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Oxidative stress, apoptosis activation and symbiosis disruption in giant clam *Tridacna crocea* under high temperature

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ABSTRACT

Giant clams are one of the most important animals in coral reef ecosystem, and its growth and reproduction are being threatened by heat stress due to global warming. In the present study, the symbiont density, the crucial enzyme activities and the transcriptome were investigated in the outer mantle of giant clam *Tridacna crocea* after the acute exposure of high temperature. The density of symbiotic zooxanthellae decreased significantly during 12–24 h, with the minimum level (7.75×10^5 cell cm^{-2}, $p < 0.05$) at 12 h after heat stress. The activities of superoxide dismutase in the heat stress group was significantly lower than that in the control group at 24 h after heat stress, while no significant change in the activities of catalase was observed during the entire stress process. The activation level of caspase3 began to increase significantly at 12 h (1.22-fold, $p < 0.05$), and reached the highest level at 24 h (1.38-fold, $p < 0.05$) after heat stress. Six paired-end libraries were sequenced in two groups, including the heat stress and control group at 12 h after heat stress. Through the assembling of 187,116,632 paired-end reads with lengths of 2×150 bp, a total of 26,676 genes were obtained which derived from giant clam. Bioinformatics analysis revealed 47 significantly upregulated and 88 significantly downregulated genes at 12 h after the treatment. There were 12 overrepresented GO terms for significantly upregulated genes, mostly related to unfolded protein binding and ATP binding, whereas no GO term was overrepresented for significantly downregulated genes. These results collectively suggest high temperature could induce excessive oxidative stress through the repressed antioxidant ability, the apoptosis activated by the unfolded protein response, and further the collapse of the symbiosis between host and symbiont, which has been threatening the growth and reproduction of the giant clam *T. crocea*.

1. Introduction

Giant clams live mostly in coral reefs in the tropical Indo-Pacific through the symbiosis with the dinoflagellate *Symbiodinium*, colloquially known as zooxanthellae [1]. The elaborately symbiosis endows giant clams with the successful adaptation to well-lit and oligotrophic waters in coral reef ecosystem. Unlike the intracellular symbiosis in stony corals, symbiotic zooxanthellae reside extracellularly inside a tubular system permeating the outer mantle of giant clams [2]. The symbionts provide giant clams with nutrients through photosynthesis, which can constitute up to 100% of the host's energy requirements [3]. In exchange, giant clams provide the symbionts with essential nutrients such as inorganic carbon, nitrogen, and phosphorus in support of their metabolism and growth [4].

Giant clams play various ecological roles in coral reef ecosystem, whereas populations of wild giant clams have been declining all over the world [5–9]. The shells of giant clams can provide substrate for epibionts and contribute to the complexity of the coral reef structure, while their mantle cavities harbour substantial commensal and ectoparasitic organisms [10,11]. Furthermore, giant clams can expel *Symbiodinium*, faeces and gametes to feed other organisms in the coral reef ecosystem [9]. Because the shifts of the mean annual sea surface temperature due to global warming have caused the outbreak of coral bleaching that increase both in frequency and severity, coral reef ecosystem has also been degrading over the past three decades [12]. The destruction of environmental condition in coral reef ecosystem has threaten the growth and reproduction of giant clams [13], and therefore their conservation, reproduction and adaptation have been paid more attentions for the restoration and protection of coral reef ecosystem [14].

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When giant clams suffer from heat stress, their symbiosis with zooxanthellae can collapse through the disordered metabolism, further resulting in their bleaching. It has been observed that the exposure to increased water temperature could cause significant respiration increase with a high photosynthetic rate in giant clam *Tridacna squamosa* [15], and induce the change of fatty acid diversity and the expression upregulation of genes involved in lipids in giant clam *Tridacna maxima* [16]. Heat stress in combination with an irradiance increase has been reported to cause the loss of symbiont, the retention of small symbiont and the chlorophyll content decrease of remaining symbiont in giant clam *Tridacna gigas*, which further resulted in the collapse of the clam-zooxanthella symbiosis, and finally the bleaching of giant clam [17]. Furthermore, it has also been considered that heat stress could lead to two sequential cell death processes for the symbionts in giant clam *Tridacna maxima* [16], and total mortality of trochophores in giant clam *Tridacna squamosal* [18–20]. These studies have described the responses of giant clam to heat stress in the morphology and metabolism level, however, the detailed physiological mechanism underlying the heat stress response of giant clam and their bleaching remains unclear.

Giant clam *Tridacna crocea* is a species of bivalve in the family Cardiidae. It is native to the Indo-Pacific region, and its typical habitat is embedded in massive corals. The giant clam has been reported to suffer from the serious bleaching and even death, and this mass bleaching accompanied with high sea surface temperatures [17]. To understand systemically the potential effects of high temperature on the physiology and symbiosis of giant clam, the symbiotic zooxanthella density, the crucial enzyme activities and the metatranscriptome were investigated in the giant clam *T. crocea* after the acute exposure to high temperature. The present study would provide insights to further understand the physiological mechanisms underlying heat stress response of giant clam and its potential environmental adaptability.

2. Materials and methods

2.1. Giant clam

The giant clams *T. crocea* (Shell length: 8–10 cm) were collected and cultured in flow-through aquaria (ca. 300 L) filled with natural seawater (Temperature: 26 °C; Salinity: 34) in a facility located at Hainan University. Cultures were illuminated with metal halide bulbs in a 12 h/12 h light-dark cycle for two weeks to acclimatize in laboratory conditions.

2.2. Acute heat stress exposure

A total of 25 giant clams were employed in the heat stress experiment. Ten giant clams were incubated at 32 °C, which were referred hereafter as the heat stress group, while another ten giant clams remained at 26 °C and used as the control group. Subsequently, five clams were sampled randomly in each group at 12 and 24 h of incubation, while five clams at 0 h were sampled as the blank group (no treatment). The outer mantles (containing zooxanthellae) of 3 giant clams each from the heat stress and control groups after 12 h treatment were collected and stored immediately in liquid nitrogen for subsequent RNA extraction and metatranscriptome sequencing. Meanwhile, outer mantles from 5 giant clams were prepared in each group at each time point and used for evaluating the density of symbiotic zooxanthellae and the activities of crucial enzymes.

2.3. The density determination of symbiotic zooxanthellae

Variation in the density of symbiotic zooxanthellae after acute heat stress was determined by the method described by Maboloc et al. with few modifications [21]. Briefly, the outer mantles sampled were cut into smaller pieces, and were homogenized using a tissue homogenizer. After filtered in a 100 μm sized mesh sieve to remove the cell debris, the homogenates with symbionts were centrifuged at 1500 × g, 4 °C for 10 min, and the supernatants were used to determine enzyme activities of giant clams. The harvested symbionts were resuspended in the filtered seawater after the washing three times, and counted using a Neubauer hemocytometer (QIUJING, China). The surface area of the outer mantle was determined using the scaled photo method [22], and the density of symbiotic zooxanthellae was defined as the ratio of their number to the surface area of the outer mantle (cell cm^{-2}).

2.4. Activity assay of antioxidant enzyme

The activities of superoxide dismutase (SOD, JIANCHENG, A001) and catalase (CAT, JIANCHENG, A007) in the supernatants were measured using commercial kits, according to the manufacturer's protocols. Total SOD activity was determined by the hydroxylamine method [23], where 1 SOD activity unit was defined as the enzyme amount causing 50% inhibition in 1 mL reaction solution. Total CAT activity was detected using spectrophotometry to measure the yellowish complex compound generated after the reaction between hydrogen peroxide and ammonium molybdate. Here, 1 CAT enzyme activity unit referred to the amount of enzyme needed to degrade 1 mmol hydrogen peroxide per second. The concentration of total protein in the supernatant was quantified by BCA method [24]. SOD and CAT activities in the supernatants were the ratio of the total enzyme activity unit to the total protein, and the results were expressed as U mg^{-1} protein.

2.5. Activity assay of caspase3

The caspase3 activities in the supernatant was measured by Caspase-3 Colorimetric Assay Kit (KeyGEN BioTECH) according to the instruction. Briefly, the supernatants of all samples were diluted firstly to the same protein concentration. Then, 50 μL supernatants were added in the reaction mixture containing 50 μL reaction buffer and 5 μL substrate. After an incubation in the dark at 37 °C for 4 h, the color change was detected spectrophotometrically at the wavelength of 405 nm. The activity of caspase3 was defined as the absorbance of the reaction solution at 405 nm (ABS_{405}), and the activation level of caspase3 in the mantle of giant clams was defined as the ratio of ABS_{405} in samples to that of the blank group.

2.6. Deep sequencing of outer mantle metatranscriptomes

Total RNA was isolated from outer mantle samples of giant clams following the TRIzol reagent (Invitrogen) protocol. The extracted total RNA was quantified by Nanodrop 2000 (Thermo Scientific) at 260/280 nm (ratio > 2.0) and its integrity was checked with Agilent 2100 Bioanalyzer (Agilent Technologies). Six paired-end fragment libraries (2 × 150 bp) were constructed and sequenced on an Illumina HiSeq X platform according to the manufacturer's instructions (BGI, Shenzhen, China). The generated raw sequencing reads were deposited at the NCBI Short Read Archive (SRA) under Accession No. SRP125867.

2.7. Assembly and differentiation of transcripts in the outer mantle of giant clams

Raw reads from six transcriptome libraries were first processed using the Fastx-toolkit pipeline (http://hannonlab.cshl.edu/fastx_toolkit/index.html) to evaluate sequencing quality, and remove low quality reads and adaptor sequences. The obtained clean reads were merged to assemble transcripts and genes, using the software package Trinity (http://trinityrnaseq.github.io/) [25]. Giant clam and zooxanthella transcripts were further differentiated via the homologous alignment method with the BLAST software as previously reported with several modifications [26]. Briefly, the sequences of all proteins encoded by the genome of oyster *Crassostrea gigas*, zooxanthella *Symbiodinium microadriaticum* (clade A1), *Symbiodinium minutum* (clade B1),

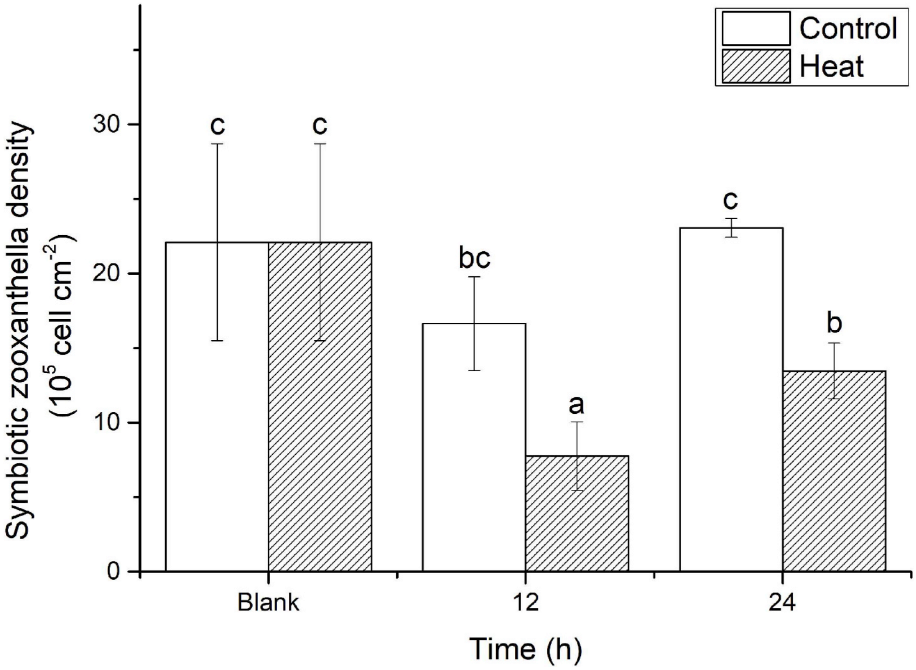

Fig. 1. Density change of the symbiotic zooxanthellae in the outer mantle of the giant clam *Tridacna crocea* after heat stress. Vertical bars represent the mean ± S.D. (N = 5), and bars with different letters are significantly different ($p < 0.05$).

Symbiodinium goreaui (clade C1) and *Symbiodinium kawagutii* (clade F1) were downloaded, and merged to construct a protein BLAST database. The assembled transcripts were aligned to the protein database via local BLASTX program (evalue < 0.00001, max_target_seqs = 1). Transcripts with encoded proteins sharing higher sequence similarity with that from oyster *C. gigas* than *Symbiodinium* species were regarded as giant clam-derived transcripts. Furthermore, transcripts were defined as originating from *Symbiodinium* that encoded proteins with higher sequence similarity with proteins from the four *Symbiodinium* species.

2.8. Identification of differentially expressed genes

All assembled transcripts served as reference sequences for reads mapping. The alignment of paired-end reads to the reference sequences was performed using the RSEM and bowtie2 software [27]. DESeq2 software was used to estimate transcript abundances and identify differentially expressed genes between the heat stress and control groups [28]. Based on the differentiated transcripts, two differentially expressed gene lists of the giant clam *T. crocea* were retrieved in the outer mantle after heat stress, including significantly upregulated and downregulated genes.

2.9. GO overrepresentation of differentially expressed genes in giant clam

The likely encoding proteins of all giant clam genes were retrieved using the software package TransDecoder (http://transdecoder.github.io/). Their transcripts and proteins were aligned by local BLASTX and BLASTP search to SwissProt databases (max_target_seqs = 1). The potential domains of these proteins were predicted through a Pfam search. After the homology search and protein identification, signal peptide and transmembrane region were further predicted using the SignalP 4.1 (http://www.cbs.dtu.dk/cgi-bin/nph-sw_request?signalp) and TMHMM (http://www.cbs.dtu.dk/cgi-bin/nph-sw_request?tmhmm) softwares, respectively. All obtained results were submitted to the Trinotate SQLite database, following the procedure of the Trinotate 3.1.0 software (http://trinotate.github.io/). Finally, functional annotation and GO terms were assigned to all giant clam genes.

GO overrepresentation analysis was implemented via the hypergeometric test with filter value of 0.05. The significantly upregulated or downregulated genes in giant clams were selected as test set, while all assembled giant clam genes were used as the reference set. The BiNGO tool in Cytoscape software was employed to calculate the overrepresented GO terms in the network and displayed them as a network of significant GO terms [29].

2.10. Statistical analysis

All data were presented as means ± standard deviation (SD). All of the data were subjected to one-way analysis of variance (one-way ANOVA) followed by multiple comparison (Tukey) to determine significant differences among the treatments and controls. Differences were considered significant at $p < 0.05$.

3. Result

3.1. Temporal variation in the density of symbiotic zooxanthellae after acute heat stress

The density of symbiotic zooxanthellae decreased significantly in the outer mantle of the giant clam *T. crocea* after acute heat stress. It decreased significantly during 12–24 h and reached the minimum level (7.75×10^5 cell cm^{-2}, $p < 0.05$) at 12 h after heat stress (Fig. 1). No significant differences were detected in the density of symbiotic zooxanthellae between the control group and blank group during the whole stress process.

3.2. Activity changes of SOD and CAT in the giant clam mantle after acute heat stress

For SOD activity, significant alteration in the heat stress group was observed only at 24 h (169.92 U mg^{-1}, $p < 0.05$) of stress, which was significantly lower than that in the control group (Fig. 2). Furthermore, there was no significant difference in the CAT activities between the heat stress and control groups for the entire duration of the experiment (Fig. 3).

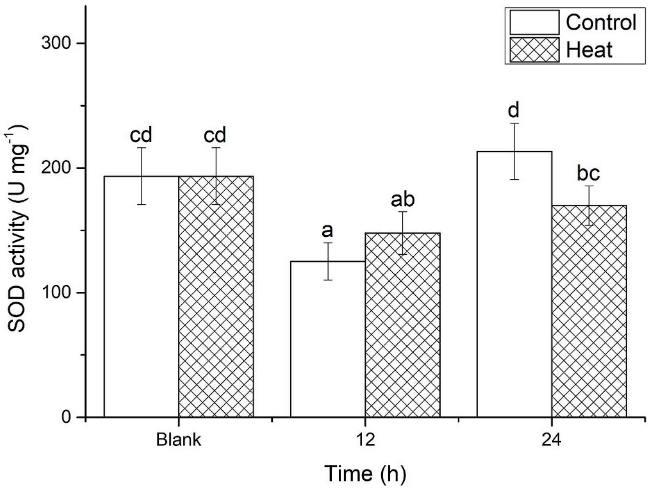

Fig. 2. Temporal activities of superoxide dismutase (SOD) in the outer mantle of the giant clam *Tridacna crocea* after heat stress. Vertical bars represent the mean ± S.D. (N = 5), and bars with different letters are significantly different ($p < 0.05$).

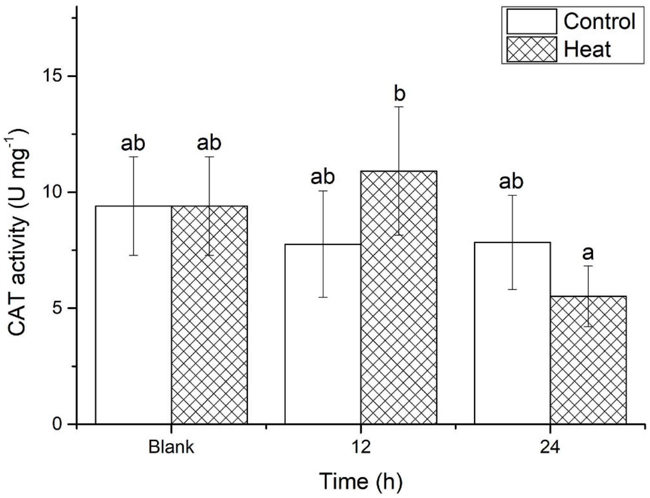

Fig. 3. Temporal activities of catalase (CAT) in the outer mantle of the giant clam *Tridacna crocea* after heat stress. Vertical bars represent the mean ± S.D. (N = 5), and bars with different letters are significantly different ($p < 0.05$).

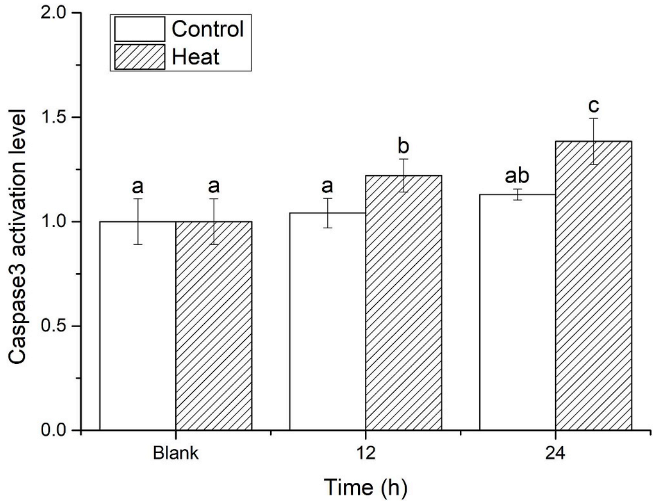

Fig. 4. Activation level alteration of caspase3 in the outer mantle of the giant clam *Tridacna crocea* after heat stress. Vertical bars represent the mean ± S.D. (N = 5), and bars with different letters are significantly different ($p < 0.05$).

Table 1
Mapping statics of six mantle transcriptomes.

Library	Total reads	Mapped reads	Mapped rate
Control_1	30,471,983	19,080,558	62.7%
Control_2	31,678,953	19,832,380	62.6%
Control_3	31,205,142	17,321,691	55.5%
Heat_1	30,886,135	17,700,831	57.3%
Heat_2	31,194,579	22,000,485	70.5%
Heat_3	31,679,840	19,875,563	62.7%

Table 2
Assembly statics of six mantle transcriptomes.

	Total	Giant clam	Zooxanthella
Assembled transcripts	1,080,251	63,205	318,120
Assembled genes	528,267	26,676	153,887
Transcript N50 (bp)	929	1460	1569
Gene N50 (bp)	811	1567	1487

3.3. Alteration of caspase3 activation level in the giant clam mantle after acute heat stress

The activation level of caspase3 in the heat stress group increased significantly in the outer mantle of the giant clam *T. crocea*, compared by those in the blank and control groups. It began to increase significantly at 12 h (1.22-fold, $p < 0.05$) after heat stress, and reached the highest level at 24 h (1.38-fold, $p < 0.05$) (Fig. 4). Furthermore, there was no significant difference in the caspase3 activation level between the control and blank groups.

3.4. The assembly and differentiation of transcripts in the giant clam mantle

After filtering of low-quality and adaptor sequences, there was a total of 187,116,632 paired-end reads obtained from all six metatranscriptome libraries. The number of reads in each library is summarized in Table 1. All six libraries were merged and submitted to the Trinity software for *de novo* transcriptome assembly. A total of 1,080,251 transcripts (N50 = 929 bp) were obtained, which corresponded to 528,267 genes (N50 = 811 bp). These transcripts were aligned to the BLAST protein database from *C. gigas*, *S. microadriaticum*, *S. minutum*, *S. goreaui* and *S. kawagutii*. Among all assembled transcripts, 63,205 transcripts (26,676 genes, transcript N50 = 1460 bp, gene N50 = 1567 bp) whose encoded proteins shared higher homology with oyster *C. gigas*, and designated as transcripts of the giant clam *T. crocea*. Likewise, the encoded proteins of 318,120 transcripts (153,887 genes, transcript N50 = 1569 bp, gene N50 = 1487 bp) shared higher homology with *Symbiodinium* species, which were regarded as the transcripts of *Symbiodinium* (Table 2).

3.5. Identification of differentially expressed genes

The high-quality reads in the six metatranscriptome libraries were processed in RSEM software for alignment to all assembled transcript sequences. The mapped rates ranged from 55.5 to 70.5% for the 6 libraries (Table 1). The counts of mapped reads in each gene were further submitted to the DESeq2 software to identify differentially expressed genes. After library calibration, the expression levels of all obtained genes were compared between the heat stress (treatment) and control groups, which revealed 2578 differentially expressed genes (FDR < 0.05), including 1198 significantly upregulated and 1380 significantly downregulated genes. For the giant clam *T. crocea*, there were 47

Table 3
Differentially expressed genes in symbiotic association and giant clam *Tridacna crocea* after heat stress.

	Symbiotic association	Giant clam
Total assembled genes	528,267	26,676
Differentially expressed genes	2578	135
Significantly upregulated genes	1198	47
Significantly downregulated genes	1380	88

significantly upregulated and 88 significantly downregulated genes retrieved according to the differentiated genes (Table 3).

3.6. Functional annotation of the differentially expressed genes after acute heat stress

A total of 47 significantly upregulated genes and 88 significantly downregulated genes were observed in the outer mantle of the giant clam *T. crocea* after heat stress. The GO overrepresentation of these differentially expressed genes in the control/stress comparison was further analyzed at multiple GO levels.

For the 47 significantly upregulated genes, there were 12 major overrepresented GO terms (Fig. 5), including *ATP binding* (GO:0005524, 7.38E-03), *adenyl ribonucleotide binding* (GO:0032559, 7.38E-03), *adenyl nucleotide binding* (GO:0030554, 9.57E-03), *purine nucleoside binding* (GO:0001883, 9.57E-03), *nucleoside binding* (GO:0001882, 9.57E-03), *unfolded protein binding* (GO:0051082, 9.57E-03), *ribonucleotide binding* (GO:0032553, 1.56E-02), *purine ribonucleotide binding* (GO:0032555, 1.56E-02) and *purine nucleotide binding* (GO:0017076, 3.07E-02) in the Molecular Function category, and *determination of adult lifespan* (GO:0008340, 7.38E-03), *multicellular organismal aging* (GO:0010259, 9.50E-03) and *protein folding* (GO:0006457, 4.56E-02) in the Biological Process category. Furthermore, there was no overrepresented GO terms for the 88 significantly downregulated genes in the outer mantle of the giant clam *T. crocea* after heat stress.

4. Discussion

It is similar with stony coral that giant clam will be able to suffer from the bleaching after the exposure to environmental changes [30]. High temperature is one of the most important factors for the bleaching of giant clam, which has threatened its survival and reproduction [31]. The bleaching is also caused by the collapse of the symbiosis between giant clam and zooxanthella, and characterized by the subsequent loss of symbiotic zooxanthellae [32]. In the present study, the change in the density of symbiotic zooxanthellae was determined in the outer mantle of the giant clam *T. crocea* to illustrate the effect of high temperature on the symbiosis of giant clam and zooxanthella. The symbiont density decreased significantly after heat stress, compared by those in the blank and control groups. It revealed that the heat stress could cause the decline of symbiont density in the outer mantle of the giant clam, demonstrating that the balance of clam-zooxanthella symbiosis could be disrupted by heat stress. The symbiosis collapse was similar with the observation in stony coral [33,34]. It was reported that when the temperature exceeds a certain degree, the enzymes participating in photosynthetic reaction will cease functioning, which can result in a lack of available oxygen and the accumulation of waste products in the mantle. The clam then would respond by expulsing the zooxanthellae from its tissue so as to reduce its metabolism [35,36]. In addition to this, it might also result from excessive oxidative stress during heat stress, which was supported by the recent report that the expressions of genes involved in ROS pathways were upregulated in giant clam *T. maxima* under high temperature [16]. The excessive oxidative stress would result in the expulsion of symbiotic zooxanthellae from the outer mantle of the giant clam. The rest of symbionts might not produce enough nutrient substances containing energies to meet the demand of the host, which would threat the growth and reproduction of the giant clam *T. crocea*.

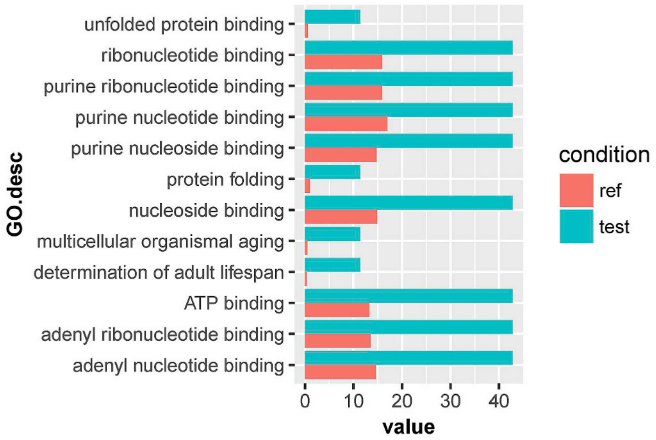

Fig. 5. Overrepresented GO terms of the 47 significantly upregulated genes at 12 h after heat stress. A total of 12 GO terms were overrepresented for these significantly upregulated genes. “Ref” referred to the proportion of genes annotated to a GO term in all reference gene of the giant clam *Tridacna crocea*, while “test” referred to the proportion of genes annotated to that GO term in all significantly upregulated genes.

The temporal activities of crucial enzymes including antioxidases and caspase3 were determined in the outer mantle of the giant clam *T. crocea* after heat stress, to further elucidate its physiological response to heat stress. Both SOD and CAT are main antioxidases in the redox system, and catalyze the deoxidation of superoxide radical and hydrogen peroxide, respectively. Results showed that the SOD activity at 24 h after heat stress was significantly lower than that in the control group, while no significant change in the CAT activities was observed during the entire stress process. It demonstrated that the SOD and CAT activities did not increase significantly in the giant clam *T. crocea* after heat stress. However, it was inconsistent with the previous observations where the expressions of antioxidase genes were upregulated in giant clam *Tridacna maxima* after heat stress [16]. The disparity in the observations could result from the differences in seawater temperature used and the sampling time, since the upregulated expression was observed between 3 and 13 days after heat stress (29 °C). The present results revealed that the antioxidant ability could be repressed in the outer mantle of the giant clam during the early stage of heat stress (32 °C). Environmental stressors generally induce the level of reactive oxygen species through respiratory burst in the internal environment of the organism. Therefore, the repression of antioxidant ability implied that there was excessive oxidative stress in the outer mantle after heat stress, which could also be supported by the overrepresented GO terms related to unfolded protein binding in the transcriptome analyses. Furthermore, the unfolded protein response might be activated, similar with the observation of the heat stress response in mollusc *Mytilus galloprovincialis* and *Laternula elliptica* [37,38].

Caspase3 is the key executory enzyme and final effector for apoptosis to occur [39]. The activation level of caspase3 was surveyed to understand the apoptosis status of the outer mantle in the giant clam *T. crocea* after heat stress. It increased significantly during 12–24 h, with the highest level at 24 h after heat stress. The result revealed that heat stress could induce the apoptosis of the outer mantle in the giant clam *T. crocea* [40]. It could be inferred from antioxidase results that the induction of apoptosis was caused by excessive oxidative stress and unfolded protein response in the outer mantle of the giant clam after heat stress. The unfolded protein response is a cellular stress response related to the endoplasmic reticulum, and has been found to be conserved amongst most eukaryotes [41,42]. If these unfolded or misfolded proteins are not repaired within a certain time span or the disruption is prolonged, the unfolded protein response would commit the cells to a pathway of apoptosis [43]. These results suggested that heat stress could also induce excessive oxidative stress owing to the repression of antioxidant ability, which further activate the apoptosis through the unfolded protein response in the outer mantle of the giant clam *T. crocea*. The induced apoptosis of outer mantle would influence negatively the supply of essential nutrients such as inorganic carbon, nitrogen, and phosphorus by giant clam to symbiotic zooxanthellae, which could limit their metabolism and result in its expulsion.

To investigate systematically physiological alterations of the giant clam *T. crocea*, their outer mantle metatranscriptomes were analyzed after subjecting to high temperature. A total of 135 differentially expressed genes were identified in the giant clam, demonstrating that their transcriptome had been remodeled after heat stress, which could be involved in the heat stress response and symbiont expulsion in the giant clam *T. crocea*. GO overrepresentation analyses of these differentially expressed genes were conducted to further understand the transcriptional change of the giant clam after heat stress. A total of 12 overrepresented GO terms for the 47 significantly upregulated genes were related mainly with unfolded protein binding and ATP binding. It demonstrated that acute heat stress could induce the production of unfolded protein and the utilization of ATP in the giant clam *T. crocea*. The emergence of unfolded proteins could result from the massive oxygen free radicals produced by the respiratory burst under environmental stress, such as elevated temperatures [44,45]. In general, molecular chaperones such as heat shock proteins can bind to the unfolded or misfolded proteins, and help these proteins regain nature conformation and recovery of its biological activity with the energy supply from ATP [46]. Therefore, some GO terms related with ATP binding were also overrepresented for the significantly upregulated genes in the present study, further revealing that the repair mechanism of unfolded proteins was activated in the giant clam *T. crocea* after heat stress. Furthermore, no overrepresented GO term was observed for significantly downregulated clam genes at 12 h after heat stress. These results together suggest that high temperature could induce excessive oxidative stress and apoptosis through negatively regulating antioxidant ability and unfolded protein response, and further bring about the expulsion of symbiont from the outer mantle, which would threaten the growth and reproduction of the giant clam *T. crocea*.

Acknowledgments

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