

mRNA expression of CYP4 in marine polychaete *Marphysa sanguinea* in response to benzo[a]pyrene

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Abstract

Rapid amplification of cDNA ends (RACE) and real-time polymerase chain reaction (RT-PCR) were carried out to analyze the *CYP4* gene expression in polychaete *Marphysa sanguinea* exposed to benzo[a]pyrene (BaP) in this study. The full length of *MsCYP4* cDNA was 2 470 bp, and it encoded 512 amino acids. The deduced amino acid sequence showed 47% identity with CYP4F from frog *Xenopus tropicalis* and shared high homology with other known CYP4 sequences. To analyse the role of CYP4 in protecting *M. sanguinea* from BaP exposure, three BaP groups were established: 0.5, 5 and 50 µg/L. Polychaetes were sampled after 3, 7 and 12 d. At 0.5 µg/L, the effect of BaP on *MsCYP4* gene expression increased with time prolonged. *MsCYP4* gene expression curve showed U-shaped trend with time in 5 and 50 µg/L BaP groups. Therefore, *MsCYP4* gene may play an important role in maintaining the balance of cellular metabolism and protecting *M. sanguinea* from BaP toxicity.

Key words: *Marphysa sanguinea*, CYP4, benzo[a]pyrene, toxicity effect

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1 Introduction

Polycyclic aromatic hydrocarbons (PAHs), which are composed of two or more aromatic nuclei, have been regarded as a primary pollution source in marine coastal environment (Martins et al., 2013). The oil spill in the Gulf of Mexico (2010) as well as in the Bohai Bay of China (2011), and ship wrecks such as the *Prestige* accident in 2002, the *Erika* accident in 1999 and the *Volganefi-139* accident in 2010 have caused several millions of crude oil leaking into the sea, and its main content PAHs made serious impact on the marine environment in those areas. PAHs are persistent, toxic, and contain carcinogenic compounds that can attack DNA by forming PAH-DNA adducts and cause disorders in marine animals (Tsai et al., 2007). These compounds can also be transferred throughout the food chain and threaten higher levels of organisms, through biomagnification; as a result, various disorders, such as decreased fertility, obesity, and abnormal organ development, occurred (Kraugerud et al., 2012; Lyche et al., 2011, 2013). Low-molecular-weight PAHs volatilize into the atmosphere, but high-molecular-weight PAHs subside in water and adsorb on the surface of sediment particles; the adsorbed PAHs then accumulate in estuarine sediments and absorb by the benthonic animal (Barakat et al., 2011; Tobiszewski and Nami-ęńnik, 2012).

Cytochrome P450 (CYP) is a superfamily of protoheme oxygenase that catalyze a high range of reactions, such as phase I oxidation, production, and reduction of endogenous substances,

including vitamins, steroids, fatty acids, and prostaglandins. These enzymes also play an essential role in metabolizing xenobiotics, such as PAHs, to detoxified forms, which are hydrophilic, or to harmful reactive intermediates (Bach et al., 2005; Scornaienchi et al., 2010). Study showed that CYP1A1 plays a protective role against oral BaP toxicity in aorta ROS production contributed to BaP-exacerbated atherosclerosis, but no CYP1A has been identified in invertebrates (Uno et al., 2014). CYP4 have been proven to be responsible for CYP1A-like responses, and more importantly these enzymes were widely found in bacteria, fungi, plants, vertebrates and invertebrates; hence, CYP4 constitute a diverse and large number of subfamilies, such as CYP4B, CYP4F, CYP4T, CYP4V, CYP4X, and CYP4Z (Kirischian and Wilson, 2012). Meanwhile, a high diversity of *CYP4* genes has also been determined in marine invertebrates such as mussels, oysters, shrimp and polychaete (Guo et al., 2013; Zanette et al., 2010). The CYP4 enzymes present a high degree of structural diversity and comprise numerous homologous members among invertebrates. The protective role of CYP4 against BaP has also been explored in other marine invertebrates, including clam *Ruditapes philippinarum*, polychaete *Perinereis nuntia*, *Perinereis aiubhitensis*, mollusk *Perna viridis*, and scallop *Chlamys farreri*, the results showed that *CYP4* mRNA expression was significantly induced in different PAHs-polluted environments (Miao et al., 2011; Pan et al., 2011; Zhou et al., 2010a, b). CYP4 play an important role in metabolism of PAHs, Pan et al. (2011) demonstrated that *CYP4*

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mRNA expression was induced significantly in the digestive gland of the clam *Ruditapes philippinarum* by exposure to 0.2 µg/L BaP. Chen et al. (2012) proposed that the expression pattern of *CYP4* in PAH-exposed polychaete *Perinereis aibuhitensis* changes with time in a dose-dependent manner which petroleum hydrocarbon and combined toxicity treatments elevated the mRNA level of *PaCYP4*.

Polychaetes, which possess various types of *CYP4*, have been used as a toxicological animal model. Jørgensen et al. (2005) revealed two *CYP4* gene sequences in *Nereis virens*. *CYP4BB2*, *CYP423A1* and *CYP424A1* have been cloned in *Perinereis nuntia* (Zheng et al., 2013). Polychaetes are important geographically distributed marine invertebrates in mudflats and estuarine sediments. They exhibit strong tolerance and bioavailability to many persistent pollutants (Lewis and Watson, 2012). PAHs can accumulate in their bodies after a long-term exposure. Thus, the polychaete is often considered an indicator organism in the assessments of coastal sediment pollution and model species in toxicological studies (Alam et al., 2010; Dean, 2008; Musale and Desai, 2011). The polychaete *Marphysa sanguinea* is an important commercial species widely distributed along the coasts of Southeast Asia (Zhao et al., 2016). This species is an essential carrier for the transfer of contaminated sediments to higher trophic levels and key point to detoxifying PAHs in the food chain of marine ecosystems (Onozato et al., 2012). The relationship between organic chemicals, such as PAHs, and the responses to the stress of *CYP4* gene expression in *M. sanguinea* should be analyzed to monitor PAHs pollution and to establish a theoretical basis for understanding the regulation of the body's detoxification signal transduction system. As PAHs concentrations in aquatic environmental increased because of oil spill, the effects on benthic invertebrates should be experimented, especially on polychaete (Feng et al., 2009; Hong et al., 2012; Jernelöv, 2010; Sim et al., 2010). In our study, the full-length cDNA of *CYP4* from *M. sanguinea* was cloned, and the expression of *MsCYP4* mRNA under BaP exposure was examined. This study aimed to elaborate the relationship between the varying levels of *CYP4* gene expression in *M. sanguinea* and PAHs pollution concentration. Our study could also provide a basis for the investigation of stress-related responses, ecological assessment of petroleum pollution, and identification of potential biomarkers of organic pollutants in polychaete.

2 Materials and methods

2.1 Animals and exposure experiment

Segment worms *M. sanguinea* (1.5–2.5 g wet weight) were collected in tidal sand flat sediments from Dalian Heishijia coast (39°N, 121°E), water salinity was 32, pH 8.25±0.10, temperature (14±0.5)°C. The animals were transferred from the field to the laboratory with sediments and acclimated in seawater and mud for a week. The seawater was changed daily and kept at a temperature of (14±0.5)°C.

According to People's Republic of China sea water quality standard and our previous study (Song et al., 2011), three BaP (Sigma, America) concentrations were used: 0.5, 5 and 50 µg/L. A solvent control group (100 µL/L acetone) and a blank group were also set. A total of 0.05 g BaP powder was dissolved in 100 mL acetone to prepare 0.5 g/L stock solution. And then stock solution was added to 500 mL seawater to forming final concentration. Five polychaetes were placed into each plastic beaker. Three replicates were used for each treatment. The seawater was changed daily during the experiment and experimental condi-

tions (salinity, pH, temperature, and density) were the same as those used for acclimation. Three individuals were sampled in each group at 3, 7 and 12 d during exposure, and the body wall was frozen for RNA extraction.

2.2 Cloning of the full-length cDNA of *CYP4* from *M. sanguinea*

2.2.1 Degenerate primers

Primer Premier 5.0 software was used for designing primers. Two degenerate primers (F1 and R1) for *MsCYP4* were designed using CDS conserved regions from known polychaete *P. aibuhitensis* gene (GenBank: HM126463) which had been compared with other invertebrates. Gene-specific primers (F2, R2, F3, and R3; Table 1) were subsequently designed on the basis of the obtained cDNA partial sequence.

Table 1. Primers in experiment

Primer	Sequences (5'-3')
F1	ATGTTTGAAGGYCATGAYAC
R1	AATKYTGNCRRATRCARTT
F2	GACAACATAG CCTGGTCAGA
F3	AGGATACACAGTCCAGTTTTAC
R2	GTTCCATTAGGGATCACTCT
R3	TGTGTATCCTCAGACTTTCTTT
<i>β-actin</i> F	GGGCTACTCCTCACCACCA
<i>β-actin</i> R	CGAAGTCCAGAGCAACATAG
F4	TGTAGATATGCTGCTTGATGCC
R4	CCTCTCTGCATCTTTCTTGGTG
UPM-L	CTAATACGACTCACTATAGGGCAAGCAGTGGTAT-CAACGCAGAGT
UPM-S	CTAATACGACTCTATAGGGC

Note: Y=CT; R=AG; N=ATCG.

2.2.2 cDNA synthesis

The individual total RNA was extracted from the pieces of body wall in the first third of *M. sanguinea* using RNAiso Plus (Takara, Shiga, Japan) according to the manufacturer's instructions and purified using Recombinant DNase I. Approximately 500 ng/µL RNA was used as template, and the first-strand cDNA synthesis was carried out using TaKaRa RNA PCR Kit (AMV) Ver. 3.0. Subsequently, cDNA was diluted and stored at -20°C.

2.2.3 cDNA cloning

Polymerase chain reaction (PCR) was conducted to obtain the *MsCYP4* fragments. F1 and R1 were used in a 25 µL of reaction volume containing 5× PCR buffer (5 µL), primers (10 µmol/L, 0.5 µL each), cDNA (5 µL), TaKaRa Ex Taq HS (0.15 µL), and PCR-grade water (13.85 µL). The PCR program was set at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 51°C for 30 s, 72°C for 30 s, and the final extension step at 72°C for 10 min. The PCR products were gel-purified, cloned into the pMD™-18 T vector (TaKaRa, Shiga, Japan), and transformed into the *Escherichia coli* competent cell DH5α (TaKaRa, Shiga, Japan). Positive recombinants were identified via blue-white color selection in ampicillin-containing LB plates and sequenced by Sangon Technology Co. Ltd. (Shanghai, China).

2.2.4 Rapid amplification of cDNA 3' and 5' ends (3' and 5' RACE)

To obtain the full-length *MsCYP4* cDNAs, the 5' and 3' RACE PCR technique was used. Four gene-specific primers (F2, R2, F3 and R3; Table 1) were designed on the basis of the fragments obtained from homology cloning.

RNA was reverse-transcribed to first-strand cDNA using 3'-Full RACE Core Set Ver. 2.0. The 3' end of CYP4 cDNA was amplified using TaKaRa LA Taq[®] with GC Buffer via nested PCR. In the first round of nested PCR, F2 and UPM-L were used as primers. The reaction volume was 50 μ L containing 2 μ L of cDNA, 8 μ L of 1 \times cDNA Dilution Buffer II, 2 μ L of each 10 mmol/L primer, 25 μ L of 2 \times GC Buffer I, 0.5 μ L of TaKaRa LA Taq[®] (5 U/ μ L) and 10.5 μ L of PCR-grade water. The reaction condition was as follows: 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 2 min, and a final extension at 72°C for 10 min. In the second round of nested PCR, F3 and UPM-S primer were used as primers. The reaction volume was 50 μ L containing 1 μ L of outer PCR product, 8 μ L dNTP (2.5mM each), 2 μ L of each 10 mmol/L primer, 25 μ L of 2 \times GC Buffer I, 0.5 μ L of TaKaRa LA Taq[®] (5 U/ μ L) and 11.5 μ L of PCR-grade water. The amplification profile was 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 2 min, and a final extension at 72°C for 10 min.

The 5' end of CYP4 cDNA was synthesized through nested PCR using 5'-Full RACE Kit (Takara, Shiga, Japan). After Alkaline phosphatase (CIAP) and TAP treatment, RNA was ligated using the 5'-RACE adaptor and reversed to first-strand cDNA. In the first round of nested PCR, R2 and UPM-L were used as primers. The reaction volume was same as 3'-RACE outer PCR except the use of primers and 5' cDNA instead of 3' cDNA. The reaction condition was as follows: 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 2 min, and a final extension at 72°C for 10 min. In the second round of nested PCR, R3 and UPM-S were used as primers. The reaction volume was same as 3'-RACE inner PCR except the use of primers and 5' cDNA instead of 3' cDNA. The amplification profile was 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 2 min, and a final extension at 72°C for 10 min.

2.2.5 Homology analysis

The resulting sequences were analyzed for similarity with other known sequences using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>). The deduced amino acid sequence was analyzed using the Expert Protein Analysis System (<http://www.us.expasy.org/tools>). Alignment of multiple sequences was also analyzed using the CLUSTAL W (<http://www.ebi.ac.uk/clustalW>). The functional sites and motifs in amino acid sequences were predicted using Motif Scan (<http://www.hits.isbsib.ch/cgi-bin/PESCAN>) and Expasy (<http://www.au.expasy.org/prosite/>). Phylogenetic analysis was conducted via Mega 5.0 software using the neighbor-joining algorithm.

2.3 Quantitative real-time PCR analysis

The total RNA in each treatment was extracted from the body wall using TaKaRa RNAiso[™] Plus kit and reverse-transcribed into single-strand cDNA using PrimeScript[®] RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara, Shiga, Japan). cDNA was stored at -20°C.

Gene-specific primers for *MsCYP4* (F4, R4) were designed for real-time PCR using the Primer Premier 5.0 software. The β -actin housekeeping gene was used as reference gene for internal standardization. The total amplification volume was 20 μ L, which included SYBR[®] Premix Ex Taq[™] II (10 μ L), forward and reverse primer (0.8 μ L each), ROX reference dye (0.4 μ L), 1:10 diluted cDNA (2 μ L), and DEPC-treated water (6 μ L). The PCR protocol was 95°C, for 30 s followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Each amplification product generated a single peak in the dissociation curve analysis, which demonstrated the specificity of the PCR products.

2.4 Statistical analysis

The 2^{- $\Delta\Delta$ CT} method was used to analyze the expression level of mRNA from *M. sanguinea*. All data were expressed as means \pm SE. Differences among groups were analysed by one-way ANOVA and Duncan's multiple range Test using the SPSS 18 at a significance level of $p < 0.05$.

3 Results

3.1 Identification of *MsCYP4* full-length cDNA

The full-length cDNA of *MsCYP4* contains 2 470 nucleotides, including a 3' untranslated region (UTR, 822 bp), 5' UTR (109 bp), and an open reading frame of 1 539 nucleotides that is predicted to encode a protein of 512 amino acids with predicted molecular mass of 59.36 kDa and theoretical isoelectric point of 8.12 (Fig. 1). The sequence of *M. sanguinea* CYP4 cDNA is named *MsCYP4* and submitted to NCBI GenBank (GenBank ID: Kf203131). Conserved structural patterns are included in this deduced amino acid sequence. The CYP4-conserved cysteine heme-binding domain, FxxGxRxCxG, is located at the amino acid residues 453–466 aa. The conserved motif ExxR within the K-helix and WxxxR within the C-helix are presented at the amino acid residues 379–382 aa and 141–145 aa, respectively. The CYP4 family characteristic sequence of 13-residues EVDTFMFEGHDTT in the I-helix with the function of proton transfer during monooxygenation. The deduced amino acid sequence shared similarity with known CYP4 enzymes and was highly homology to CYP4F (47%) from *Xenopus tropicalis* and other CYP family 4 (42%–45%) from invertebrate and vertebrate (Fig. 2). *Marphysa sanguinea* CYP sequence belonged to the CYP family 4 due to the p450 domain (272–1582 aa). Phylogenetic tree was constructed to demonstrate the relationship of this deduced amino acid sequence with other CYP proteins (Fig. 3).

3.2 mRNA expression of *MsCYP4* under BaP exposure

The dose-dependent pattern of *MsCYP4* in response to BaP exposure was examined (Fig. 4). Solvent control group showed similar response with blank control group, and no significant difference was observed, which indicated that acetone had no influence on *M. sanguinea* as a solvent. The transcripts in *M. sanguinea* exposed to 0.5 μ g/L BaP were increased gradually during the exposure period and significantly upregulated to 2.92-, 6.73-, and 7.99-fold, which showed a significant difference with the control ($p < 0.01$). The maximal response was observed at Day 3 in 5 and 50 μ g/L, and the mRNA expression levels in 5 and 50 μ g/L concentrations were 6.46- and 3.33-fold of those in control which showed significant difference ($p < 0.01$). Dropping abruptly at Day 7, the mRNA expression levels were 1.26- and 1.18-fold of those in control and showed no significant difference ($p > 0.05$). The transcripts of *MsCYP4* gene increased slightly by 2.98- and 1.54-fold at Day 12 in 5 and 50 μ g/L groups, respectively.

4 Discussion

CYP4 enzymes play an important role in oxidative metabolism of endogenous and exogenous chemicals. These enzymes have diverse functions and structures in both vertebrates and invertebrates, and take on critical roles in phase I detoxification systems (Baldwin et al., 2009). CYP4 can be induced by PAHs and its metabolites in polychaetes (Zheng et al., 2013). Inhabiting in sediment *M. sanguinea* can accumulate and digested much of PAHs component. Thus, they are widely used as biomarkers in detecting marine pollution caused by PAHs (Han et al., 2014). Here, to understanding the relationship between PAHs and CYP4

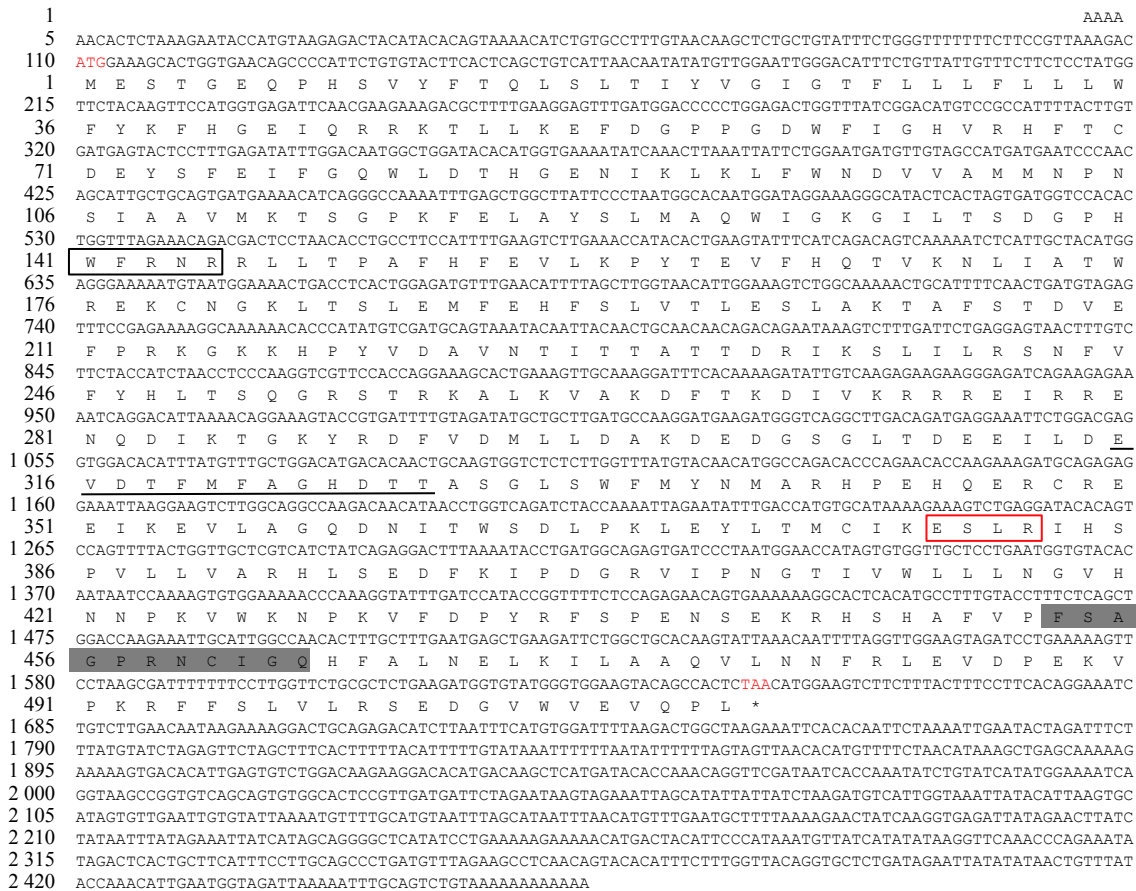


Fig. 1. Full-length cDNA and deduced amino acid sequences of *Marphysa sanguinea* CYP4. The start and stop codons are shown in red. A heme-binding domain (FxxGxxxCxG) is shaded, C-helix motif is boxed in black, K-helix motif is boxed in red, characteristic conserved domain of CYP4 EVDTFMFEGHDTT is underlined.

activity in *M. sanguinea*, the *MsCYP4* gene was cloned, and the expression pattern was detected.

In the current study, the full-length cDNA of *MsCYP4* gene was identified and characterized for the first time. This sequence contains 2 470 bp that encodes 512 amino acids. The deduced amino acid sequence shares a number of common characteristics with other members of the CYP4 family: heme-binding domain (FxxGxxxCxG) serving as the fifth ligand to heme iron (Position 453–466) (Xu et al., 2015), and cysteine residue playing an important part in the structure of binding oxygen; highly conserved domain WxxxR (Positions 141–145) in the C-helix at N-terminal, which can interact with hemoglobin (Liu et al., 2010); 13-residue (EVDTFMFEGHDTT) domain in the I-helix of CYP4 family, which is involved in substrate oxidation reaction and proton transfer (Positions 322–327) (Pan et al., 2011); and conserved domain EXXR (Positions 379–382) in the K-helix, which stabilize the heme core structure (Chen et al., 2014). The multiple sequence alignment analysis showed that the CYP4 amino acid sequence of *M. sanguinea* had similarity with CYP4s in other species. Numerous alignments corresponding to high 40% similarity confirmed that this *M. sanguinea* sequence belongs to the CYP4 family (Zheng et al., 2013). This result was in accordance with the findings of Rewitz et al. (2004).

Deposit-feeding invertebrates, such as polychaetes, can be sensitive to the biotransformation of pollutants in sediments. Experiment showed that after the excrement of *M. sanguinea* had

stood on the sediment for 2 h, the PAHs concentrations decrease to half, and this reduction may be caused by microorganisms' chemical changes or enzyme changes in the excrement of *M. sanguinea* (Onozato et al., 2012). Bach et al. (2005) found 20% of total PAHs to be polar metabolites in *Capitella* sp. I after 15 d exposure to 30 (µg fluoranthene)/(g sediment), however, *Capitella* sp. S only 3% of total fluoranthene were present as polar metabolites. The PAHs tolerance was different between two different *Capitella* species.

Jørgensen et al. (2008) indicated that the biotransformation process of PAHs was similar in both vertebrates and invertebrates which included two stages. In the first stage, CYP enzymes catalyze PAHs into water-soluble metabolites. The second stage is glutathione S-transferase detoxification. Many studies have investigated the induction after exposure to PAHs in marine animals. PAHs have upregulation effects on the mRNA expression of *CYP4* genes in polychaete *N. virens*, *C. capitata*, and *P. nuntia* (Nelson, 2011; Ohtsuki et al., 2012; Won et al., 2013; Zheng et al., 2013). The changes indicated that the *CYP4* gene expression in polychaete was inducible by PAHs. Miao et al. (2011) showed that exposure to BaP, *CfCYP4* was significantly decreased in the gill and digestive gland of scallops. Nelson (2011) proposed that two *CYP4* genes in fungi have low expression level under PAHs exposure, and the low expression level may be caused by the low control ability of CYP4 enzymes. In the present study, *MsCYP4* mRNA expression level was also induced under BaP exposure.

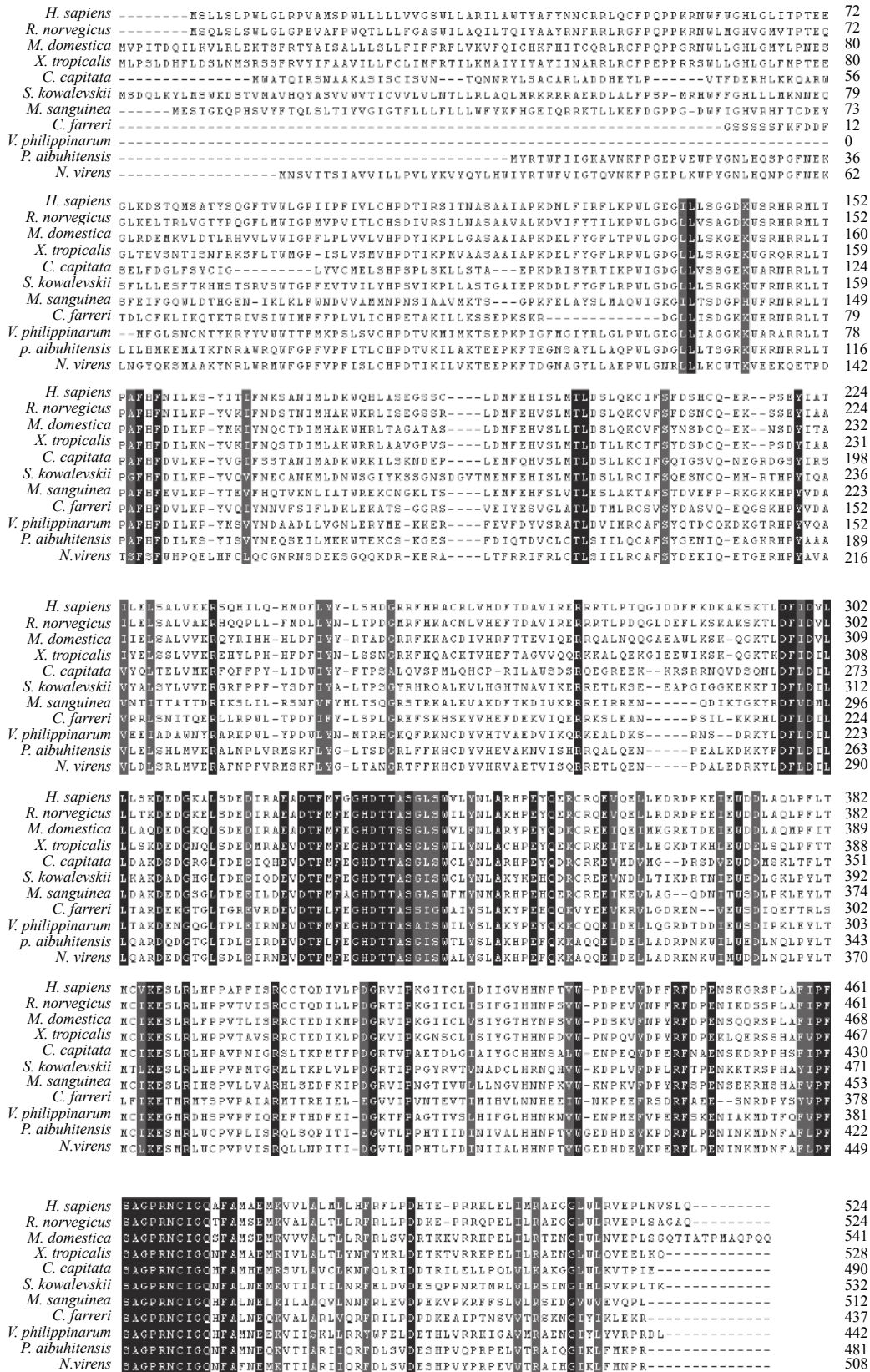


Fig. 2. Multiple alignment of CYP4 protein. Amino acid residues that are conserved in at least 50% sequence are shaded, and similar amino acids are shaded in dark, including *Homo sapiens* (AAH35350.1), *Rattus norvegicus* (NP_062569.2), *Monodelphis domestica* (XP_001367719.1), *Xenopus tropicalis* (NP_001015810.1), *Capitiella capitata* (AAS87604.1), *Saccoglossus kowalevskii* (XP_002730972.1), *Marphysa sanguinea* (HM126463), *Chlamys farreri* (ACL80141.1), *Venerupis philippinarum* (ACM16804.2), *Perinereis aibuhitensis* (ADI52567.1), and *Nereis virens* (AAR88241.2).

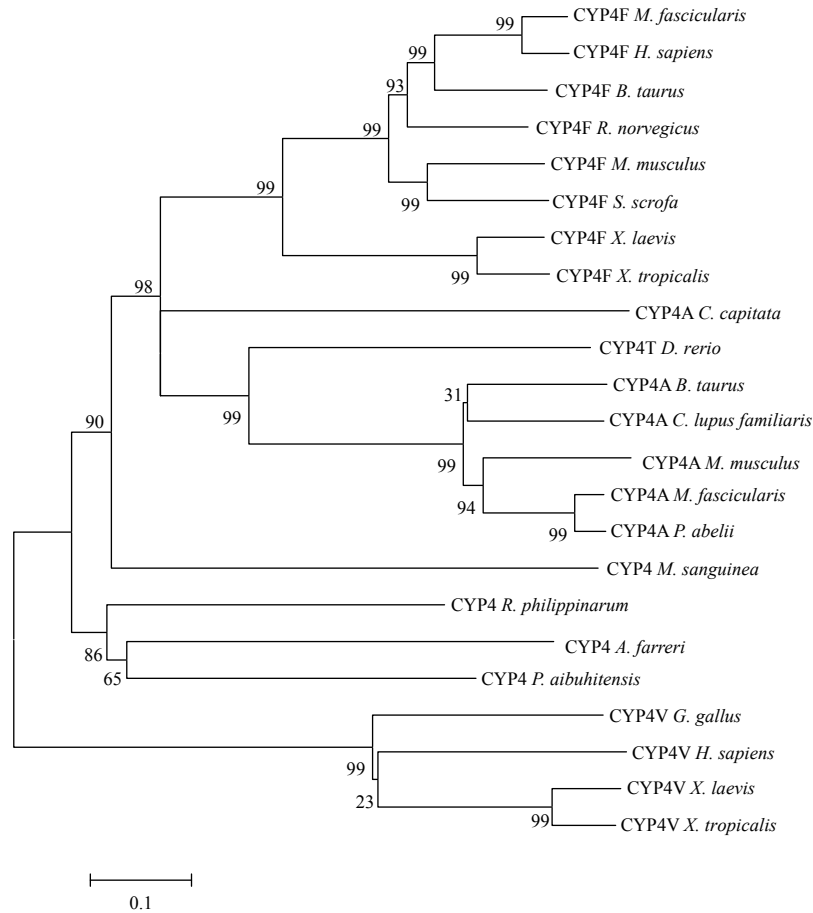


Fig. 3. Phylogenetic neighbor-joining tree of the 512 amino acid sequences of CYP4 from *M. sanguinea* with other species. The other CYP4 were as follow: *Macaca fascicularis* (NP_001270193.1), *Homo sapiens* (AAH35350.1), *Bos taurus* (NP_001030214.1), *Rattus norvegicus* (NP_062569.2), *Mus musculus* (NP_570952.1), *Sus scrofa* (NP_001231565.1), *Xenopus laevis* (NP_001091388.1), *Xenopus tropicalis* (NP_001015810.1), *Capitella capitata* (AAS87604.1), *Bos taurus* (NP_001071376.1), *Canis lupus familiaris* (NP_001041499.1), *Mus musculus* (NP_758510.2), *Macaca fascicularis* (NP_001270811.1), *Pongo abelii* (NP_001126777.1), *Danio rerio* (NP_954686.1), *Gallus gallus* (NP_001001879.1), *Xenopus laevis* (NP_001086053.1), *Xenopus tropicalis* (NP_001072667.1), *Homo sapiens* (EAX04624.1), *Azumapecten farreri* (ACL80141.1), *Ruditapes philippinarum* (ACM16804.2), and *Perinereis aibuhitensis* (ADI52567.1).

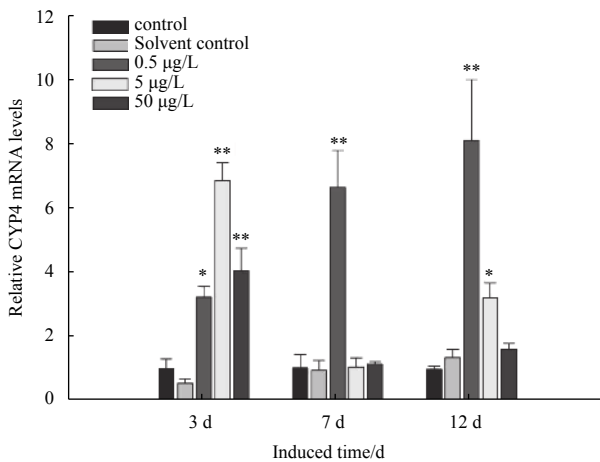


Fig. 4. Relative expression of CYP4 mRNA under benzo[a]pyrene exposure in *M. sanguinea*. Samples were treated with different concentrations (0.5, 5 and 50 µg/L) for 12 d. * Significant differences among concentrations within sampling times ($p < 0.05$); ** extremely significant differences among concentrations within sampling times ($p < 0.01$).

The transcripts of *MsCYP4* mRNA increased with exposure time in 0.5 µg/L dose group, and a 7.99-fold induction of *MsCYP4* mRNA expression was observed after 12 d. The *MsCYP4* mRNA expression firstly showed an increasing trend, then decreased, and finally increased slightly in 5 and 50 µg/L dose group. These different expression trends with other species may be related to the polychaete tolerance of PAHs. The *MsCYP4* mRNA expression was increased within the tolerance of the PAHs which explained the rising trend of low concentration groups. The downward trend of *MsCYP4* mRNA expression may result from the excessive DNA damage under high concentration of BaP exposure or toxicity effect. Many researchers refer to this phenomenon as induced saturation, that is, when the inducer reached a certain concentration, the gene expression levels would not increase but decrease (Fisher et al., 2003).

Pan et al. (2011) studied the CYP4 mRNA expression in different tissues and showed no notable change in CYP4 mRNA expression in gill of clam *R. philippinarum* exposed to BaP; on the contrary, the CYP4 mRNA expression was induced significantly by 0.2 µg/L BaP ($p < 0.05$) in the digestive gland of the clam. The different expression levels may be caused by the sampling tissues the experiment used. In the current study, only body wall

was used as sample, and further experiment about *CYP4* mRNA expression in different tissues is needed. Moreover, the detoxification mechanism of *CYP4* in polychaete should be further studied.

In conclusion, we cloned the full length of *CYP4* cDNA from *M. sanguinea* and examined the mRNA expression of *MsCYP4* under BaP exposure. The deduced amino acid sequence shared high homology with other proteins in the *CYP4* family. Our results proposed a biomarker to detect PAHs pollution in marine environment and indicated that *M. sanguinea* may be a promising species that can detect coastal environment pollution.

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