Transcriptional Responses of Flavin-Containing Monooxygenase Genes in Scallops Exposed to PST-Producing Dinoflagellates Implying Their Involvements in Detoxification

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Flavin-containing monooxygenase (FMO) is one of the most prominent xenobiotic metabolic enzymes. It can catalyze the conversion of heteroatom-containing chemicals to polar, readily excretable metabolites and is considered an efficient detoxification system for xenobiotics. Bivalves can accumulate paralytic shellfish toxins (PSTs) produced by dinoflagellates, especially during outbreaks of harmful algal blooms. Exploring *FMO* genes in bivalves may contribute to a better understanding of the adaptation of these species and the mechanisms of PSTs bioavailability. Therefore, through genome screening, we examined the expansion of *FMO* genes in two scallops (*Patinopecten yessoensis* and *Chlamys farreri*) and found a new subfamily (FMO_like). Our expression analyses revealed that, in both scallops, members of the FMO_N-oxide and FMO_like subfamilies were mainly expressed from the D-stage larvae to juveniles, whereas the FMO_GS-OX subfamily genes were mainly expressed at and prior to the trochophore stage. In adult organs, higher expressions of *FMO*s were observed in the kidney and hepatopancreas than in other organs. After exposure to PST-producing algae, expression changes in *FMO*s occurred in hepatopancreas and kidney of both scallops, with more members being up-regulated in hepatopancreas than in kidney for *Alexandrium catenella* exposure, while more up-regulated *FMO*s were found in kidney than in hepatopancreas of *C. farreri* exposed to *A. minutum*. Our findings suggest the adaptive functional diversity of scallop *FMO* genes in coping with the toxicity of PST-producing algae.

Keywords: scallop, flavin-containing monooxygenase, paralytic shellfish toxins exposure, transcriptional responses, detoxification

INTRODUCTION

Organisms have developed extensive molecular mechanisms to protect against the toxicity of various xenobiotic compounds, producing a variety of enzymes and proteins in response to xenobiotic exposure (Li et al., 2007; Raghunath et al., 2018). Flavin-containing monooxygenase (FMO), a prominent family of monooxygenases in eukaryotes (Naumann et al., 2002; Huijbers et al., 2014), is a major contributor to oxidative xenobiotic metabolism and is as important as cytochrome P450 (CYP450) (Manikandan and Nagini, 2018). FMO was first discovered in the 1960s in hepatic microsomes in pigs as an NADPH- and O_2-requiring activity that converted N, N_0-dimethylaniline to N-oxide (Ziegler and Pettit, 1964). To date, FMO homologs have been found in a range of organisms, including bacteria (Chen et al., 2011), fungi (Suh et al., 2000), marine invertebrates (Boutet et al., 2004), insects (Naumann et al., 2002; Sehlmeyer et al., 2010), and teleost fish (Peters et al., 1995; Larsen and Schlenk, 2001). *FMO* genes have been studied widely in mammals that possess five *FMO* genes (e.g., human *FMO1-FMO5*) (Cashman and Zhang, 2006). In unicellular eukaryotic organisms, such as yeast, there is only one *FMO* isoform (Eswaramoorthy et al., 2006), and in insects, there are two [e.g., in *Drosophila melanogaster* (Scharf et al., 2004)] or three [e.g., *FMO1-FMO3* in *lepidoptera* (Sehlmeyer et al., 2010)]. Conversely, plants have various FMO genes. For example, there are 29 FMO genes in *Arabidopsis thaliana* (Schlaich, 2007).

FMOs utilize a tightly bound FAD prosthetic group, NAD(P)H, and molecular oxygen to create monooxygenate or otherwise oxidize substrates, producing water and $NAD(P)^+$ as byproducts (Poulsen and Ziegler, 1995; Krueger and Williams, 2005). Generally, FMOs act on a large variety of nitrogen-, sulfur-, selenium-, or iodine-containing compounds, but carbon, phosphorus, and other elements are also amenable to FMO-mediated oxidation (Başaran and Can Eke, 2017). FMOs transfer hydroxyl groups to these substrates, rendering them more polar and more readily excretable (Cashman, 1995), and thus are thought to participate in the detoxification of foodstuffs and other xenobiotics (Hodgson et al., 1995). In humans, substantial evidence has shown that FMOs play an important role in the detoxification of tertiary amines [e.g., trimethylamine (Cholerton and Smith, 1991) and (S)-nicotine (Park et al., 1993)]. In mice, one FMO is involved in the detoxification pathway of l-methyl-4-phenyl-l,2,3,6-tetrahydropyridine (Cashman, 1989; Chiba et al., 1990; Di-Monte et al., 1991). A role for detoxification of xenobiotics by FMOs has also been observed for insects, including the European cinnabar moth (*Tyria jacobaea*), which utilizes senecionine N-oxygenase, an FMO, to efficiently N-oxidize pyrrolizidine alkaloids (PAs), which are toxic to unspecialized insects and vertebrates, thereby allowing larvae to feed on PA-containing plants (Naumann et al., 2002). A relatively high level of FMO activity has also been observed in guinea pigs, which can also efficiently detoxify PAs (Miranda et al., 1991). In bivalves, which are continuously exposed to non-biological contaminants (e.g., industry, agriculture, and urban effluents) (Boutet et al., 2003), FMOs may function in a tissue-specific toxin-responsive manner with differences in *FMO* expression between tissues in the stress response induced by hydrocarbons in Pacific oysters (*Crassostrea gigas*), revealing that FMO was upregulated after exposure to hydrocarbons in the digestive gland on day 7, while it was not expressed in the gill (Boutet et al., 2004).

In addition to non-biological contaminants (Boutet et al., 2003), bivalves are also exposed to a variety of biological contaminants. As filter-feeding organisms that mainly feed on microalgae, bivalves can accumulate large amounts of paralytic shellfish toxins (PSTs) produced by dinoflagellates, especially during the outbreak of harmful algal blooms (Andres et al., 2019). PSTs are a class of potent neurotoxins that reversibly block voltage-gated sodium channels in the cell membrane to prevent the occurrence of neuronal signals, eventually leading to muscle paralysis (Boullot et al., 2017). The contamination of bivalves by PSTs negatively impacts sanitary quality and often leads to the closure of related industries (Farabegoli et al., 2018). Although bivalves showed high PSTs resistance mainly because of PST-resistant amino acids in the voltage-gated sodium channel (Li et al., 2017), PSTs accumulation changes bivalve behavior and physiology (Shin et al., 2018). Many studies on the response of bivalves to PSTs stress found that *CYP450* and *GST*, which are related to detoxification, were significantly upregulated in bivalves after exposure to PST-producing algae (Garcia-Lagunas et al., 2013; Lou et al., 2020), indicating that the detoxification process might be a strategy bivalves employ to cope with the effects of PST accumulation (Garcia-Lagunas et al., 2013). However, whether *FMO* genes are involved in this process remains unknown.

Yesso scallops (*Patinopecten yessoensis*) and Zhikong scallops (*Chlamys farreri*) are important aquaculture species in the two main sea areas (yellow sea and Bohai sea) in China (Li et al., 2017; Wang et al., 2017; Yang et al., 2019), which are both economically important, and the two species have been reported to accumulate PSTs (Li et al., 2017; Hu et al., 2019; Liu et al., 2020). Therefore, they are widely used to study PST accumulation and transformation (Hu et al., 2019; Xun et al., 2020). Previous studies have indicated that the hepatopancreas and kidney are the most toxic organs in scallops exposed to PSTs (Li et al., 2017). In this study, *FMO* genes were identified and characterized, and their responses were systematically assessed in the hepatopancreas and kidney after exposure to two different PST-producing dinoflagellates (*Alexandrium catenella* and *Alexandrium minutum*) in two scallops (*P. yessoensis* and *C. farreri*). We found a new subfamily of FMOs that has not been identified previously and indicated the involvement of bivalve *FMO*s in the response to the accumulation of PSTs. Our results will contribute to a better understanding of the functional diversity and adaptive evolution of *FMO* genes in bivalves in coping with toxic algae.

MATERIALS AND METHODS

Genome-Wide Identification and Characterization of *FMO* Genes in Scallops

Candidate *FMO* genes have been identified in the genome (Li et al., 2017; Wang et al., 2017) and transcriptome (Hou et al., 2011; Wang et al., 2013) sequences of both *P. yessoensis* and *C. farreri*. The orthologous FMO sequences of some representative species, including humans (*Homo sapiens*), frogs (*Xenopus laevis*), zebrafish (*Danio rerio*), mice (*Mus musculus*), fruit flies (*Drosophila melanogaster*), Pacific oysters (*C. gigas*), and nematodes (*Caenorhabditis elegans*), from the NCBI[1], and Uniprot[2] databases were downloaded and used as queries to perform whole-genome and transcriptome-based blast with an *e*-value threshold of 1E-05. Then, the coding sequences were translated into amino acid sequences using an ORF Finder[3], and the predicted FMO proteins were aligned to KEGG[4], Uniprot (see text footnote 2), and the non-redundant database of the NCBI (see text footnote 1) using BLASTP (*e*-value set: 1E-05) to confirm the completeness and accuracy of the identified *FMO*s. The translated amino acid sequences were further analyzed using SMART tools (Schultz et al., 1998) and subjected to the NCBI Conserved Domains Database [5]to identify the conserved domains. Finally, the physicochemical characteristics of the identified proteins, including the number of amino acids, molecular weight, and theoretical isoelectric point, were calculated using the ProtParam tool[6].

Phylogenetic and Evolutionary Analysis of *FMO* Genes

The amino acid sequences of FMOs from selected organisms, including humans (*H. sapiens*), original chickens (*Gallus gallus*), frogs (*X. laevis*), zebrafish (*D. rerio*), amphioxus (*Branchiostoma floridae*), sea urchins (*Strongylocentrotus purpuratus*), freshwater snails (*Biomphalaria glabrata* and *Pomacea canaliculata*), and pacific oysters (*C. gigas*), were obtained from the NCBI database (**Supplementary Table 1**), and were combined with the FMOs identified in scallops to construct a phylogenetic tree. A phylogenetic analysis was conducted using MEGA7 (Kumar et al., 2016) to construct a neighbor-joining tree using the p-distance and maximum likelihood methods. We eliminated positions containing gaps and missing data, and tested the robustness of the results *via* a reanalysis of 1,000 bootstrap replicates.

To measure the selective pressure in the individual codons of the amino acid sequences, we applied codon-based site models in the PAML 4.9 package (Yang, 2007) with a maximum likelihood ratio test, which estimates the non-synonymous (dN)

and synonymous (dS) substitution ratios (dN/dS or ω) variation among the codons. The codon-based site model assumes that different branches of the phylogenetic tree are under the same selection pressure. However, different amino acid positions experience different selection pressures, indicating that different positions of the same sequence have different ω values. Different ω values indicate different selective pressures, in which 1, < 1, and > 1 represent neutral evolution, purifying selection/negative selection, or positive selection, respectively. In our study, three pairs (M0 vs. M3, M1a vs. M2a, and M7 vs. M8) of models were applied. Model M0 (one ratio) assumes that all sites have a constant ω ratio, whereas model M3 (discrete) assumes that there are three classes of ω. The null model, M1a (neutral), allows two classes of codon sites for ω, wherein 0 < ω < 1 and ω = 1, whereas the alternative model, M2a (selection), allows one additional site class with ω > 1. The M7 (beta) model assumes that ω (0 < ω < 1) at all sites belongs to the matrix (0, 1) and fits a beta distribution with 10 categories, whereas the alternative model, M8, also has a category with ω > 1. If model M8 (M2a) fitted the data significantly better than model M7 (M1a), we applied a Bayes Empirical Bayes estimation method in the PAML 4.9 package (Yang, 2007) to identify the sites estimated to be under positive selection.

Expression Profiles of *FMO* Genes in Developmental Stages and Adult Organs/Tissues of Scallops

The expression profiles of *FMO*s in *P. yessoensis* (*PyFMO*s) and *C. farreri* (*CfFMO*s) during the developmental stages and in adult tissues were analyzed and represented in the form of RPKM (reads/kilobase of exon model/million mapped reads) based on the RNA-Seq data from previous studies (Li et al., 2017; Wang et al., 2017). The developmental stages include zygotes, 2–8 cell stage, blastula, gastrula, trochophore, D-stage larva, umbo early larva, umbo middle larva, umbo post larva, creeping larva, and juvenile scallop (**Supplementary Table 2**) and the adult tissues include the mantle, gill, gonad, kidney, hepatopancreas, smooth muscle, striated muscle, and foot. The body size of the sampled scallops were summarized in **Supplementary Table 3**. The heat map of the expression levels was drawn using custom R scripts to visualize the expression patterns of *FMO*s in *P. yessoensis* and *C. farreri*.

Expression Regulation of *FMO* Genes in Scallops After Exposure to PST-Producing Dinoflagellates

Scallops were previously subjected to PST-producing dinoflagellates, in which *C. farreri* was fed with *A. catenella* (ACDH strain) and *A. minutum* (AM-1 strain) (Li et al., 2017), and *P. yessoensis* was fed only *A. catenella* (ACDH strain). Briefly, two-year-old *P. yessoensis* and *C. farreri* were collected from Zhangzidao Group Co., Ltd. (Dalian, Liaoning Province, China) and Xunshan Group Co., Ltd. (Rongcheng, Shandong Province, China), respectively. Individual scallops were acclimated by feeding non-toxic algae (*Isocrydid galbana*) in pre-filtered seawater (30 spu; pH = 7.9) at 12–13°C for 3 weeks in our

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[1]https://www.ncbi.nlm.nih.gov/
[2]https://www.uniprot.org/
[3]https://www.ncbi.nlm.nih.gov/orffinder/
[4]http://www.kegg.jp/
[5]https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi
[6]http://www.expasy.org/tools/protparam.html

laboratory. The two toxic strains of dinoflagellates, *A. catenella* (ACDH strain) and *A. minutum* (AM-1 strain), which have been confirmed to produce PSTs (analogs C1 and C2 in ACDH, and GTXs, mainly GTX1-4 in AM-1), were cultured independently (Navarro et al., 2006) at 20°C under a light-dark cycle with 14 h light and 10 h dark (light intensity of 6,000 lx) in 10 L Erlenmeyer flasks (Guillard and Ryther, 1962), and collected when the cell density reached 3×10^5 cells/mL in the exponential growth phase. The acclimated scallops were individually cultured in a flat-bottom beaker with pre-filtered seawater (30 spu; pH = 7.9) at 12–13°C, and half-volume of the seawater were changed every day. A total of 3 L of dinoflagellate cells were fed to each acclimated scallop once a day at a density of 2,000 cells/mL. The exposure experiments lasted for 15 days, and scallop tissues were sampled at six time points, including days 0 (samples collected immediately before feeding *A. catenella* and *A. minutum*), 1, 3, 5, 10, and 15, in which three scallops were randomly selected at each time point. For each individual scallop, the total RNA was extracted from the hepatopancreas and kidney using the traditional phenol-chloroform method, following the guidelines described by Puissant and Houdebine (1990), and RNA-Seq libraries were constructed using the NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, United States) according to the manufacturer's instructions, and then subjected to the Illumina X-ten platform with the mode of paired-end 150 bp. The expression levels of all *FMO*s were normalized and represented in the form of RPKM. The differentially expressed genes between the exposure and control groups were determined using the R package edgeR (Robinson et al., 2010) with $p < 0.05$ and $| \log_2 FC| > 1$. TBtools (Chen et al., 2020) was used to draw heat maps with the $\log_2 FC$ values.

RESULTS AND DISCUSSION

Identification and Characterization of *FMO* Genes in *Chlamys farreri* and *Patinopecten yessoensis*

Through genome and transcriptome mining, 15 and 13 *FMO* genes were identified in *P. yessoensis* and *C. farreri*, respectively. Based on the phylogenetic analysis, the *FMO*s were classified into three subfamilies (**Figure 1**), including FMO_N-oxide, FMO_GS-OX, and FMO_like, wherein the gene numbers of these subfamilies were 8, 4, and 3 in *P. yessoensis*, and 7, 3, and 3 in *C. farreri*, respectively (**Table 1**). FMO_N-oxide was the only FMO subfamily that was present in both mammals and other animals, but FMO_GS-OX was not found in mammals, probably because of gene loss. Expansion of the two subfamilies was observed in mollusks, including scallops, when compared with vertebrates (**Figure 1** and **Table 1**). Note that the FMO_like subfamily, which was identified for the first time in this study, was only found in scallops. As it was absent in other mollusks, including bivalve oysters, FMO_like might have evolved independently from the other two FMO subfamilies in scallops. A previous study characterized the long-term evolution of this FMO family and found that this family was

strongly selected for purification at the protein level (Hao et al., 2009). Coastal marine organisms are continuously exposed to a wide variety of contaminants from industrial, agricultural, and urban effluents (Boutet et al., 2004). Thus, the diversity of *FMO* members in scallops may be a defense mechanism to cope with different chemical pollutants in the marine environment.

The basic information regarding the *PyFMO*s and *CfFMO*s, including gene name, length of coding sequence (CDS), length of amino acid sequence, exon number, isoelectric point, molecular weight, and SwissProt annotation, is summarized in **Supplementary Table 4**. The CDS lengths of *PyFMO*s and *CfFMO*s varied from 1,224 to 1,758 bp and 1,287 to 3,084 bp, respectively, encoding 407 to 585 and 428 to 1,027 amino acids, respectively (**Supplementary Table 4**). The predicted molecular weight of the FMO proteins ranged from 49.00 to 65.90 kDa with PIs ranging from 5.40 to 9.11 in *P. yessoensis* and 49.20–117.00 kDa with *PI*s from 6.44 to 9.21 in *C. farreri* (**Supplementary Table 4**). Various exon/intron organization patterns of *FMO* genes are present in *P. yessoensis* and *C. farreri* (**Figure 2**), wherein the number of exons ranged from 3 to 10, and 3 to 12, respectively (**Figure 2** and **Supplementary Table 4**).

A protein structure analysis indicated that all *PyFMO*s and *CfFMO*s were composed of three conserved domains (**Figure 3**). The FAD-binding domain (GXGXXG), which was present in all the species examined, has been shown to be critical for their correct structure, catalytic function (Eswaramoorthy et al., 2006), and the FMO-identifying motif (FxGxxxHxxxY/F) (Eswaramoorthy et al., 2006), which interacts with the flavin of FAD (**Figure 3**). In addition to these two domains, the NADPH binding motif (GXGXXG/A) was detected at the C-terminal of the scallop FMO_N-oxide subfamily, but not in the other two subfamilies (**Figure 3**). Such circumstance was also observed in *A. thaliana* with this motif only detected in some members of all the FMO proteins (Eswaramoorthy et al., 2006), while the mechanism of the lack of this domain in some FMO proteins must be further studied. Identical characteristic sequences of three conserved domains have also been reported in other organisms (Choi et al., 2003; Eswaramoorthy et al., 2006; Schlaich, 2007).

Selective Pressure Analysis of the Scallop *FMO* Genes

Gene replication and expansion by acquiring new genes or modifying existing genes driven by positive selection could provide support for the emergence of new functional genes (Kulmuni et al., 2013), which could possibly help organisms adapt to complex living conditions (Zhang et al., 2012; Ellegren, 2014). To analyze the selective pressure of the expanded scallop *FMO* genes at the protein level, we used the codon models implemented in PAML to evaluate their ω values in the two subfamilies (FMO_N-oxide and FMO_GS-OX) present in both vertebrates and scallops. We observed that the FMO_N-oxide and FMO_GS-OX subfamilies in *P. yessoensis* and *C. farreri* were under variable selection pressure with *p*-values of 0 (less than 0.05), indicating the rejection of the M0 model (single ratio) and support of the M3 model (discrete). These results demonstrate
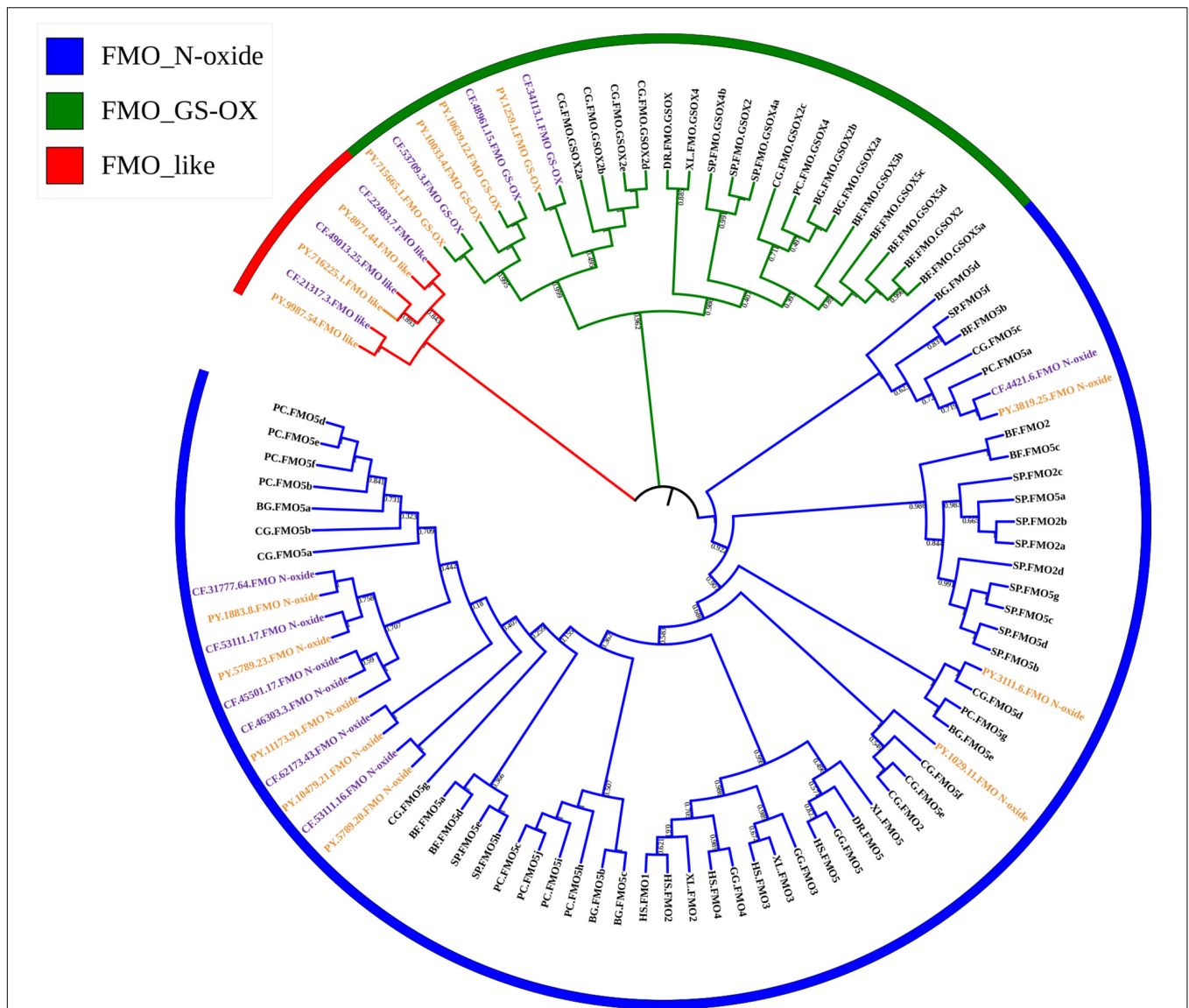
FIGURE 1 | Phylogenetic tree of flavin-containing monooxygenase (FMO) amino acid sequences of selected organisms. The trees were constructed using the neighbor-joining (NJ) method with bootstrapping of 1,000 pseudo replicates. HS, *Homo sapiens*; GG, *Gallus*; XL, *Xenopus laevis*; DR, *Danio rerio*; BF, *Branchiostoma floridae*; SP, *Strongylocentrotus purpuratus*; BG, *Biomphalaria glabrata*; PC, *Pomacea caniculata*; CG, *Crassostrea gigas*; CF, *Chlamys farreri*; PY, *Patinopecten yessoensis*.

that *P. yessoensis* and *C. farreri* allow for different ω ratios of the two subfamilies. The *P*-value of the chi-square comparison test between the M7 and M8 models of the two subfamilies was 0 in *P. yessoensis* (**Table 2**), indicating the statistical significance of allowing positive selection for M8 in *P. yessoensis*, while in *C. farreri,* the *P*-values were 0.95 and 0.96, displaying no statistical significance (**Table 2**). These results suggest the presence of positively selected sites in the FMO_N-oxide and FMO_GS-OX subfamilies in *P. yessoensis*. We further analyzed the sites of the two subfamilies under positive selection (ω > 1) in the M8 model using the Bayes Empirical Bayes approach in PAML and observed 9 and 11 positively selected sites in the FMO_N-oxide and FMO_GS-OX subfamilies, respectively, with

a posterior probability > 95% (**Table 2**). The expansion of *FMO* genes in scallops may reflect the requirement of a certain selection pressure for such genes as a strategy of molecular sequence evolution, which may be an important way to adapt to the complex and variable marine environment.

Spatiotemporal Expression Pattern of Scallop *FMO* Genes

Previous studies have found stage-dependent expressions of *FMO*s during early organogenesis in *Oryzias latipes* that were regulated by developmental factors (Kupsco and Schlenk, 2017), suggesting the involvement of *FMO*s in early development. In
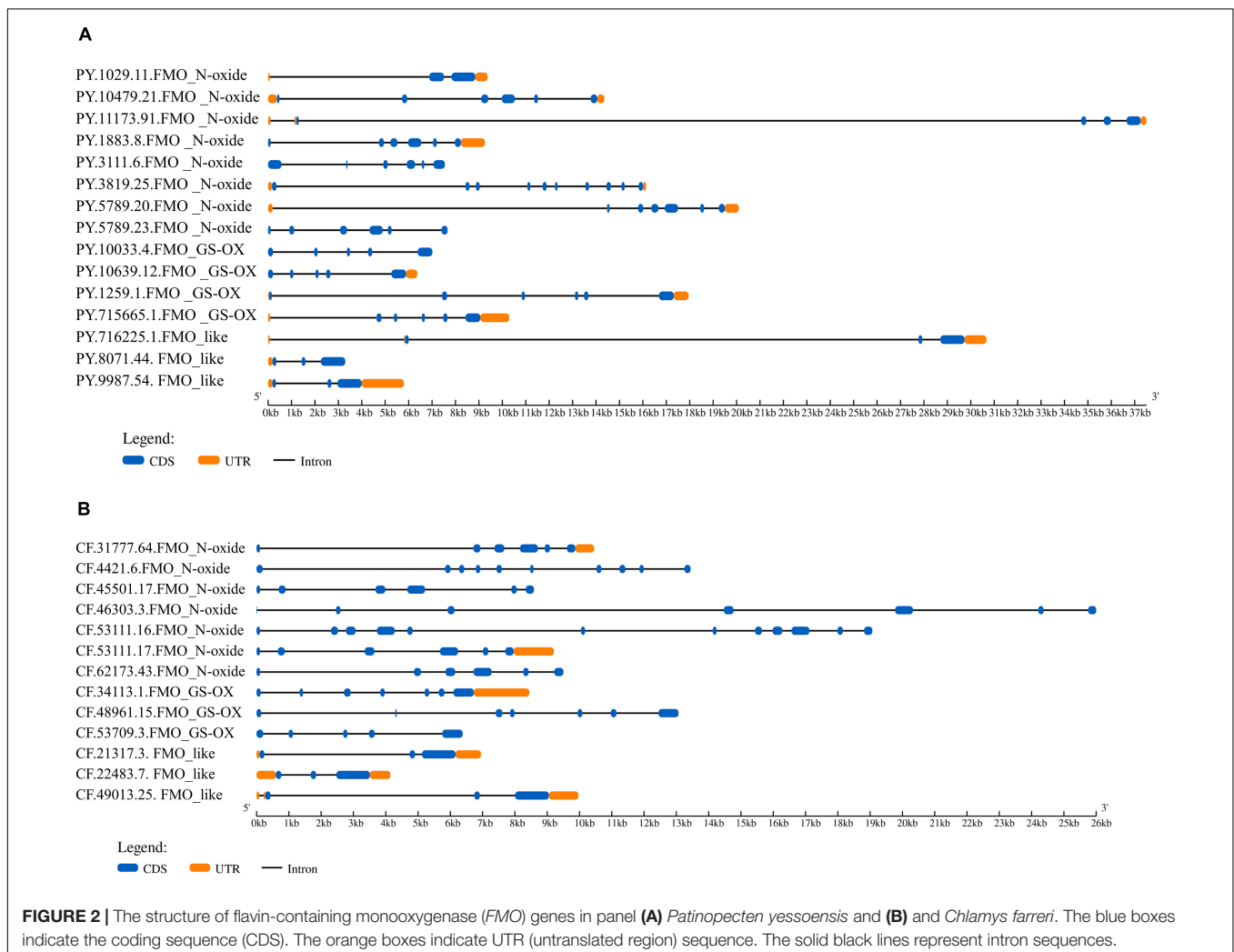
TABLE 1 | Copy numbers of flavin-containing monooxygenase (FMO) subfamilies among selected vertebrate and scallop genomes.
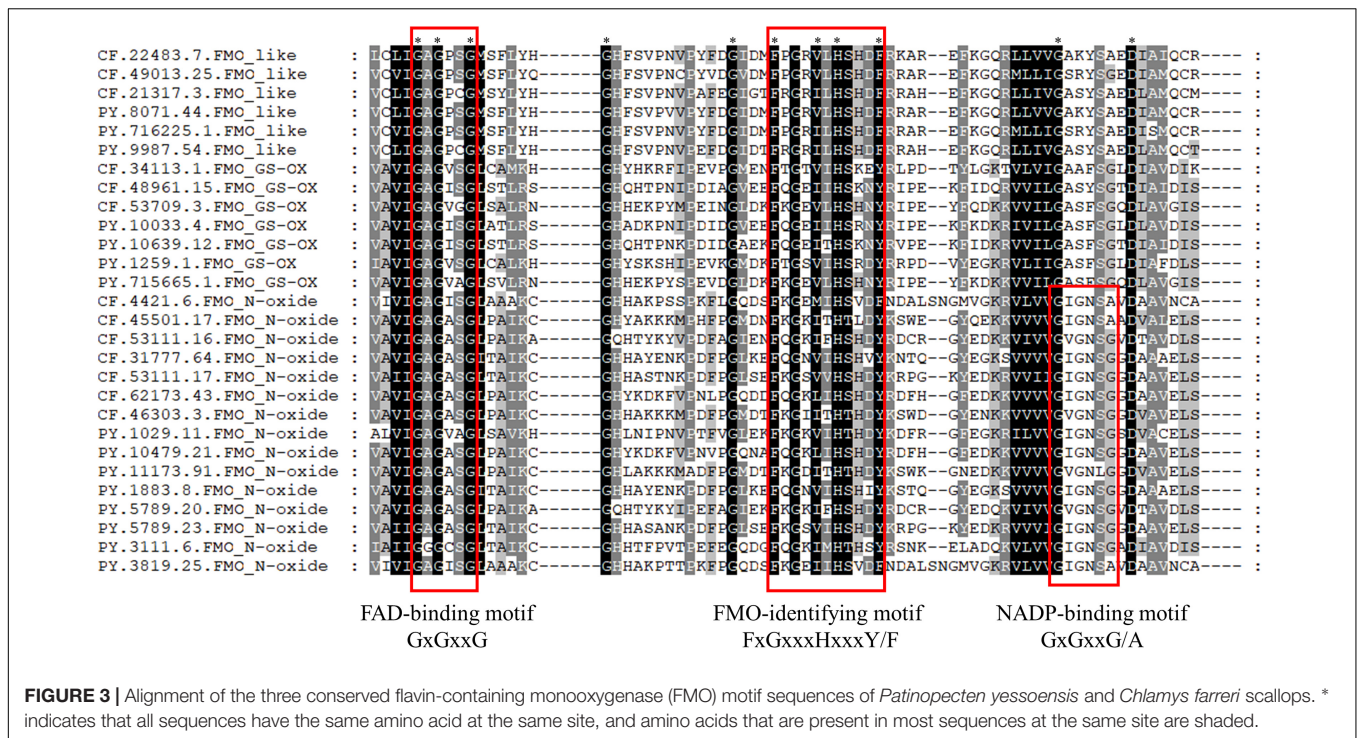
	Human	Chicken	Frog	Zebrafish	Yesso scallop	ZhiKong scallop
FMO_N-oxide	5	3	3	1	8	7
FMO_GS-OX	0	0	1	1	4	3
FMO_like	0	0	0	0	3	3

this study, the expression patterns of *PyFMO* and *CfFMO* genes during different developmental stages (**Figure 4**) were analyzed. In general, most members of the FMO_N-oxide and FMO_like subfamilies in the two scallops were expressed during the post-trochophore stages from D-stage larvae to juveniles, while genes of the FMO_GS-OX subfamily were mainly expressed prior to and at the trochophore stage (**Figure 4**). *PyFMO* and *CfFMO* genes showed, to some extent, different stage-specific expressions. For *PyFMO*s, two members (PY.1259.1 and PY.10033.4) of the FMO_GS-OX subfamily and one member (PY.716225.1) of the FMO_like subfamily were mainly expressed from the zygote to the blastula stages, while two members

(PY.1029.11 and PY3111.6) of the FMO_N-oxide subfamily and one member (PY.8071.44) of the FMO_like subfamily were expressed at the trochophore larval stage. The remaining eight members played a major role in post-larval development (**Figure 4A**). Regarding *CfFMO*s, one member (CF.45501.17) of the FMO_N-oxide subfamily and one member (CF.48961.15) of the FMO_GS-OX subfamily showed elevated expression at multiple developmental stages, while one member (CF.22483.7) of the FMO_like subfamily and two members (CF.53709.3 and CF.34113.1) of the FMO_GS-OX subfamily were highly expressed at only one or two developmental stages, specifically prior to and at the D-stage (**Figure 4B**). The remaining eight members were highly expressed in the middle and late umbo stages (**Figure 4**).

In adult scallops, higher *FMO* expressions were detected mostly in the kidney and hepatopancreas, wherein, five and six members of *PyFMO*, and eight and three members of *CfFMO* were highly expressed, respectively (**Figure 5**). Abundant *FMO* transcripts in the kidney and liver (or hepatopancreas) were also found in many other organisms, including humans (Cashman and Zhang, 2006) and marmosets (*Callithrix jacchus*)

FIGURE 2 | The structure of flavin-containing monooxygenase (*FMO*) genes in panel **(A)** *Patinopecten yessoensis* and **(B)** and *Chlamys farreri*. The blue boxes indicate the coding sequence (CDS). The orange boxes indicate UTR (untranslated region) sequence. The solid black lines represent intron sequences.

FIGURE 3 | Alignment of the three conserved flavin-containing monooxygenase (FMO) motif sequences of *Patinopecten yessoensis* and *Chlamys farreri* scallops. * indicates that all sequences have the same amino acid at the same site, and amino acids that are present in most sequences at the same site are shaded.

(Uehara et al., 2017). The kidney and hepatopancreas are crucial organs for detoxifying chemicals (Metian et al., 2008; Banni et al., 2010) and removing metabolic waste (Bourioug et al., 2018; Wakashin et al., 2018) in mollusks. Previous studies in scallops have indicated that the hepatopancreas and kidney are two major toxic organs of bivalves (Tian et al., 2010; Li et al., 2017). The scallop *FMO*s with abundant expression in the kidney and hepatopancreas may participate in similar xenobiotic detoxification processes observed in other organisms (Phillips and Shephard, 2017). In addition, some other *FMO*s in scallops exhibited high expression in the mantle, gills, and

gonads (**Figure 5**), implying their functions in detoxification in these tissues.
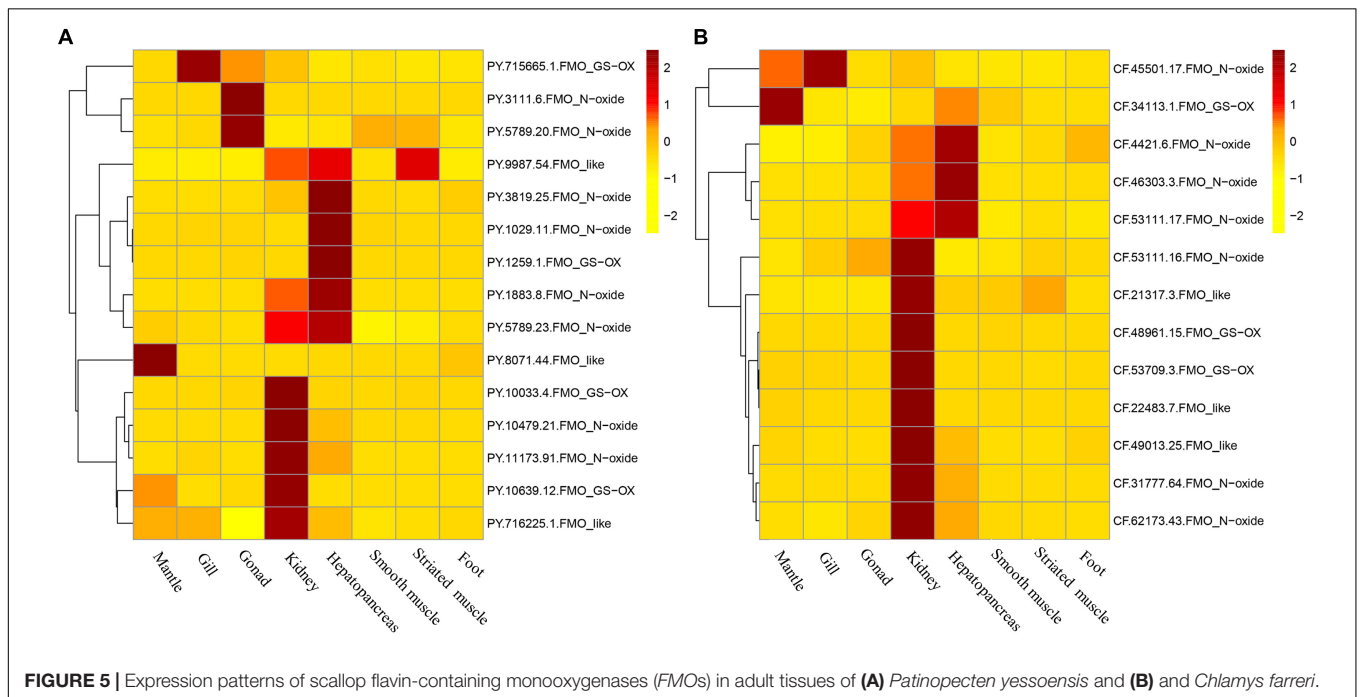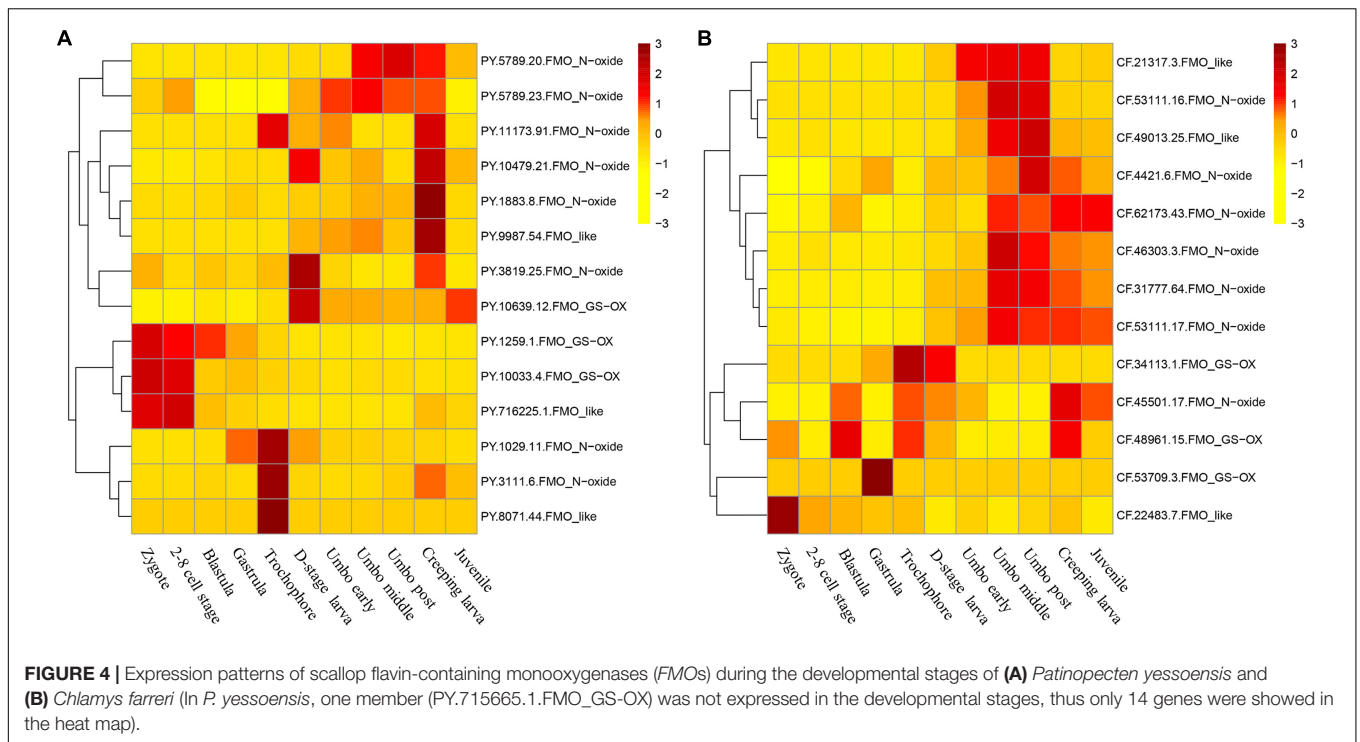
Expression Regulation of Scallop *FMO*s After Toxic Dinoflagellates Exposure

To evaluate the possible involvement of scallop *FMO*s in coping with PST-producing algae, we examined their expression changes after exposure to toxic dinoflagellates in the hepatopancreas and kidney, which are the two most toxic organs with respect to PSTs accumulation (Li et al., 2017). For *P. yessoensis* exposed to *A. catenella*, a total of 7 *PyFMO*s were found to be significantly regulated ($|\log 2FC| \geq 1$, p-value < 0.05) in the hepatopancreas (**Figure 6**), which is the main organ for PSTs accumulation (Li et al., 2017). Of these, 6 were upregulated, and 2 (PY.11173.91 and PY.8071.44) responded acutely on days 1 and 3, respectively, whereas the other *PyFMO*s responded chronically on days 5 and 10. For each of the three subfamilies, two upregulated *PyFMO*s were found (**Figure 6**). The only down-regulated *PyFMO* (PY.1883.8) was from the FMO_N-oxide subfamily and was found on day 10 (**Figure 6**). Meanwhile, in the kidney, wherein PSTs can be transformed into derivatives with higher toxicity (Li et al., 2017; Liu et al., 2020), only two *PyFMO*s (PY.1259.1 and PY.9987.54) were significantly regulated (**Figure 6**). Specifically, one (PY.1259.1) was from the FMO_GS-OX subfamily, which was down-regulated on days 3 and 5, and then upregulated on day 10, and one (PY.9987.54) was from the FMO_like subfamily, which was rapidly upregulated on day 1 and showed continuous upregulation throughout the entire exposure experiment, implying its vital role in responding to PST stress (**Figure 6**). Thus, the *PyFMO*s regulated in the hepatopancreas and kidney were different.

TABLE 2 | Site model analysis for the expanded scallop flavin-containing monooxygenase (FMO) subfamilies.

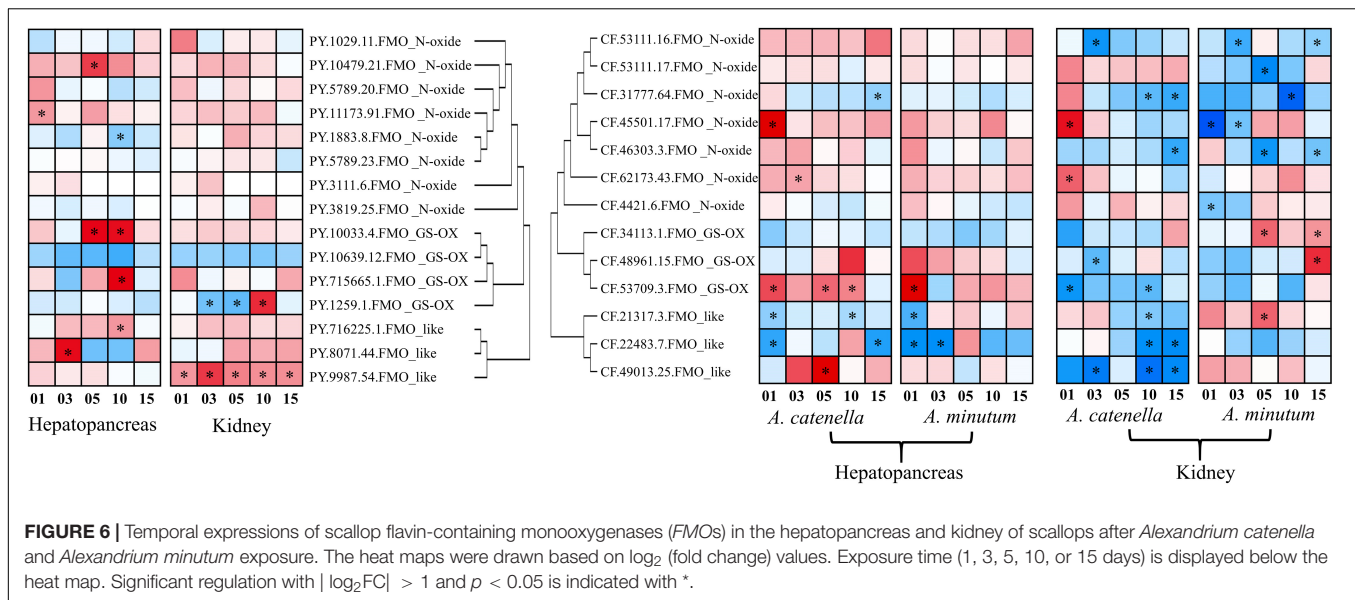| Scallop	FMO subfamily	No. copies	LRT statistics (*p*-value) (No. sites Pr > 95%)		
			M0 vs. M3	M1a vs. M2a	M7 vs. M8
Yesso scallop (*Patinopecten yessoensis*)	FMO_N-oxide	8	**138.7(0)**	**46.5(0)**	**34.6(0)(9)**
	FMO_GS-OX	4	**153.7(0)**	**46.5(0)**	**55.4(0)(11)**
ZhiKong scallop (*Chlamys farreri*)	FMO_N-oxide	7	**553.9(0)**	0(1)	0.08(0.95)
	FMO_GS-OX	3	**553.9(0)**	2.0(0.99)	0.08(0.96)

LRT is the likelihood ratio test statistic for M0 vs. M3, M1a vs. M2a, and M7 vs. M8 according to the PAML package.
Significant results with p < 0.05 are in bold.
Pr, posterior probability for sites under positive selection.

FIGURE 4 | Expression patterns of scallop flavin-containing monooxygenases (*FMO*s) during the developmental stages of **(A)** *Patinopecten yessoensis* and **(B)** *Chlamys farreri* (In *P. yessoensis*, one member (PY.715665.1.FMO_GS-OX) was not expressed in the developmental stages, thus only 14 genes were showed in the heat map).

FIGURE 5 | Expression patterns of scallop flavin-containing monooxygenases (*FMO*s) in adult tissues of **(A)** *Patinopecten yessoensis* and **(B)** and *Chlamys farreri*.

We further examined the expression changes of *FMO*s in the hepatopancreas and kidney of *C. farreri*, another massively cultured scallop in China. In addition to *A. catenella*, another dinoflagellate, *A. minutum* was also used to feed *C. farreri* to assess the response differences of *CfFMO*s under exposure to different PSTs. Generally, more *CfFMO*s were significantly regulated in the kidney than in the hepatopancreas

(**Figure 6**). In the hepatopancreas, a total of 7 *CfFMO*s showed significant responses after exposure to the two toxic dinoflagellates, among which 3 showed similar acute responses with both diets, including 1 (CF.53709.3) upregulated and 2 down-regulated (CF.21317.3 and CF.22483.7). The other four regulated *CfFMO*s only significantly responded to *A. catenella* exposure. In particular, two members (CF.45501.17

FIGURE 6 | Temporal expressions of scallop flavin-containing monooxygenases (*FMO*s) in the hepatopancreas and kidney of scallops after *Alexandrium catenella* and *Alexandrium minutum* exposure. The heat maps were drawn based on $\log_2$ (fold change) values. Exposure time (1, 3, 5, 10, or 15 days) is displayed below the heat map. Significant regulation with $|\log_2 FC| > 1$ and $p < 0.05$ is indicated with *.

and CF.62173.43) of the FMO_N-oxide subfamily and one (CF.49013.25) of the FMO_like subfamily were significantly upregulated, respectively, in acute and chronic manners, and one gene (CF.31777.64) of the FMO_N-oxide subfamily was significantly downregulated in a chronic manner on day 15.

In the kidney, all the *CfFMO*s showed significant responses after exposure to toxic *A. catenella* or *A. minutum*, most of which were significantly downregulated and showed dinoflagellate-type dependent responses. In particular, the two upregulated *CfFMO*s after *A. catenella* exposure were found on day 1, both of which are from the FMO_N-oxide subfamily. For *A. minutum* exposure, the three upregulated members were from the other two subfamilies and exhibited chronic responses. These results suggest the PSTs- and organ-dependent responses of *CfFMO*s. Note that one member (CF.45501.17) showed consistent acute upregulation in both the kidney and hepatopancreas after exposure to *A. catenella*, which could be a good candidate indicator gene for the accumulation of PSTs.

CONCLUSION

In this study, we performed the first systematic analysis of the FMO gene family in scallops *P. yessoensis* and *C. farreri*, and observed the expansion of this family possibly with a bivalve- or scallop-specific gene duplication event. In addition, considerable structural diversity, conserved domains, and motifs were identified, and stage- and tissue-specific expression patterns were revealed. Furthermore, the genes from the expanded subfamily of *P. yessoensis* may be more inclined toward positive selection and may be involved in lineage-specific adaptation. After exposure to two different toxic *Alexandrium* species, the regulation patterns of the scallop *FMO*s presented time-, algal strain-, and organ-dependent expression patterns. These findings will contribute to a better understanding of the evolutionary origin of the FMO gene

family and the detoxification strategies of bivalves in response to algal biotoxins in complex marine environments.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

XH conceived and designed the study. ML and JS performed the experiments. LK, PL, YL, and HW participated in the data analysis. LK and PL wrote the manuscript. YL and XH revised the manuscript. All authors contributed to the article and approved the submitted version.

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