GENOME-WIDE IDENTIFICATION AND CHARACTERIZATION OF CATALASE (*CAT*) GENE IN ZHIKONG SCALLOP REVEALS GENE EXPANSIONS AFTER EXPOSURE TO THE PST-PRODUCING DINOFLAGELLATE, *ALEXANDRIUM MINUTUM*

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Abstract

Bivalves can accumulate high concentrations of paralytic shellfish toxins (PSTs) produced by toxic algae that may induce oxidative stress. Catalase (CAT) is one of the important antioxidant enzymes involved in scavenging the high level of ROS and plays a significant role in the protection of aerobic organisms against oxidative stress by degrading hydrogen peroxide. In the present study, a total of two *CAT*s were identified in the *Chlamys farreri* genome. Sequence characterization revealed *CfCAT* protein sequences contained proximal heme-ligand signature sequence (^{354}RLFSYSDTH^{362}), two N-glycosylation sites (^{440}NFS^{442} and ^{482}NFT^{484} domains), the proximal catalase active site signature (^{64}FNRERIPERVVHAKGGGA^{81}), the peroxisome-targeting signal in the C-terminus ^{495}QKL^{497} and 12 amino acids (H^{89}, N^{171}, F^{154}, S^{122}, R^{112}, N^{142}, Y^{325}, K^{169}, I^{311}, W^{277}, Q^{331}, and Y^{260}), which were identified as the putative residues involved in NADPH binding. Eight amino acids: R^{320}, H^{364}, R^{365}, N^{369}, F^{409}, R^{432}, Y^{404}, and R^{457} were identified as the heme-binding site residues. Three conserved catalytic amino acids (H^{166}, N^{148}, and Y^{137}) and catalase signature sequences were essential for the structure and function of *CfCAT*s. The homology of deduced amino acid sequences revealed that *CfCAT*s had high identity with catalases from other mollusks. RNA-Seq data analysis revealed there were no regulation patterns of scallop *CAT*s were significantly induced in the kidney after exposed to *Alexandrium minutum* (AM-1). Gene expression analysis in the scallop revealed all *CAT*s being predominantly expressed in the mantle, gill, muscle, and hepatopancreas after feeding the scallop with PST-producing dinoflagellates. All these results indicate that CAT involves an important role in counteracting oxidative stress in *C. farreri* by PST accumulation. The tissue-, species-, and toxin-dependent expression pattern of scallop *CAT*s might be involved in their functional diversity in response to toxin exposure.

Keywords *Chlamys farreri*; catalase; gene expansion; paralytic shellfish toxins (PSTs); *A. minutum*; expression profiling

Introduction

Reactive oxygen species (ROS) are generated as a by-product of aerobic metabolism where the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are formed when molecular oxygen chemically oxidizes electron carriers (Chaitanya, Shashank et al. 2016). Through the per-oxidizing of cellular proteins, nucleic acids, lipids, enzyme deactivation, down-regulation of redox-sensitive processes, and signaling pathways, the excessive production of ROS in cells can harm a variety of cellular components and immune dysfunction. Antioxidant enzymes are crucial in protecting organisms from the potentially harmful consequences of oxidative stress caused by various environmental stresses (Arockiaraj, Easwvaran et al. 2012). Oxidative stress is caused by the over-production of ROS and damage to various organs has occurred as a critical factor for organisms responding to environmental challenges. (Li, Wang et al. 2022). All aerobic species have developed enzymatic defense mechanisms against ROS including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and peroxiredoxins (Prx) to prevent oxidative stress. Among these systems, catalase (EC 1.11.1.6) is a major enzyme of the enzymatic antioxidant system that catalyzes the breakdown of hydrogen peroxide (H_2O_2) to water and oxygen and is essential to life. This enzyme is ubiquitous among aerobic

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organisms and plays an important role in protecting cells from oxidative damage by ROS (Ken, Lin et al. 2000).

Additionally, the expression level of CAT in cells can also affect many other biological processes (Wu, Li et al. 2016). Studies on the structure and regulation of catalase genes and proteins have been reported in plants (McClung 1997), bacteria (Storz and Tartaglia 1992), and mammals (Park, Kim et al. 2004). To date, more studies about catalase have focused on aquatic organisms including fish, crustaceans, and mollusks (Gao, Ishizaki et al. 2016). Bivalve mollusks, like other aquatic invertebrates, are continually exposed to possibly harmful xenobiotics and a wide range of toxic stressors in their environment. In mollusks, cloning and characterization of CAT gene sequences have been reported for the scallop *Chlamys farreri* (Li, Ni et al. 2008), abalone *Haliotis discus discus* (Ekanayake, De Zoysa et al. 2008), oyster *Crassotrea hongkongensis* (Zhang, Fu et al. 2011), pearl oyster *Pinctada fucata* (Guo, Zhang et al. 2011), mussel *Cristaria plicata* (Xilan, Gang et al. 2011), and clam *Meretrix meretrix* (Wang, Yue et al. 2013). Previous studies on the effects of heavy metal exposure, bacterial challenge, and PST exposure on antioxidant enzyme responses, particularly CAT responses, have already been conducted in various bivalve species (Gao, Ishizaki et al. 2016). But few studies have documented how toxins or exposure to harmful algae species alters the gene expression in bivalves.

Filter-feeding bivalves can accumulate paralytic shellfish toxins (PSTs) produced by marine dinoflagellates through the consumption of algae. The genus Alexandrium spp which is strongly associated with harmful algal blooms (HABs) and are the most potent marine biotoxins (Li, Sun et al. 2017). Saxitoxin (STX) and its variants which can reversibly bind the voltage-gated Na+ channels (NaV) of excitable cell membranes and block the conduction of nerve signals are the main components of the class of neurotoxins known as paralytic shellfish toxins. This stress on the body's metabolism and paralysis of the neuromuscular system is caused by this blockage of nerve signals. (Bricelj, Connell et al. 2005, Li, Sun et al. 2017, Wang, Liu et al. 2021).

Scallops, as filter feeders may consume toxic dinoflagellates that can accumulate high levels of neurotoxins in their tissues, tolerate a higher concentration of PSTs, and retain these toxins for a more extended period than other bivalve species (Tan and Ransangan 2015). Based on the genomic resources, the present study performed systematic identification of *CAT* genes in the commercially important bivalve species known as the Zhikong scallop, *Chlamys farreri* (Jones et Preston, 1904, also known as Chinese scallop) which is farmed as aquaculture in China (Li, Sun et al. 2017). After being challenged by the PST-producing dinoflagellate, *Alexandrium minutum*, the expansion of scallop *CAT* genes and their tissue-specific expression were revealed and examined using RNA-seq datasets. These results will help understand the molecular mechanisms in *C. farreri* after exposure to *A. minutum*, and could be used to protect this commercially important species from oxidative stress and PST-induced antioxidant responses in bivalves.

Materials and Methods

Sample Collection

The Zhikong scallop, *C. farreri* was collected from Xunshan Group Co., Ltd. (Rongcheng, Shandong Province, China) and Zhangzidao Group Co., Ltd. (Dalian, Liaoning Province, China), respectively. To analyze the effects of PST-producing dinoflagellate, two-years-old adult scallops of *C. farreri* were acclimated in filtered and aerated seawater at 12–13°C and depurated for three weeks by feeding non-toxic algae, *Isochrysis galbana* (7.5×10^5 cells/mL) (Romero - Geraldo and Hernández - Saavedra 2014) as the control group then maintained

separately with aeration during the exposure experiments (Escobedo-Lozano, Estrada et al. 2012).

Genome-Wide Identification and Sequence Analysis of *CAT* Genes in *C. farreri*

The transcriptome and whole-genome sequence databases (Li, Sun et al. 2017, Hu, Li et al. 2019) of *C. farreri* were searched to identify the *CAT* genes using all available *CAT* protein sequences of invertebrates and vertebrates, including *Homo sapiens, Mus musculus, Xenopus tropicalis, Bos taurus, Rattus norvegicus, Danio rerio, Gallus gallus* and some mollusks species: *Pinctada fucata, Unio tumidus, Haliotis discus hannai, Crassostrea gigas, C. hongkongensis, Argopecten irradians, Mimachlamys nobilis, Hyriopsis cumingii, Meretrix meretrix, Sus scrofa,* and *Cristaria plicata, Azumapecten farreri* queries from NCBI, Ensembl, and OysterBase online databases to obtain candidate *CAT* sequences. ORF finder was used to translate the candidate *CAT* sequences and the predicted *CAT* proteins were aligned to public online databases including UniProt. The predicted amino acid sequence was conducted by BLASTP against the NCBI non-redundant (Nr) protein sequence database (e-value set: 1E-05). All candidate *CAT*s with a significant BLAST hit were obtained and the presence of *CAT* domain was further verified by Conserved Domains Database and SMART tools. The compute pI/MW tool was used to predict the pI value and MW (kDa). "Multiple EM for Motif Elicitation" (MEME) version 5.4.1 was used to find conserved motifs in *CfCAT* proteins. The physicochemical properties of *CAT* proteins of scallops were performed using the ProtParam tool. The subcellular localization of *CAT* proteins was analyzed through ProtParam, CELLO (Yu, Chen et al. 2006), EukmPLoc 2.0 (Chou and Shen 2010), and WoLF PSORT II (Horton, Park et al. 2006). The percentages of similarity and identity of full-length amino acid sequences between *CfCAT* and *CAT* proteins from other organisms were calculated using the Sequence Identity and Similarity (SIAS) tool. The exon/intron structure analysis of *CAT* was carried out from the scallop genome GFF3 gene annotation file using the Gene Structure Display Server. The secondary structure of *CAT* proteins was predicted by JPred 4 server program (Drozdetskiy, Cole et al. 2015) and the SOPMA program (Geourjon and Deleage 1995). The tertiary structure of scallop *CAT* proteins was predicted using the Phyer2 tool.

Multiple Sequence Alignment and Phylogenetic Analysis of *CAT* Gene Family

Multiple sequence alignments of *CAT* proteins were performed using Clustal W (Larkin, Blackshields et al. 2007), then edited by Genedoc software (version 2.7.0) (Nicholas 1997). The *CAT* protein sequences from scallops and other representative species were employed for phylogenetic analysis. The phylogenetic tree of *C. farreri CAT*s was constructed with MEGA 7.0 by using the maximum likelihood (ML) method with bootstrap values as 1000 replicates were applied as the best-fit model LG + G + I model (LG model and Gamma distribution with Invariant sites)(Kumar, Stecher et al. 2016).

Expression analysis of *CAT* genes in scallops exposed to PST-producing dinoflagellate, *A. minutum*

The strains of *A. minutum* were cultivated independently in F/2 medium with a light-dark cycle of 12:12 h, then harvested when the cell density approached 5×10^4 cells/mL in the exponential growth phase by centrifugation of 2500 g/10 min (Hwang and Lu 2000, Navarro, Munoz et al. 2006, Garcia-Lagunas, Romero-Geraldo et al. 2013). Then, each scallop was fed once a day with 3 L volume with a final cell density of 2.5×10^3 cells/mL during the feeding experiments. There were 6 groups with 3 individuals of scallops collected and fed with the PST-producing dinoflagellate, *A. minutum* (AM-1 strain) on days 0, 1, 3 (acute response), 5, 10, and 15 (chronic response) exposure. The mantles, gills, muscles, kidneys, and hepatopancreas of the scallop were dissected, washed with sterile seawater, and frozen at -80°C for subsequent RNA

extraction. Total RNA was extracted from these tissues using the conventional guanidinium isothiocyanate method (Hu, Bao et al. 2006). RNA-Seq libraries were constructed using the NEB Next mRNA Library Prep Kit following the manufacturer's instructions and were subjected to PE125 sequencing on the Illumina HiSeq 2000 platform. The RNA-seq reads were mapped to the *C. farreri* genome using Tophat (ver 2.0.9) (Trapnell, Pachter et al. 2009), and the expression of all *CAT* genes was normalized and represented in the form of RPKM. The fold changes in *CAT* expression for each test point were calculated as $(RPKM_{test}\text{-}RPKM_{control})/RPKM_{control}$ (Cheng, Xun et al. 2016). Significant differences between the experimental and control groups were determined using an independent-samples T-Test ($p < 0.05$, $p < 0.01$, and $p < 0.001$, $n = 3$). Statistical analyses were carried out with GraphPad Prism 5.0 software (Mavrevski, Traykov et al. 2018).

Results

A total of two *CAT* genes were identified in *C. farreri* genomes including *CfCAT*1 and *CfCAT*2. Basic information regarding their genome position, encoding protein length, intron number, protein characterization, and sub-cellular localization prediction were analyzed. According to sequence analysis, the coding sequences of *CfCAT*s ranged from 1494 to 1563 bp in length and encoded proteins from 497 to 520 amino acids (aa). The predicted molecular weight (MW) of *CAT* proteins ranged from 56.26 kDa to 59.12 kDa with average theoretical pIs ranging from 7.22 to 8.66 in *C. farreri*, respectively. The instability index of *CAT* proteins was predicted to be stable (instability index ≥40). According to the variable of isoelectric point (pI) values, two *CfCAT*s were alkaline amino acids in character. The aliphatic index of *CfCAT* conserved proteins ranged from 58.87 to 61.01. The values of the grand average of hydropathicity (GRAVY) showed all *CAT* proteins in scallops were found to be hydrophilic proteins based on a grand average of hydropathicity (GRAVY) analysis where the hydrophobic protein: $0 < GRAVY < 2$; hydrophilic protein: $-2 < GRAVY < 0$. The sub-cellular predictions showed that the *CfCAT*1 and *CfCAT*2 proteins were mainly localized in peroxisomal. As a result of various exon/intron organization patterns, a total of 11 introns were identified in both *CfCAT* genes. About 44.42% of *CfCAT*1 and 43.66% of *CfCAT*2 were composed of regular secondary structural elements, which accounted 28.46% and 26.76% for α-helices, 15.96% and 16.90%, for β-sheet, as well as 49.62% and 50.91% for the random coil. To understand the active site structure-function relationships, the predicted tertiary structure of *CfCAT*s proteins was shown in Fig. 1 (a and b). The four antiparallel β-strands dominated by α-helices are found in *CfCAT*1 and *CfCAT*2, respectively.

Conserved Structures of *CAT* Genes in *C. farreri*

Multiple sequence alignment of scallop *CAT* proteins with their homologs in other selected species revealed the presence of conserved structural domains (Fig. 2). Two CAT family signature sequences were found in the deduced amino acid sequence of *CfCAT*s that contained the catalase proximal heme-ligand signature motif, ^{354}RLFSYSDTH3^{62} was completely conserved as both amino acid composition and location in both *CfCAT*s. A highly conserved catalase active site signature motif, ^{64}FNRERIPERVVHAKGGGA^{81}, was also found in both *CfCAT*s with two synonymous amino acid substitutions (D-65 to N and A-79 to G). Two putative N-glycosylation sites, ^{440}NFS^{442} and ^{482}NFT^{484} domains, and the peroxisome-targeting signal in the C-terminus ^{495}QKL^{497} were predicted in *CfCAT*s. The C-terminal tri-peptide, Gln (Q), Lys (K), and Leu (L) were identified as the putative internal peroxisomal targeting signal PTS1 in *CfCAT* proteins. Twelve amino acids: H^{89}, N^{171}, F^{154}, S^{122}, R^{112}, N^{142}, Y^{325}, K^{169}, I^{311}, W^{277}, Q^{331}, and Y^{260} were identified as the putative residues involved in NADPH binding and three conserved catalytic amino acid residues, H^{166}, N^{148}, and Y^{137} were close to α5″, in β7′, and β6 in

all species. Eight amino acids: R^{320}, H^{364}, R^{365}, N^{369}, F^{409}, R^{432}, Y^{404}, and R^{457} were identified as the heme-binding site residues.

Phylogenetic Relationship of *CAT*s between Bivalves and Other Organisms

BLAST analysis showed that the deduced amino acid sequences of *CfCAT*1 and *CfCAT*2 exhibited a high identity with those of other bivalve mollusks CATs with *A. irradians* (78.31%, 68.82%), *M. nobilis* (66.6%, 91.16%), *P. fucata* (80.23%, 66.06%), *M. meretrix* (66.79%, 62.44%), *C. hongkongensis* (64.87%, 62.85%), *C. plicata* (78.69%, 66.86%), *H. discus discus* (72.74%, 62.04%), *C. gigas* (65.06%, 62.04%), and *A. farreri* (96.16%, 70.68%). The phylogenetic analysis of *CAT* amino acid sequences from 17 selected species was conducted (Fig. 3). Each member of *CAT* in scallops clustered into well-supported separate clades with its orthologues of other species. The phylogenetic analysis showed that a clear clade division of *CAT* proteins was found between vertebrate *CAT* members, and their corresponding *CAT* orthologous in bivalve mollusks. The scallop *CAT* members can be classified into a major clades. *CfCAT1* and *CfCAT2* were firstly clustered to the origin of the main bivalve branch and closely positioned to the bivalve scallop (*A. farreri*, *A. irradians*, and *M. nobilis*) *CAT* sub-clusters. Bivalve mollusks, oysters, and fish catalase sub-clusters were observed within the main bivalve cluster. The most relationship of the evolution of *CfCAT*s was that of marine bivalves, the next was freshwater bivalves, oysters, and fish, then the last was vertebrates.

Expression Regulation of Scallop *CAT*s after Toxic Dinoflagellate Exposure

To understand the antioxidant defensive mechanism of scallop *CAT*s in response to PST-producing algae challenge, RNA-Seq analysis was performed to determine the expression pattern of *CAT* genes in the mantle, gill, muscle, kidney, and hepatopancreas of *C. farreri* after exposed to the PST-producing dinoflagellate, *A. minutum* (AM-1 strain). *CfCATs* were significantly differently expressed ($P< 0.05$) according to their RPKM values. The two *CfCAT* genes were regulated in all tissues but they exhibited different expression patterns with fold changes higher than 0.5 at least one test point. The relative mRNA expression of *CfCAT*1 showed both up and down-regulation at different time points (days 1, 3, and 10) in the mantle with an increase in fold changes (1.8, -0.4, and 0.3, ($P<0.05$)) compared with the control group, while being acutely up-regulated on day 1 in muscle with 4.34-fold, $P<0.01$ during exposure experiment (Fig. 4 (a)). Similarly, the inductive expressions of *CfCAT*2 were observed after AM-1 exposure in these organs at days 3 and 5 (fold changes: 2.21 and 0.83, $P<0.05$). A chronic induction of *CfCAT*2 was detected at day 15 in the gill and hepatopancreas (fold changes: 0.83 and 0.52, $P<0.05$) after AM-1 challenge (Fig. 4 (b)). Whereas, the expression profiles of *CfCAT*s showed little change in expression levels in the kidney between control and exposure groups after being exposed to toxic algae throughout the whole experiment. They were likely to be constitutively expressed and were assumed to be not toxin-inducible.

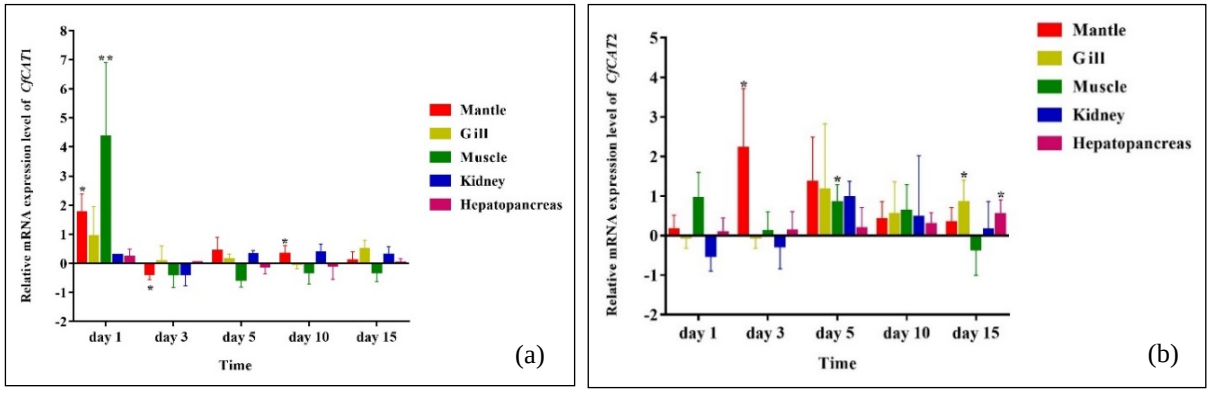

Figure 4 The expressions of (a) *CfCAT*1 and (b) *CfCAT*2 in the mantle, gill, muscle, kidney, and hepatopancreas were measured by RNA-Seq datasets after AM-1 challenge. Bars represented means ± SE; n = 3 (each group/each time point). *P < 0.05 vs control group at the same time. Different asterisks indicate significant differences between groups ($P < 0.05$, $P < 0.01$, and $P < 0.001$).

Discussion

The catalase (CAT) gene family members of *C. farreri* were identified and characterized at the genomic level. The whole-genome identification of the *CAT* gene was conducted in a scallop, then two copies of *CAT* gene were observed. The varied number of introns contributes to the variation in the length of *CAT* genes in scallops which were organized into 11 introns. Multiple introns of *CfCAT*s showed that these genes have a strong relationship between the evolution of gene structures and the sub-functionalization of proteins encoded by the genes (Lynch and Conery 2000). For understanding the protein charge stability, the theoretical pI is the pH at which a particular molecule carries no net electrical charge (Gasteiger, Hoogland et al. 2005). The *CfCAT*s were found to be alkaline/basic in characters showing the proteins which separate based on their relative content of acidic and basic residues, whose value is represented by the isoelectric point (pI). All *CAT* proteins in scallops were predicted to be stable where the instability index is ≤40), and the aliphatic index of a protein is regarded as a positive factor for the increase in the thermo-stability of globular proteins (Enany 2014). The high aliphatic index of *CfCAT*s indicated that these proteins are thermo-stable over a wide range of temperatures. Most of the amino acid residues have stronger hydrophilicity, whereas the N-end and C-end regions have hydrophobicity (Xu, Yu et al. 2017). The values of GRAVY in all *CAT*s in scallops are between -2 and 0, classified as hydrophilia proteins. The functional analysis features of proteins determined by subcellular localization which provides information about the biological and cellular functions (Ebersbach, Galli et al. 2008). The subcellular localization prediction indicated that all *CfCAT* proteins were predicted in peroxisomal locations. The catalase of *C. maenas* and *M. galloprovincialis* also were localized in the peroxisomes stated by (Orbea, Dariush Fahimi et al. 2000). The tertiary structure is primarily due to interactions between R groups of amino acids that make up the protein (Lumry and Eyring 1954). The three-dimensional predicted protein structure of *CfCAT*s showed their active site position.

The similarity and identity of *CfCAT*s amino acid sequences among marine bivalves are quite high ranging from 95.06% and 96.16% to 10.62% and 5.75% (*A. farreri* and *H. cumingii*), freshwater bivalve, *C. plicata* (83.11% and 78.69%) in *CfCAT*1 while the ranging from 89.37% and 91.16% to 12.14% and 7.09% (*M. nobilis* and *H. cumingii*), *C. plicata* (67.93% and 66.86%) in *CfCAT2* reflecting the high conservation of *CAT*s in bivalve mollusks. While *CAT*s in vertebrates are much less conserved with the identity ranging from 10.81% and 4.41% in *CfCAT1* where to 9.48% and 4.21% with *X. tropicalis* in *CfCAT2*. This result assumed that the

molecular evolution of *CAT*s was consistent with the species taxonomy. The proximal heme-ligand signature sequence, the proximal active site signature, and the signal sequences were identified in *CfCAT*s and *CAT*s of bivalves By multiple sequence alignments (Li, Ni et al. 2008, Gao, Ishizaki et al. 2016, Xia, Huang et al. 2016). It was supposed that the *CAT* proteins of *C. farreri* encode a putative peroxisomal catalase via the internal peroxisomal targeting signal (PTS1), QKL which was revealed to direct the interaction between catalase protein and PTS1 receptor PEX5 in the cytosol (Abu-Romman 2016). Twelve conserved amino acid residues were identified in the structure of *CfCAT*s which belong to the NADPH-binding catalase family (Putnam, Arvai et al. 2000). There were 15 amino acid residues in *MyCAT*, ten amino acids in *AwCAT* were identified in the sequence as NADPH binding sites and nine of them were completely conserved with human *CAT* and *CfCAT*s (Li, Ni et al. 2008). The *CfCAT*s first clustered with bivalve *CAT*s indicating that scallop *CAT*s are widely distributed with their corresponding *CAT*s orthologous from other species and evolutionarily conserved and may be derived from a common ancestor.

Up- and down-regulated gene expressions of *CfCAT*s in the tissues of scallops were observed. The up-regulation of *CfCAT*s expression displayed a time-dependent pattern in response to *A. minutum* exposure. For the inducible *CfCAT* copies, an immediate up-regulation was observed at 1-3 days, which was the scallops' acute response in the presence of the toxic dinoflagellate, and then a decrease of expression was observed at 5 days. However, sub-chronic exposure (10-15 days) may reflect the accumulating effect of prolonged PST exposure. We observed the significant inductive expression of *CfCAT*s in mantle and gill after *A. minutum* exposure, as these two organs are the first organs of directly large contact with the toxic dinoflagellate cells during the filtration process (García-Lagunas, de Jesus Romero-Geraldo et al. 2016). Furthermore, muscle is the most important tissue of protein deposition for the metabolism of nutrients, immune responses, and energy storage in response to Alexandrium cells during filtration (Sun, Xuan et al. 2015) where the significant expression up-regulation of *CfCAT*s was observed after toxic dinoflagellate exposure. It was suggested that the hepatopancreas is the major organ involved in the bioaccumulation and detoxification response to PST challenge occur (Huang, Peng et al. 2018), so the transcriptional up-regulation of *CfCAT*2 was detected in hepatopancreas after oxidative stress induced by AM-1 exposure. If the generation of ROS is under a low level, the antioxidant enzyme expressions should be up-regulated that contribute to enduring the oxidative stresses whereas the down-regulation of *CfCAT*1 in the mantle is associated with increased ROS production and oxidative damage by toxic exposure (Radak, Suzuki et al. 2016). The importance of catalase induction at the transcriptional level as an adaptive antioxidant defense system response in bivalves under algal toxin exposure. Sub-chronic exposure might be caused by strong oxidative stress in tissues that are frequently in contact with PST-producing dinoflagellate. The regulation of *CAT* genes revealed that these genes play a protective role in the defense against oxidative stress and the adaptative responses of scallops caused by AM-1 challenge. The functional variety of scallop *CAT*s is involved in the tissue-specific regulation of *CAT*s that might contribute to adaptation response to the harmful effects of PST-producing algae and could be as tissue-responsive indicator genes with PSTs challenge.

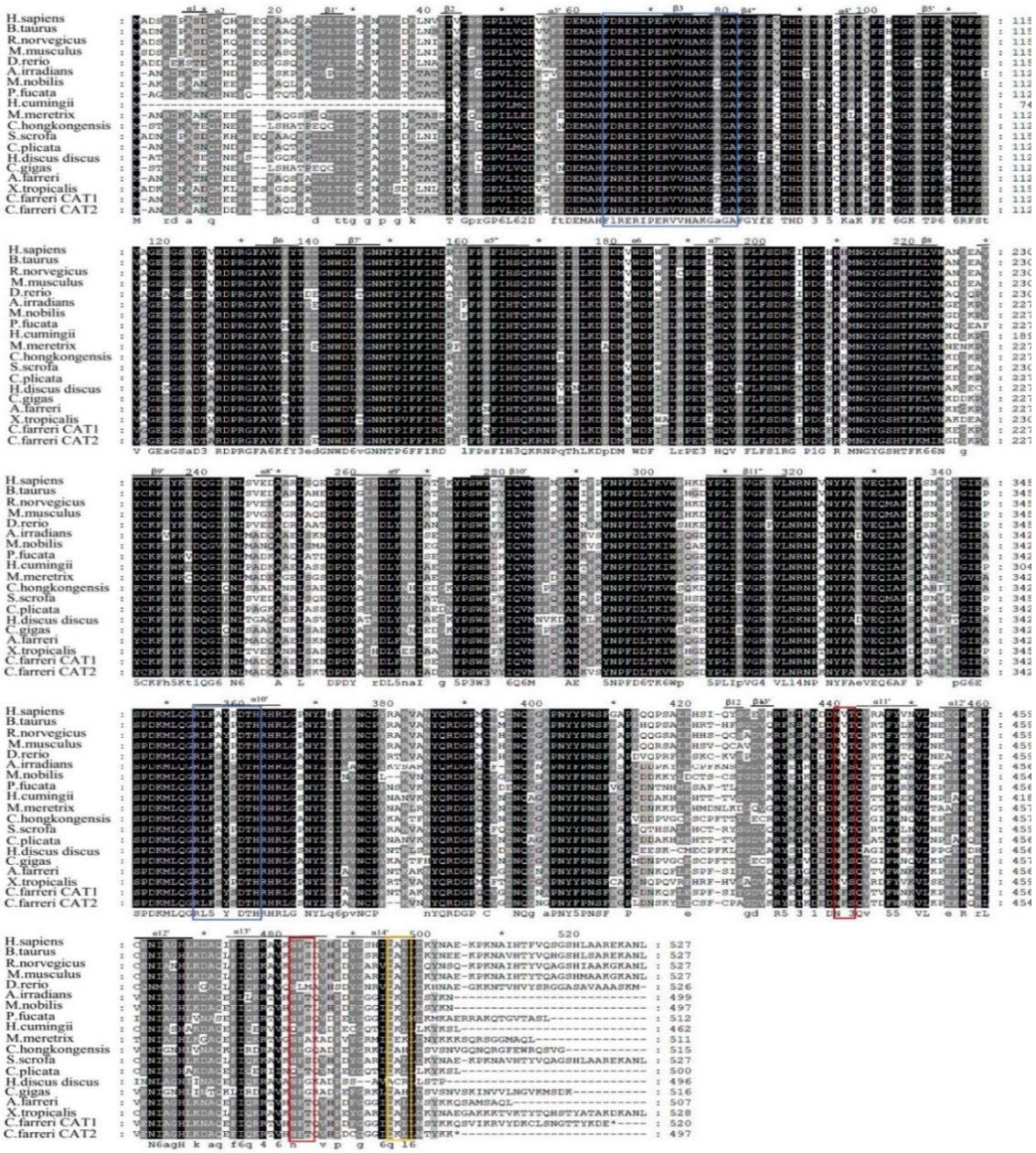

Figure 2 Multiple sequence alignment of *CfCAT*s in scallops with other homologous vertebrates and mollusks catalase amino acid sequences. The proximal active site signature and the proximal heme-ligand signature are boxed in blue color. The conserved catalytic amino acids (H^{71}, N^{144}, and Y^{354}) are shown in the down arrow. Peroxisome targeting signal (QKL) is boxed in yellow color. The predicted secondary structure is indicated by α or β (α is α-helices and β is β-sheets).

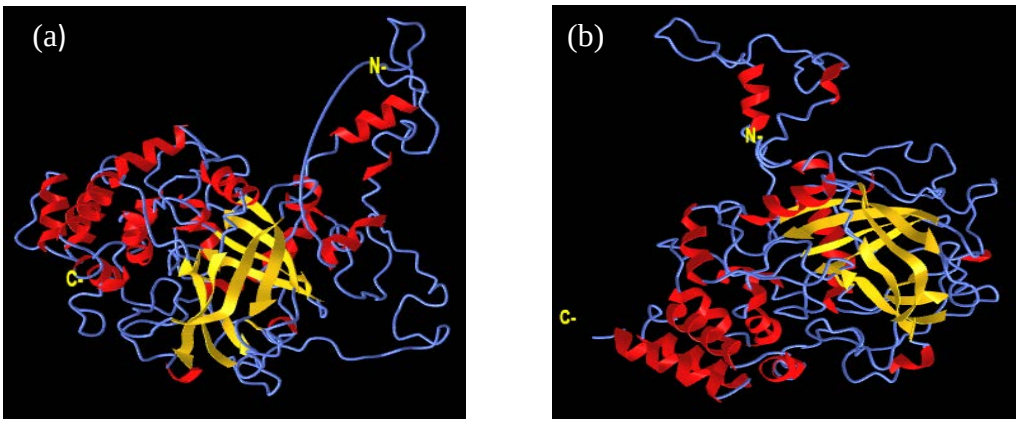

Figure 1 Three-dimensional predicted protein structures of (a) *CfCAT*1 and (b) *CfCAT*2. Models were visualized by color from N to C terminus and organized in order as gene sequences. Different sub-family proteins have similar protein models. Red ribbons represent α-helices and yellow arrows indicate β-sheets.

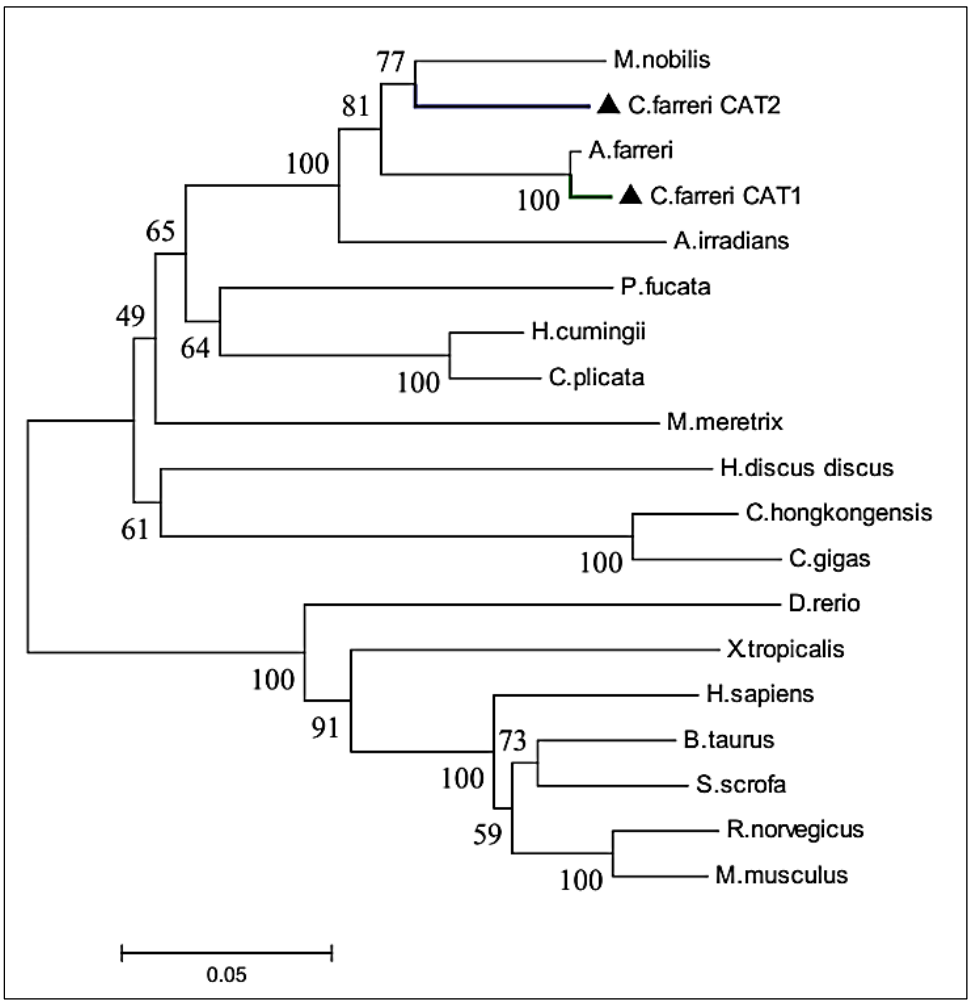

Figure 3 Phylogenetic tree of *CAT* proteins from *C. farreri* and other selected organisms. The tree was constructed using the maximum-likelihood (ML) method with LG + G + I module. Numbers at the branch point of the node represent the value resulting from 1000 replications. Branches of *CfCAT*1 and *CfCAT*2 proteins are marked with black triangles.

Conclusion

Two *CfCAT* genes were scanned and systematically characterized from the *C. farreri* genome that belongs to the NADPH-binding catalase family. After ingesting PST-producing algae, no regulated expression of *CfCAT*s was observed in the kidney of scallop, but inducible *CfCAT*1 and *CfCAT*2 were either up-or down-regulated in the mantle, gill, muscle, and hepatopancreas with acute and chronic regulation after *A. minutum* exposure. These results indicated that the response of scallop *CAT*s to PST-producing dinoflagellate was dependent on scallop species and tissues. We found diversified responsive profiles of scallop *CAT* genes after the toxic algae challenge, suggesting the catalase implied in the protective role of oxidative stress which provides a better understanding of mollusks' defensive mechanisms against the harmful effects of PST accumulation which might contribute to the adaptive evolution of scallop.

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