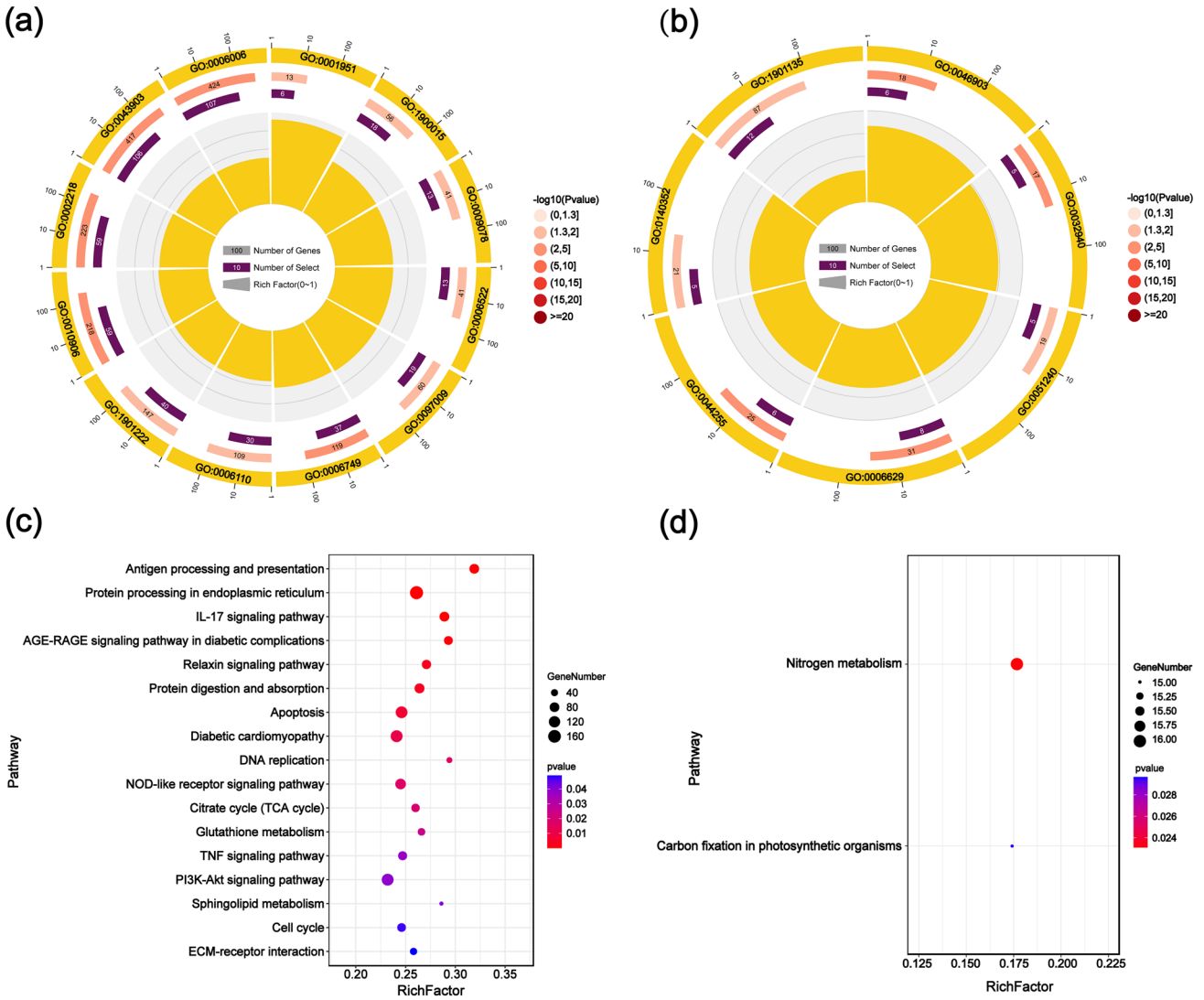

Fig. 5 The summary of DEGs enrichment analyses, coral host's results were shown on the left, and the symbiotic algae's results were on the right. **a**, **b** The main terms involved under the biological process classification in GO enrichment analysis. From outside to inside, the graph had four circles. The first circle was the enriched terms id, and the outermost layer was the number of genes; as for the second circle, it represented the number of enriched terms in the background genes and the *p*-value (longer bars corresponded to more genes, and smaller values corresponded to redder colors); the third circle showed the total number of differential genes. The fourth circle was the rich factor value of each category (the background auxiliary line represented 0.1 for each small cell). **c**, **d** The major KEGG pathway-related information was shown by scatter plots

cycle had similarly increased expression, such as dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complexes (*DLAT*s), homogentisate 1,2-dioxygenase-like genes (*HGD*s), and glyceraldehyde-3-phosphate dehydrogenases (*GAPDH*s) in HP group. By contrast, genes participating in coral innate immunity had significantly lower expression levels in HP group, like the heat shock proteins (*HSP*s) and its regulatory element (heat shock factors, *HSF*s), protein kinase C (*PKC*), multifunctional intracellular tumor necrosis factor receptor-associated factor 6 (*TRAF6*) and its nuclear transcription factor kappa B subunit 1 (*NFKB1*) as well as the tyrosinase-like genes (*TYR*s). In zooxanthellae, genes in energy metabolism encoding common transporter proteins, including ABC transporters and ammonium transmembrane transporters (*AMT*s), were upregulated in the HP group. A correlation analysis of the gene expressions in the coral host and zooxanthellae showed that, host energy metabolism-related genes were positively correlated with the genes in zooxanthellae which were related to transporter proteins (Fig. S5). In particular, coral host glycolysis-associated genes *GAPDH*s were significantly and positively correlated with transporter protein-related genes ABC transporters and *AMT*s in zooxanthellae. In

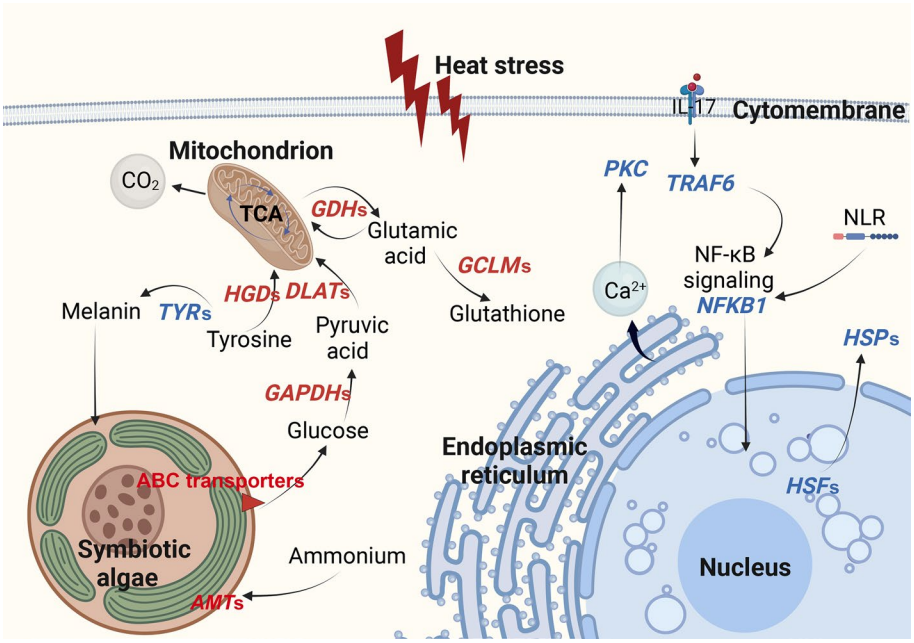

Fig. 6 Diagram of the integrated regulatory network in *P. decussata* during heat stress (Created by BioRender). The red characters represented upregulated genes, while the blue characters represented downregulated genes in the HP group

contrast, innate immunity-related genes *TYR*s in host were significantly negative correlated with ABC transporters and *AMT*s in zooxanthellae (Fig. S5).

Validation of selected DEGs expression using qRT-PCR

To determine the validity of the DEGs identified in the RNA-sequencing data, we randomly selected five DEGs of coral host, including *GDH*, valosin-containing protein (*VCP*), *TRAF6*, *NFKB1*, and SMAD family member 4 (*SMAD4*). Results of the qRT-PCR were consistent with those of the RNA sequencing, demonstrating that the RNA-sequencing data were reliable (Fig. S6).

Discussion

High-quality *P. decussata* transcripts were obtained

SMRT sequencing, with its high accuracy, reliability, long-read length, and fast sequencing speed, is useful in the absence of reference genomes (Jayakumar and Sakakibara 2017; Cheng et al. 2021; Xu et al. 2021). For scleractinian corals, which have a unique symbiosis with zooxanthellae, SMRT sequencing could also help to reveal the genetic bases that maintain the symbiotic relationship between coral host and their algal symbionts (Guo et al. 2021). In this study, a full-length transcriptome of *P. decussata* was obtained with 40,369 full-length isoforms, substantially more than the 38,365 isoforms in *Montipora foliosa* reported by Liu et al (2021) and 38,663 isoforms in *Pocillopora damicornis* (Guo et al. 2021).

After functional annotations, most of the coral host (96.04%) and symbiotic algae (97.44%) isoforms were annotated, and large numbers of both isoforms were involved in immunity and metabolic processes (Fig. S2). In general, the formation and maintenance of coral symbionts involve membrane recognition and innate immune processes between coral host and zooxanthellae, as well as material exchange between them (Liu et al. 2021). All the isoforms revealed are important resources for the genetic manipulation of *P. decussata*. In addition, CDS (45,251 of coral host and 3590 of symbiotic algae), TFs (2156 and 44, respectively), SSRs (3400 and 134, respectively), and lncRNA (633 and eight, respectively) were found in this study, and will be highly valuable as a reference for future research.

Heat stress affected the energy metabolism of the coral host and zooxanthellae

Most of the energy to maintain coral symbioses originates from zooxanthellae photosynthesis (Lichtenberg et al. 2016), whereas nitrogen availability from the coral host determines the homeostasis of the zooxanthellae community in coral cells (Rädecker et al. 2015). The most obvious feature of a coral symbiotic relationship breakdown was an imbalance in energy exchange between symbiotic partners. But it was easily negligible that, energy metabolism might have been altered after the coral symbiont experienced thermal stress, and both the coral host and its symbiotic algae exposed to a starvation threat, although bleaching has not yet occurred (Ezzat et al. 2019; Rädecker et al. 2021). Therefore, the energy budget within coral holobiont might play an important role in maintaining the stability of the

symbiotic relationship. And differences in energy utilization and exchange might be responsible for the variant symbiotic states within *P. decussata* species under extreme heat stress.

In the present study, the significantly enriched pathway TCA cycle (Fig. 5c) was the major energy-yielding metabolic pathway in the organism's cells (Bender 2003), which might provide clues to how coral holobiont overcome starvation in the context of increasing difficulty in assimilating nutrients during heat stress (Ezzat et al. 2019). The different operations of the TCA cycle in coral host under starvation conditions could underlie the balance or disruption of the symbiotic relationship. In the current study, *GDH*s and *GCLM*s of coral host were upregulated in the HP group, indicating that the amino acid glutamate might help coral host cope with heat stress since it was related to both GSHs involved in amino acid transport (Meister 1982) and served as an energy source. As well as facilitating glutamate anabolism, GDHs catabolized glutamate to fuel the TCA cycle (Roberts et al. 2001); during glutamate catabolism, the ammonium that is broken down could be assimilated by symbiont algae (Rädecker et al. 2021) which could overcome the nitrogen limitation of symbionts. And, in the HP group, the increased transcript level of *AMT*s in symbiont algae might indicate the efficient ammonium utilization. In addition, in comparison to bleached BP group, symbiotic algae in HP group might still suffer from some degree of nitrogen limitation due to the maintenance of their populations, which was also reflected by the upregulation of *AMT*s (Xiang et al. 2020). Appropriately, the most significant pathway was "Nitrogen metabolism," which was found in the KEGG enrichment analysis of DEGs in the symbiotic algae (Fig. 5d), indicating the importance of nutritional interactions for both the host and its symbionts (Xiang et al. 2020). ABC transporters, which were widely found in eukaryotes, played an important role in the transmembrane transport of substances (Rees et al. 2009), and in the HP group, the upregulation of genes encoding ABC transporters in zooxanthellae might predict that surviving symbiotic algae in unbleached corals still had a capacity in transferring energy substances to the host (Wu et al. 2023). Symbiont thereby maintained the exchange of energy with the coral host, as transferring glucose to the host (Goreau et al. 1973; Rosic et al. 2014).

Corals allocate energy more toward basic survival during periods of stress (Pei et al. 2022), and algal-derived glucose is involved in the host TCA cycle through glycolysis to provide energy to the host. CO_2 produced after the consumption of glucose contributes to symbiont photosynthesis (Rädecker et al. 2017). GAPDHs were the key enzyme involved in glycolysis and DLATs catalyzed the conversion of the glycolytic product pyruvate to acetyl CoA. Both genes were significantly upregulated in the HP group, indicating that coral holobionts might survive starvation by utilizing glucose in addition to glutamate. And, in coral host, *HGD*s was upregulated in the HP group, showing that tyrosine degradation was involved in the maintenance of coral health during heat stress (Parthasarathy et al. 2018). Therefore, differences in energy metabolism might be responsible for variant high temperature sensitivity within *P. decussata* species.

Regulation of innate immunity in coral host under heat stress affected symbiotic relationships

Cnidarians have innate immune mechanisms comprising TFs and immune signaling pathways (Mansfield and Gilmore 2019) to cope with oxidative stress (Janeway and Medzhitov 2002). Additionally, these pathways managed beneficial microbes and supported mutualistic microbial symbioses.

As TFs, HSFs are a family of DNA-binding proteins that regulate gene expression at the level of transcription, and NF-κB is an evolutionarily conserved TF that promotes many immune and inflammatory processes. In previous studies, the upregulation of *HSF1* and its downstream targets was shown to be a strong and rapid response of corals to endoplasmic reticulum stress induced by increasing heat (Gomez-Pastor et al. 2018; Cleves et al. 2020a). Moreover, *HSF1* is involved in the thermal tolerance of coral host (Cleves et al. 2020b), although no significant changes in HSF-mediated transcription of HSPs within the range of heat tolerance were previously reported (Shitaoka et al. 2020). In this study, *HSF1* and its downstream *HSP* genes were consistently downregulated in the HP group. This might mean that these unbleached corals experienced less endoplasmic reticulum stress than the bleached corals. Previous study has found similar result that endoplasmic reticulum function might be impaired in naturally thermal bleached *P. decussata*, compared to unbleached counterparts (Zhang et al. 2022). Furthermore, combined with the fact that the abnormal high temperature event did not bleach the HP group corals and the role of *HSF1* as an indicator of thermal tolerance, these unbleached corals might have differential thermal tolerance compared to the bleached corals. As noted by Beere et al (2000) and Gupta et al (2010), the upregulation of HSPs could safeguard corals from heat stress by preserving the protein structure, thereby impeding the formation of apoptosomes. Therefore, it could be inferred from the indicator of bleaching, HSPs (Moghaddam et al. 2021), that bleached corals might maintain cellular function through high expression of HSPs, whereas unbleached corals appeared to show a different response tendency relative to bleached corals in this regard. This might portend a limited ability of unbleached *P. decussata* to respond to elevated temperatures by further modulating physiology (Franzellitti et al. 2018) following extreme thermal events, although these corals might differ from bleached counterparts in their thermal tolerance.

The NF-κB signaling pathway might have an important role in protecting *P. decussata* from bleaching. Nod-like receptors (NLR), a class of cytosolic pattern recognition receptors involved in microbial recognition and host defense in coral host (Franchi et al. 2009), activate the NF-κB signaling pathway. In this study, most of the DEGs enriched in the NLR signaling pathway were downregulated. TRAF6, which is a signaling protein upstream of NF-κB involved in innate immunity (van de Water et al. 2015), was downregulated in the HP group, as was *PKC*s, which acts synergistically with NF-κB in the apoptotic pathway (Liu et al. 2023). NF-κB-regulated activation of innate immunity and apoptotic pathways has been proposed to have a major role in bleaching (Weis 2008); in addition, its expression was downregulated in the symbiotic sea anemone *Aiptasia* compared with aposymbiotic anemones (Mansfield et al. 2017). Therefore, the downregulation of genes involved in NF-κB signaling suggested that the HP group might have the advantage of maintaining a symbiotic relationship in terms of gene expression and regulation compared to the BP group, which might be one of the reasons for the visual difference in *P. decussata*.

Similarly, the melanin synthesis pathway is a commonly used immune pathway in invertebrates. In cnidarians, tyrosinases synthesize melanin and participate in innate immunity (van de Water et al. 2018; Bailey et al. 2019). In the current study, *TYR*s was significantly downregulated in the HP group, suggesting that the mechanism of rejecting pathogens or symbionts had a relatively low level of transcription in non-bleached *P. decussata* when experiencing heat stress, further demonstrating the symbiotic status of these corals. Innate immunity in invertebrates is also related to energy metabolism processes involving the TCA cycle (Lanz-Mendoza and Contreras-Garduno 2022), and tyrosine might indirectly regulate the innate immune process by stabilizing coral host energy utilization through involvement in the TCA cycle.

Overall, the establishment and maintenance of a symbiotic relationship depended on host innate immunity. The maintenance of symbiotic relationships might favor coral resistance to high temperatures, which would be critical in the future to further understand the variation in thermal tolerance of individual corals.

Conclusion

In the present study, SMRT sequencing and next-generation sequencing were used to investigate the molecular differences between unbleached and bleached *P. decussata* coral after natural heat stress. The transcriptome of the scleractinian coral *P. decussata* was sequenced by SMRT sequencing technology, and GO and KEGG analyses determined that this coral had the capacity to regulate life activities, metabolism, and respond to stress, whereas its symbiotic algae are particularly outstanding in terms of metabolism. This provided a basis for further studies on coral symbiosis. In addition, *P. decussata* showed different health conditions under heat stress probably because of its altered energy utilization and innate immunity. In terms of energy metabolism, coral host that successfully survived after heat stress provided energy by upregulating amino acid metabolism, while releasing ammonium for symbiont utilization, which helped to maintain the energy-substance exchange in the symbiotic relationship and further strengthened the energy budget of coral symbiont. Moreover, in unbleached coral, the downregulation of *HSF*s, NF-κB signaling pathway-related genes, and *TYR* in the host weakened the innate immunity of *P. decussata*, which helped the symbiont to maintain a regular symbiotic relationship under unfavorable conditions. Overall, the current study revealed molecular mechanisms underlying the different responses of *P. decussata* to prolonged heat stress, providing a theoretical basis for understanding and evaluating thermal tolerance, and predicting survival strategies of *P. decussata* under future oceanic environmental changes.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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