The role of catalase in the immune response to oxidative stress and pathogen challenge in the clam *Meretrix meretrix*

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**ABSTRACT**

Catalase (CAT) can effectively eliminate H$_2$O$_2$ and maintain the redox balance of immune system, which is essential for innate immunity. A catalase gene was cloned and its potential role in immune system was investigated in the clam, *Meretrix meretrix*. The catalase (*MmeCAT*) gene had an open reading frame of 1533 bp encoding 511 amino acids which showed high identity with that of molluscs. The distribution of *MmeCAT* in clam tissues was examined and the mRNA, protein expression and CAT activity paralleled with each other, with the highest expression in hepatopancreas. In response to H$_2$O$_2$ challenge, *MmeCAT* mRNA showed significantly higher expression at 12 h and 24 h post-challenge in experimental clams than in control clams (*P* < 0.05). Meanwhile, the protein expression in experimental clams was increased to about 3 times as much as that in control clams at 6 h post-challenge. After injection with a *Vibrio parahaemolyticus*-related bacterium (MM21), the expression of *MmeCAT* mRNA was significantly up-regulated at 12 h and 24 h post-injection (*P* < 0.05). It suggested that *MmeCAT* might be involved in the immune response to *Vibrio* infection. To better understand the role of *MmeCAT* in immune system, its mRNA expression was compared between a *Vibrio*-resistant population and a control population after immersion challenge with MM21. The continuously increased transcription in resistant population suggested *MmeCAT* could benefit the immune system of clams to defend against pathogen infection. Our study indicated that the redox balance was essential for *M. meretrix* to resist pathogen infection.

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1. Introduction

An essential component of innate immunity, the phagocyte can destroy invaded microbes by phagocytosis, during which the molecular oxygen can be converted into reactive oxygen species (ROS) to damage the microbes [12]. However, when phagocytes are strongly activated, the accumulated ROS will cause cellular damage and immune dysfunction [3]. To scavenge the excessive ROS, aerobic organisms have evolved enzymatic and non-enzymatic antioxidant systems [4–6]. Catalase (CAT, EC.1.11.1.6) is an important member of the enzymatic antioxidant system and exists in all oxygen-respiring organisms [7,8]. CAT can effectively catalyze the decomposition of hydrogen peroxide (H$_2$O$_2$) to keep the balance between de novo H$_2$O$_2$ generation and efficient elimination, which is essential for innate immunity [9]. Additionally, the up- or down-regulation of catalase can influence diverse biological processes, including cell proliferation, differentiation, migration and apoptosis [10], as the H$_2$O$_2$ involves in sensing and signaling of these biological events [11].

The CAT has been studied in various species of molluscs, and most of the research focused on CAT activity that served as the indicator of the species exposed to environmental stresses [12–15]. The full-length cDNA of CATs have been reported in scallop, *Chlamys farreri* [16], *Argopecten irradians* [ADD71945], abalone, *Haliotis discus discus* [17] and [ABF67505.1], *Haliotis diversicolor* [AEP83810], oyster, *Crasostrea hongkongensis* [18], *Crasostrea gigas* [ABS18267 and EK258222.1], freshwater mussel, *Cristaria plicata* [19], *Hyriopsis cumingii* [ADL14588] and pearl oyster, *Pinctada fucata* [20]. According to these reports, the CATs responded to H$_2$O$_2$ challenge and the purified recombinant CATs could catalyze the decomposition of H$_2$O$_2$ [17–19]. In addition, the transcription of CAT genes was induced by pathogen infection, which indicated the CATs might involved in the immune defence against pathogen infection [16,18,20].

The clam *Meretrix meretrix* is one of important commercial species in coastal areas of South and Southeast Asia and the clam culture is an important aquaculture industry in China [21]. In recent years, *Vibrio* infection has caused this commercial clam farming
heavy economic losses [22]. Understanding the defense mechanisms of *M. meretrix* is beneficial of maintaining the healthy development of this clam industry. Previous studies have indicated CATs might have immune roles in molluscs [16,18,20], and we intend to further illustrate the immune role of CAT in *M. meretrix* using the Vibrio-resistant populations as research materials, which can help us to understand the defense mechanisms of this clam.

In the present study, we cloned and analyzed the cDNA sequence of CAT (MmeCAT). The tissue distribution of MmeCAT was detected, including its mRNA expression, protein expression and enzyme activity. Clams from a natural population were challenged by H2O2 and a *Vibrio parahaemolyticus*-related bacterium (MM21) [22]. The two kinds of challenge will help us to analyze the response of MmeCAT to H2O2 and pathogen challenge. Furthermore, the immune role of MmeCAT was investigated by comparing the expression of MmeCAT mRNA in MM21 challenged clams collected from the *Vibrio*-resistant population and the control population.

### 2. Materials and methods

#### 2.1. Experimental clams

##### 2.1.1. Natural population

Healthy adult clams (40.0 ± 5.0 mm in shell length) collected from Shandong natural population were acclimated for one week in the laboratory. Clams were maintained at 25 °C, 30‰ salinity, fed with *Isochrysis galbana* and kept in continuous aeration during the acclimation period. Eight similar size clams (42.0 ± 0.8 mm in shell length) were selected and their hepatopancreas, adductor muscle, gill, mantle and foot were dissected, respectively. Different tissues of the clams were preserved in liquid nitrogen for RNA or protein extraction.

##### 2.1.2. Selected Vibrio-resistant population and control population

The selected *Vibrio*-resistant population (09RP) and the control population (09CP) of *M. meretrix* were bred in the hatchery laboratory of Zhejiang Mariculture Research Institute (Wenzhou, Zhejiang). The production process of the two populations has been described by Wang et al. [23]. In brief, the parents of 09RP were the remained alive with NCBI local-blast tool (ftp://ftp.ncbi.nlm.nih.gov/toolbox/ncbi_tools/CURRENT/). ESTs related to a reference sequence which was deduced as a part of catalase were picked out from the cDNA database of *M. meretrix* [24]. These ESTs were assembled to a longer cDNA sequence, and the assembled cDNA sequence was analyzed using the BLASTX and BLASTP search programs (http://www.ncbi.nlm.nih.gov/blast/). Specific primers, CAT1f (5'-ATGGCAAAAAGGCAATGAGCA) and CAT2f (5'-GGGGAAACTGACTGATGATAGC), CAT1r (5'-ATGGCTGTCGACTGATGTATC) and CAT2r (5'-GCCGGAATGCTCAAGCTACTG), CAT2r (5'-CCCTCGGAAACCATCCTCAC) were used to verify this cDNA sequence by amplifying the cDNA sequence prepared by Huan et al. [24]. The PCR program was set as: 1 cycle of 94 °C for 4 min for denaturation, followed by 35 cycles of 94 °C for 20 s, 58 °C for 20 s for CAT1f and CAT1r or 53 °C for 20 s for CAT2f and CAT2r, 72 °C for 90 s, and 1 cycle at 72 °C for 10 min. PCR product of expected size was excised and extracted with the Gel Extraction Kit (Promega, USA), then subcloned into pMD19-T (TaKaRa, Dalian, China) and sequenced by Sunnybio (Shanghai, China).

The deduced amino acid sequence of MmeCAT was analyzed in ExPaSy server (http://www.expasy.org/tools/) and compared with that of other 7 CATs which showed high identities to MmeCAT in Genbank using BioEdit version 7.0.5.2. Phylogenetic analysis was constructed by comparing amino acid sequence of MmeCAT with that of other 18 CATs using MEGA version 4.0 [25] on the basis of p-distances by the Neighbor-joining method [26]. For the construction of the phylogenetic tree, all positions containing gaps and missing data were eliminated from the multiple alignments. The reliability of the tree obtained was assessed by bootstrapping using 1000 bootstrap replications [27].

### 2.6. MmeCAT mRNA expression in different tissues

The expressions of MmeCAT mRNA in different tissues were measured by SYBR Green quantitative RT-PCR using specific primer pair of CAT3f (5'-CTTCCACAAGCTGACAGATC and CAT3r (5'-TCTCCCAAATCCTGACATC). β-actin gene was amplified by specific primers (act-f 5'-TGTTCTGGATGGTCAACTATG and act-r 5'-TCCA-CATCAGCTGGGAAGGTG), which was set as the internal reference to normalize the expression levels between samples. Serial twofold dilutions of every sample (1, 1/2, 1/4 and 1/8) were amplified to determine both pairs of primer had similar efficiency using the formula $E = 10^{-1/slope}$. Four repeats of each sample were analyzed and the analysis was carried out on quantitative thermal cycler (Mastercycler ep realplex, Eppendorf, Germany) in a 10 μl reaction volume containing 2 ng template, 0.2 μM of each primers, and SYBR Green PCR master mix (Applied Biosystems). PCR parameters were 95 °C for 2 min, then 40 cycles of 95 °C for 15 s, 54 °C for 15 s and 68 °C for 1 min and 72 °C for 1 min.
for 30 s followed by the fluorescence melting curve analysis. Relative gene expression level was analyzed using the 2−△△CT method [28].

2.7. Detection of MmeCAT protein using Western blot

Total proteins of the tissues were extracted using a Total Protein Extraction Kit (BestBio, China) according to the manufacturer’s protocol. For the samples, three replicates (for the hepatopancreas of the sampled clams in H2O2 challenge test) or four replicates (different tissues of clams) were mixed together and separated electrophoretically in 12% SDS-polyacrylamide gels, then transferred onto a PVDF membrane by electroblotting at 100 V for 1 h. The membrane was blocked with 5% (w/v) skimmed milk powder solution at 4°C overnight and then incubated with 1:1000 diluted rabbit anti human CAT Polyclonal Antibody (Proteintech Group, USA) in Tris buffered saline with 0.5% Tween-20, pH 7.4 (TBST) containing 0.1% skimmed milk powder at 37°C for 1.5 h. After incubation, the membrane was washed 3 times with TBST, 10 min each, followed by the incubation with a 1:1000 diluted horseradish peroxidase-conjugated goat anti-rabbit IgG solution (Abcam, China) in TBST at 37°C for 1.5 h. After 3 washes of 10 min each in TBST, the MmeCAT band on the membrane was visualized using a DAB assay kit (Sangon Biotech Co., China). β-actin was set as the reference and it was detected using 1:1000 diluted anti-mouse β-actin monoclonal antibody (CWBio, China) and 1:1000 diluted horseradish peroxidase-conjugated goat anti-mouse IgG solution (Tiangen Biotech Co., China). The specificity of the primary antibody was confirmed (data not shown), while this antibody could also present other bands. Shorten the incubation time (about 30 s) with DAB reagent will greatly, but not totally eliminate the background bands.

2.8. CAT activity assay

Different tissues of the clams were homogenized in a chilled 20 mM Tris–HCl buffer (pH 7.1), and the homogenates were centrifuged at 1800 g for 10 min (4°C), then the clear supernatant fractions were used to investigate the CAT activity. For each tissue, there were four replicates. CAT activity was detected using the Catalase detection kit (Nanjing JianCheng Bio Inst., China). In brief, 50 μL clear supernatant fraction of a tissue homogenate was used to detect CAT activity following the manufacturer’s instructions. CAT activity is determined by the decomposition rate of H2O2. CAT can catalyze the decomposition of H2O2 and the remaining H2O2 will react with Ammonium molybdate to produce a yellow colored complex which can be measured by spectrophotometer at 405 nm. One unit of CAT is defined as the decomposition of 1 μM H2O2 (expect the non-enzymatic reaction) in system of enzymatic reaction per second. Tissue protein was determined using the Bio–Rad Protein Assay Kit (Bio–Rad Laboratories, Richmond, USA) with bovine albumin as a standard. CAT activity was expressed as CAT units per mg protein.

2.9. MmeCAT mRNA expression in hepatopancreas sampled in the H2O2 and Vibrio challenge test

The hepatopancreas of clams were used to detect MmeCAT mRNA expression under H2O2 and Vibrio challenge. The PCR procedure and primers were the same as that described in 2.6.

2.10. Statistical analysis

One-way ANOVA Tukey’s test was used to analyze MmeCAT mRNA expression and CAT activity in different samples, which was performed using PROC ANOVA command in SAS software (SAS version 8). A significance level of 0.05 was used for all tests.

3. Results

3.1. Sequence analysis of MmeCAT gene

The complete MmeCAT cDNA (GenBank accession number: JQ005875) consisted of a 106 bp 5′-UTR, a 1533 bp open reading frame (ORF), and a 560 bp 3′-UTR with a stop codon TAA (Fig. 1). It was deduced that MmeCAT encoded 511 amino acid residues with a calculated molecular mass of 58.2 kDa and an isoelectric point (pI) of 8.05. The deduced protein encoded by MmeCAT gene contained a highly conserved catalytic site motif, 61FNRERIPERVVHAKGAG77 and a proximal heme-ligand signature motif, 51PLFLYSDT358. Two putative N-glycosylation sites 34NK39 and 47NFT439 were detected and the peroxisome targeting signal in the C-terminus was 508AQL511. Three conserved catalytic amino acids, H72, N145 and Y355 were shown in Fig. 1.

Amino acid sequence alignment revealed that MmeCAT contained putative residues involved in NADP-binding (N145, H199, F195, S196, R200, N210, Y212, K234, V299, W300, Q302, Y355) or heme binding (R69, H72, R109, N145, F150, R200, N215, Y355, R362) (Fig. 2). The MmeCAT showed the highest similarities to one (with 511 amino acids) of the two CATs in oyster C. hongkongensis (80%), and had high level of identity with that of scallop C. farneri (77%) and human Homo sapiens (69%). The secondary structure elements of MmeCAT were predicted in SWISS-MODEL and MmeCAT had 14 α-helices and 11 β-sheets. H72, N145 and Y355 were located neighboring β2, on β4/5 in α10, respectively.

A phylogenetic tree was generated using the bacterial (Oceanobacillus iheyensis) CAT as the root and it showed vertebrate and invertebrate CATs formed separate clusters (Fig. 3). It was shown MmeCAT was placed in the Mollusc subcluster within the main invertebrate cluster. In that subcluster, CAT of disk abalone H. discus discus was positioned basally, with the CATs of bivalves including MmeCAT coming off next.

3.2. Tissue distribution of MmeCAT

The expression of MmeCAT mRNA was detected in five tissues, i.e. hepatopancreas, mantle, gill, foot and adductor muscle (Fig. 4A). The hepatopancreas exhibited the highest mRNA expression, yet the mRNA expression of mantle, foot and adductor muscle was at moderate level. The gill showed relatively low mRNA expression in comparison with the other tissues.

The rabbit anti human CAT Polyclonal Antibody was used to detect the CAT of M. mercenaria and it could present a single discernible band around 58KD for each tissue but gill (Fig. 4B). This results confirmed MmeCAT distributed mainly in hepatopancreas, yet scarcely in gill.

The CAT activity was assayed in different tissues and it was shown that the CAT activity was paralleled the MmeCAT mRNA expression in all tissues detected (Fig. 5). The highest CAT activity was detected in hepatopancreas. And the lowest activity was presented in gill. The CAT activity was not significantly different in adductor muscle, mantle and foot (P > 0.05).

3.3. MmeCAT expression in response to H2O2 challenge

The hepatopancreas of H2O2 challenged clams were dissected to detect the expression of MmeCAT mRNA and MmeCAT. The injection of 0.5% H2O2 decreased the MmeCAT mRNA expression in clams during early time post-challenge (Fig. 6). At 3 h post-challenge, the mRNA expression showed its lowest level in challenged clams, and it was significantly lower than that in control clams (P < 0.05). At 6 h post-challenge, the mRNA expression of the control clams showed its lowest level, when the mRNA expression began to
up-regulate in challenged clams. The mRNA expression was significantly higher in challenged clams than that in control clams at 12 h and 24 h post-challenge ($P < 0.05$).

The MmeCAT expression was investigated (Fig. 7A), and the intensity of the bands was analyzed using Quantity One (version 4.4.0) (Fig. 7B). Different from the MmeCAT mRNA expression, the protein expression varied slightly (within 0.6 time) in control clams during the 24 h, while it increased drastically in challenged clams at 6 h post-challenge, when the protein expression was about 3 times as much as that in control clams. Then, the MmeCAT expression in challenged clams was decreased to approximately half that in control clams at 12 h post-challenge. At the other sample time points, the MmeCAT expression in challenged clams was similar to that in control clams.

3.4. MmeCAT mRNA expression in response to MM21 injection

The expression of MmeCAT mRNA in hepatopancreas of both the PBS-injected clams and the MM21-injected clams is shown in Fig. 8. The MmeCAT mRNA expressions had no significant variation in the control clams during the 48 h we analyzed ($P > 0.05$). However, its expressions in the MM21-injected clams were
significantly increased at 12 h and 24 h post-injection and followed with a comeback at 36 h compared to that in control clams ($P < 0.05$).

3.5. MmeCAT mRNA expression in 09RP and 09CP in response to MM21 infection

Changes of MmeCAT mRNA expression in hepatopancreas of 09RP and 09CP in response to MM21 immersion challenge were detected (Fig. 9). MmeCAT mRNA expression was up-regulated in both 09RP and 09CP at 24 h post-challenge, like what happened in the natural population (section 3.4.). The up-regulated expression in 09CP decreased to the normal level at 48 h post-challenge, while the comeback did not happen in 09RP. Instead, the mRNA expression in 09RP still increased after 24 h post-challenge and there was a significant difference in the MmeCAT mRNA expression between 09RP and 09CP at 48 h post-challenge ($P < 0.05$).

4. Discussion

In this study, the cDNA of CAT gene in clam M. meretrix was identified. The ORF of MmeCAT has 1533 bp encoding a protein of 511 amino acids with a calculated molecular mass of 58.2 kDa that is similar to the CATs of other invertebrates and vertebrates [16]. In the deduced protein sequence, there are two N-glycosylation sites 34NKT36 and 437NFT439, and a peroxisome targeting signal 509AQL511, which suggests MmeCAT is a peroxisomal glycoprotein located in peroxisome, where is also the main location of H2O2 [29]. The fact that the CAT of mussel, Mytilus galloprovincialis locates in peroxisome has been reported [29,30]. Inside the peroxisome, the CAT acquires heme and forms an active tetrameric CAT molecule. Two highly conserved motifs, catalytic site motif, 61FNRER-IPERVVHAKGAG77 and proximal heme-ligand signature motif, 351RLFSYSDT358 are detected in the ORF. The sequence of the two motifs are nearly the same (at most two amino acids substitutions)
in reported molluscs [16–19]. Additionally, three catalytic amino acids, H72, N145 and Y355 are completely conserved among molluscs [16–19]. In the phylogenetic analysis, the mollusc CATs formed a cluster, indicating the CATs of molluscs may derived from a common ancestor [8]. Based on these results, we speculated MmeCAT could act the same as the reported CATs in molluscs.

The amino acid sequence of MmeCAT showed high identity with that of human H. sapiens (69%). As the CAT structure of human has been elucidated [31], the comparison between MmeCAT sequence and that of human can help us to uncover further informations about the function of MmeCAT. Twelve amino acids are responsible for the binding of NADPH in human [31], and 11 of them are conserved in the sequence of MmeCAT, except for the Q302. This result indicates MmeCAT belongs to the NADPH-binding CAT, which fits the statement that NADPH-binding is a signature of eukaryotes CATs [32]. The secondary structure of MmeCAT was similar to that of human, including the content of α-helix and β-sheet and the location of the catalytic residues [16]. These similarities in primary sequences and secondary structures lead us to believe MmeCAT can eliminate H2O2 with the same mechanism as human CAT.

The MmeCAT mRNA expression, MmeCAT expression and CAT activity were examined in different tissues and the three indexes were paralleled with each other. MmeCAT distributed mainly in hepatopancreas, which was related to the roles of hepatopancreas as the main metabolic tissue and the main defense tissue against oxidative stress [33]. It has been reported that the CAT activity was significantly higher in hepatopancreas than in gill [15], however the
fact that MmeCAT distributed scarcely in gill was inconsistent with the studies of other molluscs and the role of the gill that was expected to be the main tissue for the generation of ROS [17,18]. More than one type of CAT were found in *C. gigas*, *C. hongkongensis* and *H. discus* [17], so the other types of CAT might also exist in *M. meretrix*. While the CAT activity assay indicated the other CATs, even if exist, could be still scarce in gill. Besides CAT, peroxiredoxin (Prx) and glutathione peroxidase (GPx) can also effectively eliminate H\(_2\)O\(_2\) [33,34], so one potential explanation of the low MmeCAT expression in gills is that Prx or GPx or both exert the role of H\(_2\)O\(_2\) scavenger in gills of *M. meretrix*. We have observed high GPx activity in gills of *M. meretrix*, which can support this assumption [23].

As MmeCAT could act as a H\(_2\)O\(_2\) scavenger, its response to H\(_2\)O\(_2\) challenge was examined. The needle inoculation can induce some immune reaction which affect the normal physiological function in the early time of injection [35], so the expression of MmeCAT mRNA was decreased and the H\(_2\)O\(_2\) challenge accelerated the down-regulation. However, the decreased mRNA expression did not influence MmeCAT expression in the first 3 h post-challenge (Fig. 7). This result indicated the temporary decreased mRNA expression will not equally repress the protein expression and hence MmeCAT might be regulated by the post-translational mechanisms that will alter its degradation rate [34,36,37]. At 6 h post-challenge, MmeCAT mRNA expression began to up-regulate in experimental clams. Meanwhile, the protein expression in experimental clams was increased to about 3 times as much as that in control clams. Then the mRNA expression was significantly higher

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**Fig. 6.** Expression of MmeCAT mRNA in H\(_2\)O\(_2\) challenged clams and PBS-injected clams at 0, 1, 3, 6, 12, and 24 h post-challenge. Error bars represent standard deviation of three repeats. * Indicates a significantly different expression of MmeCAT mRNA (*P* < 0.05).

**Fig. 7.** Variation of MmeCAT in response to H\(_2\)O\(_2\) challenge. (A) Expression of MmeCAT in sampled clams at 1, 3, 6, 12, and 24 h post-challenge. C and E represent control group and experimental group, respectively. (B) Relative quantity of MmeCAT. The intensity of MmeCAT and ß-actin (MmeACT) bands was all quantified using Quantity one (version 4.4.0). The intensity of MmeCAT was divided by the intensity of its corresponding MmeACT, through which the relative quantity of MmeCAT was calculated.

**Fig. 8.** Expression of MmeCAT mRNA in MM21-injected clams and PBS-injected clams at 0, 6, 12, 24, 36, and 48 h post-challenge. Error bars represent standard deviation of four repeats. * Represents a significantly higher expression of MmeCAT mRNA in MM21-injected clams compared to that in PBS-injected clams at the same time post-challenge.

**Fig. 9.** Expression of MmeCAT mRNA in clams of 09RP and 09CP at 0, 6, 12, 24, and 48 h post-challenge. Error bars represent standard deviation of four repeats. * Indicates a significantly higher expression of MmeCAT mRNA (*P* < 0.05).
in experimental clams than in control clams at 12 h and 24 h post-challenge \((P < 0.05)\). These results showed MmeCAT could respond to \(H_2O_2\) challenge, which was consistent with the study in disk abalone, *H. discus discus* [17]. CAT is located in the peroxisome and \(H_2O_2\) can not be eliminated when its concentration is very low at the early time post-challenge [34]. With the diffusion of \(H_2O_2\) into cells, the accumulated \(H_2O_2\) can accelerate the degradation rate of CAT [36,37], which may result in the decreased amount of MmeCAT in challenged clams at 12 h post-challenge. At 24 h post-challenge, the transcription of MmeCAT gene kept increasing and the newly translated MmeCAT might compensate its degradation in experimental clams.

As the \(H_2O_2\) challenge could induce the expression of MmeCAT mRNA, we wonder whether the pathogen infection can also influence the transcription of MmeCAT gene. *A. vibrio* challenge test was conducted and the expression of MmeCAT mRNA was detected. It was found that the mRNA expression was relatively stable in hepatopancreas of the control clams, while its expression in the MM21-injected clams increased significantly at 12 h and 24 h post-injection, in accordance with the reports in Zhihong scallop, *C. farreri* [16]. It could be inferred from the results that MmeCAT was involved in the immune defense against pathogen infection. The pathogen infection can lead to an overexpressed \(H_2O_2\) which could be one of the factors influence the transcription of MmeCAT gene [12]. The CAT can be elevated by many transcription factors, such as AP-1, NF-kB, which may result in the decreased amount of MmeCAT in MM21-injected clams increased significantly at 12 h and 24 h post-challenge, the expression of MmeCAT mRNA was detected. It can be considered that the transcription factors or elicit stronger reactions of the transcription factors than \(H_2O_2\) can, while this assumption should be verified by further experiments.

A selected *Vibrio*-resistant population and a control population were used to better understand the immune role of MmeCAT played in *M. meretrix*. In response to MM21 challenge, the MmeCAT mRNA expression was up-regulated both in 09RP and 09CP at 24 h post-challenge, while it was still increased in 09RP not 09CP at 48 h post-challenge, from which we deduced that the increased transcription of MmeCAT gene would benefit the immune system of clams to defend against pathogen infection. It has been reported in *Drosophila* that CAT played a key role in immune system and the reduced CAT expression would cause high mortality of the infected species [9]. Pathogen infection could produce the oxidative stress which would induce apoptosis and necrosis of host cells and decrease the efficiency of immune system [42,43], so the increased MmeCAT might neutralize the oxidative stress to keep the immune system work efficiently. Once the immune system was repressed, the proliferation of pathogen would not be inhibited and the host could endure a stronger pathogen virulence which is concentration dependent [22]. Our previous study has indicated the transcription of GPx gene was up-regulated earlier in 09RP than in 09CP, which could help to maintain the redox state of immune system [23]. And the present study further elucidated that the redox balance was essential for *M. meretrix* to resist pathogen infection.

In conclusion, we found MmeCAT distributed mainly in hepatopancreas, the main defense tissue against oxidative stress. MmeCAT mRNA expression was up-regulated under \(H_2O_2\) and pathogen challenge, however, the up-regulation was more stronger in MM21 challenged clams than in \(H_2O_2\) challenged clams. The continuously increased transcription in resistant population of *M. meretrix* suggested MmeCAT could benefit the immune system of clams to defend against pathogen infection.

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