Identification of A Novel CYP4V Gene In The Polychaete *Perinereis Aibuhitensis*: Transcriptional Comparison With CYP4B Exposed To PAHs

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Abstract

Polychaete worms can biotransform polycyclic aromatic hydrocarbons (PAHs) in environments, and the cytochrome P450 (CYP) enzyme plays an important role in this process. Herein, a novel cytochrome P450 gene was identified and characterized from the polychaete worm *Perinereis aibuhitensis*. The full-length cDNA, which is named CYP4V82, is 1709 bp encoding a protein of 509 amino acids and has high similarity to CYP4V. The expression levels of *CYP4V82* and *CYP4BB4* (a CYP gene identified from *P. aibuhitensis* in a previous study, Chen et al., 2012) exposure to various concentrations of benzo[a]pyrene (B[a]P) (0.5, 2, 4, and 8 µg/L) and same mass concentrations of fluoranthene (Flu, 3.2 µg/L), phenanthrene (Phe, 2.9 µg/L), B[a]P (4.0 µg/L) were detected to identify the function of the CYP4 family in *P. aibuhitensis*. Compared with *CYP4BB4*, *CYP4V82* mRNA was minimally expressed on day 7 but highly sensitive on day 14. Notably, the expression level of *CYP4V82* and *CYP4BB4* was relatively different in response to PAHs with different benzene rings of the same concentration. The expression of *CYP4V82* in the B(a)P group was the highest, while that of *CYP4BB4* in the Phe group was relatively higher than the two other groups. These findings suggest that PAHs are associated with the induction of *CYP4V82* and *CYP4BB4* expressions, which may have different efficiencies in the detoxification of PAHs.

Introduction

The estuarine and coastal environments near areas of human habitation have been at risk due to PAHs derived from the anthropogenic effluent from domestic, agricultural, and industrial waste (Wang et al. 2008). PAHs can adsorb to particulate matter and accumulate in sediments due to their low water solubility and hydrophobic characteristic, and PAH pollution has increased in coastal areas over recent years (Ito et al. 2016; Zhou et al. 2020). For example, the total PAH concentration in the sediment in the Bohai Sea of China ranged from 276.26 ng/L to 10367.6 ng/L (Men et al. 2009), and that in the sediment of the west coast of Peninsular Malaysia ranged from 151 ng/g dw to 4973 ng/g dw (Vahab et al. 2019). The biotransformation of PAHs in estuarine and coastal organisms has elicited wide attention to adapt to stressful living conditions.

Polychaetes, which are the prey of many organisms, such as fishes and birds, are the most abundant taxa in the benthic community. Therefore, polychaetes play an important role in the recirculation of nutrients in marine ecology systems (Zheng et al. 2013). Polychaetes are continuously and directly exposed to pollutants from sediment and water column due to their deposit-feeding and sediment-dwelling living strategies, and studies have shown that some polychaetes can accumulate considerable amounts of pollutants (Jørgensen et al. 2008; Pires et al. 2016; Eom et al. 2019). These characteristics have resulted in the increasing use of these organisms as bioindicators in ecotoxicological studies (Lewis and Watson, 2012; Rhee et al. 2012). The biotransformation of organic xenobiotics contributes to the steady-state body burdens of polychaetes (Jørgensen et al. 2008). Polychaete biotransformation of PAHs is similar to the two-step process in vertebrates. PAHs are hydroxylated via phase I enzyme, and then metabolites are conjugated with the hydroxyl group via phase II enzyme and finally eliminated from the body (Danielson 2002; Jørgensen et al. 2008).

As a phase I enzyme, cytochrome P450 (CYP) plays an important role in the metabolism of endogenous (e.g., steroids and lipids) and exogenous (e.g., PAHs and other xenobiotics) compounds (Dejong and Wilson 2014). CYP1 is responsible for PAH biotransformation through aryl hydrocarbon receptor-mediated pathway in vertebrates (Chaty et al. 2004; Jørgensen et al. 2008; Won et al. 2013). Many studies in fishes have shown that the transcriptional level of CYP1A significantly increased in response to lipophilic xenobiotics (Uno et al. 2012; Kim et al. 2013a; Ferreira et al. 2016). By contrast, CYP1A has not been observed in invertebrates. Recent studies in marine invertebrates reported the involvement of CYPs from different families in detoxification in response to xenobiotics (Kim et al. 2013b; Yim et al. 2017; Han and Lee 2021). Several CYPs were also identified in marine polychaetes, and their functions were analyzed by mRNA expression studies. *CYP342A1* in *Nereis virens* belonging to CYP4 was induced by crude oil (Rewitz et al. 2004). The transcript level of *CYP4AT1* in *Capitella* sp. I was increased only in response to 3-MC, but the expression level of *CYP331A1* was induced by B(a)P and fluoranthene exposure (Li et al. 2004). Three CYP genes (*CYP432A1*, *CYP431A1*, and *CYP4DU1*) were identified in *Pereineis nuntia*, and the mRNA expression of these CYP genes was significantly increased by B(a)P and the water-accommodated fraction of Iranian crude oil (Won et al. 2013). Zheng et al. (2013) reported that three other CYP genes in *P. nuntia* (*CYP4BB2*, *CYP423A1*, and *CYP424A1*) were also significantly induced under B(a)P exposure. However, the various species in marine polychaetes showed different CYP enzyme inducibility, and identification of additional CYPs can improve the understanding of biotransformation pathway in marine polychaetes (Jørgensen et al. 2008).

Perinereis aibuhitensis is widely distributed along the Asian coasts and estuaries. This species has been used as a bioindicator of metal and organic contamination in estuaries due to its sensitivity to the xenobiotic pollutant (Wang et al. 2008; Chen et al. 2012; Lv et al. 2017; Zhang et al. 2017; Eom et al. 2019). A CYP4 gene named *CYP4BB4* was identified in this species in a previous study, and its expression pattern under petroleum hydrocarbon and deltamethrin was investigated (Chen et al. 2012). In this study a novel CYP4 homolog was identified to understand the mechanism of ecological adaptation and detoxification to pollutants in *P. aibuhitensis*, and the induction response to different kinds of PAHs was analyzed. Furthermore, the gene expression of two CYP4 in *P. aibuhitensis* was investigated by comparing each profile to evaluate the usefulness of *CYP* genes as bioindicators on PAH exposure in marine polychaetes.

Materials And Methods

Polychaete worm and adaptation

The polychaete *P. aibuhitensis* (1.5–2.5 g wet weight) was collected at low tide from Liaohe Estuary in Liaoning Province, China. The animals and the sediment in the sampling site were put in an ice cooler and transferred to the laboratory where they were cultured in glass tanks (50 cm × 40 cm × 40 cm) with seawater and origin sediment. The seawater used in this experiment was pumped from the Dalian coast adjacent to Lingshui Bay, where the experiment was conducted, and then filtered using a composite sand filter into a large reservoir tank. The animals were acclimated for one week with the animal density at ca.

100 ind. m⁻² before placement under exposure conditions. The seawater was changed completely daily during the acclimatization, and aeration was provided continuously, water temperature was 17°C, salinity was 30, and photoperiod was 12L:12D. The worms were fed excessively with a commercially formulated diet (13.0% moisture, 44.7% crude protein, 26.1% crude lipid, and 10.8% ash).

Cloning of full-length cDNA of CYP4

Total RNA was extracted from the body wall of *P. aibuhitensis* using RNAiso Plus (TaKaRa, Dalian, China) according to the manufacturer's instructions. The quality and quantity of total RNA were determined by electrophoresis in 1.0% (w/v) agarose gel and an ultramicro spectrophotometer, respectively.

The partial sequence fragment of *CYP4V82* was amplified through homology cloning technique with the primes based on the conserved amino acids of *Neanthes virens* CYP4BB1 (Genbank No: AY453407), CYP342A1 (Genbank No: AY453408), *Capitella* sp CYP4AT1 (Genbank No: AY574044), and CYP331A1 (Genbank No:AY574043) (Table 1). The reaction for *CYP4V82* partial sequence was conducted with nest PCR. The inner and outer PCR conditions were the same, and the procedure was conducted followed by 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min.

Table 1 The primer used in this experiment

Application	Primer name	Primer Sequence (5'-3')
Degenerate primer	CYP4F1	TGATACGTTTATGTTTGAGGG
	CYP4F2	ATGCAATTTCTGGGGCCAGC
	CYP4R1	ATGTTTGARGGDCAWGAMAC
	CYP4R2	AATNTTGNCCHATRCARTT
RACE primer	RACE-CYP4F2	CGGGAGTAATCGCGCTATCACC
	RACE-CYP4F3	CGCTTATTCCCCTCAGTACCAA
	RACE-CYP4R2	CGTTTTCGGTATATCTACACCA
	RACE-CYP4R3	CATACATTGGTACTGAGGGGAA
	actin- F1	TCTCGTAGTTGACAATGG
	actin- R1	CTTCAGTGTGAGGATACC
Real time PCR primer	CYP4V82-F4	ATCACGGTCTGCTCATTCC
	CYP4V82-R4	AAGAGTGCTGTATTCAAGAACC
	CYP4BB4-F1	GTGCCCCTAATCAGCCGA
	CYP4BB4-R1	GCAGTTTCTTGGCCCTGC

The full-length cDNA of *CYP4V82* based on the partial sequence fragment was obtained by the procedures of rapid amplification of cDNA ends (RACE) method using TaKaRa LA Taq® with a GC buffer Kit (TaKaRa, Dalian, China). 5' and 3' RACE-PCRs were also performed by nested PCR with the specific primer in Table 1. The inner and outer PCR conditions were the same. The reaction protocol of 3' RACE for *CYP4V82* was as follows: 94 °C for 3 min; followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min; finally, an extension step at 72°C for 10 min. The 5' RACE protocol for *CYP4V82* was as follows: 94 °C for 3 min; followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; finally, 72 °C for 10 min. The PCR products were determined by electrophoresis in 1% agarose gel, and the amplified cDNA fragments were purified using a gel extraction kit (TaKaRa, Dalian, China) and sequenced by Takara Biotechnology (Dalian) Co., LTD.

Sequence analysis

The complete cDNA of *P. aibuhitensis CYP4V82* was analyzed through ORF Finder on NCBI to find the open reading frame (ORF). The deduced amino acid sequence was obtained using the Expert Protein Analysis system (<http://www.us.expasy.org/tools>). The functional sites and motifs of CYP4V82 were respectively predicted with Expasy (<http://www.au.expasy.org/prosite/>) and Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan). The similarity of CYP4V82 sequence was examined with the BLAST program (<http://www.ncbi.nlm.nih.gov>). Multiple sequence alignment was analyzed with Clustal W software, and the phylogeny of the CYP4 family was examined using Mega 5.0 software. A bootstrap test was calculated from 1000 replicates.

PAH exposure

Sea sand was collected in the worm sampling site of origin and used in the experiment to provide refuge for *P. aibuhitensis*. The sand underwent combustion (550°C for 12 h) and acidification (10% HNO₃ [V/V]) to remove the organic matter. The handled sand was dried at 65°C for further use. This process was conducted to avoid the adsorption of organic matter to PAHs. Three kinds of PAHs, namely Phenanthrene (Phe), Fluoranthene (Flu), and Benzo(a)pyrene (B(a)P), were selected as pollutants. The Phe, Flu, and B(a)P standards were analytically pure and purchased from Sigma–Aldrich Corporation (Shanghai), U.S. The three PAHs respectively represent 3-, 4-, and 5-benzene ring PAHs. Four concentrations of B(a)P (0.5, 2, 4, and 8 µg/L) were set in accordance with the water solubility of B(a)P (3.8 µg/L, Sverdrup et al. 2002). A 16 nM Phe (2.9 µg/L), Flu (3.2 µg/L), and B(a)P (4 µg/L) was also established to demonstrate the CYP4 biotransformation to the same molecular but different ringed PAHs. The concentration of different kinds of PAHs in the exposure experiment was analyzed through Agilent 6890 gas chromatography coupled with Agilent 5975B mass spectrometry (Agilent Co., USA) to improve the concentration accuracy in the experiment. The actual concentration in each concentration group was detected before renewing the water, and the concentration of each group was as follows: 0.35 ± 0.025 µg/L B(a)P, 1.26 ± 0.024 µg/L B(a)P, 3.29 ± 0.301 µg/L B(a)P, 6.54 ± 0.167 µg/L B(a)P, 2.42 ± 0.203 µg/L Flu, and 2.04 ± 0.317 µg/L Phe. All three kinds of PAHs need a solvent carrier to dissolve in seawater. Therefore, a solvent control group (100 µL/L acetone) and a blank control group were set in this study.

Experimental worms were randomly put into 2 L plastic beakers containing 1500 ml seawater and a 2–3 cm layer of sand. Each group set three repetitions. The seawater was changed daily with the temperature maintained at $17 \pm 1^\circ\text{C}$, and commercially formulated diet was provided twice a day (9:00 and 16:00). Three individuals were sampled in each treatment at days 7 and 14 during exposure, and the samples were stored and frozen for further analysis.

Relative qualification of CYP 4 mRNA expression

The expression patterns of *CYP4BB4* (Chen et al. 2012) and *CYP4V82* obtained in this study were detected by real-time PCR to compare the mRNA expression of different *CYP4* under PAH exposure. Gene-specific primers (Table 1) were designed on the basis of the full-length cDNA of each gene. According to the report of Chen et al. (2012) the actin gene was used as a housekeeping gene in this study. The first-strand cDNA was synthesized from total RNA (500 ng/ μL) using a PrimeScript RT reagent Kit with a gDNA Eraser (TaKaRa, Dalian, China).

The quantitative real-time PCR was performed on ABI 7500 Sequence Detection System (Applied Biosystems, CA, USA). The reaction was conducted in triplicates in the total volume of 20 μL containing 10 μL of SYBR Premix Ex TaqTM II, 0.4 μL of ROX Reference Dye, 0.8 μL of each primer, 1.0 μL of cDNA, and 7.0 μL of double-distilled water. The amplification profile was 95 $^\circ\text{C}$ for 30 s, followed by 40 cycles at 95 $^\circ\text{C}$ for 5 s and 60 $^\circ\text{C}$ for 34 s. Dissociation curve analysis of amplification products was performed at the end of each PCR to confirm the specificity of the PCR product.

Statistical analysis

The $2^{-\Delta\Delta C_t}$ method was used to analyze the relative expression level of *CYP* mRNA under PAH exposure. All data were reported as mean $\pm$ S.D. The difference among B(a)P concentrations and different kinds of PAHs within a sampling time was determined using one-way analysis of variance followed by Tukey's test using SPSS19.0 software. Statistically significant difference was set at $P \leq 0.05$.

Results

Sequence analysis of CYP4 from *P. aibuhitensis*

The full-length CYP4 gene from *P. aibuhitensis* was 1709 bp, composed of an ORF encoding 509 amino acids. The theoretical isoelectric point and predicted molecular weight of this deduced protein was 7.06 and 58677.2 Da, respectively. This gene was annotated by criteria of the Cytochrome P450 Nomenclature Committee (Dr. David Nelson, personal communication) as CYP4V82 (JQ867402) and deposited in Genbank. Several conserved motifs have been identified in this sequence (Fig. 1): (a) the conserved signature sequence FxxGxxxCxxG (447–456 aa) in heme-binding domain at the C-terminal; (b) the conserved GxxT motif (EVDTFMFEGHDTT) in the I-helix (309–321 aa) in which the glutamic acid residue E316 is unique to CYP4 family (Rewitz et al. 2004; Miao et al. 2011); (c) the consensus sequence ExxR (374–377 aa) within the K-helix and the WxxxR motif (125–129 aa) in the C-helix.

The protein structure homology modeling of this novel CYP4V82 was predicted in Fig. 2. The conserved secondary structure of CYPs was also recognized. CYPs started with α -helix followed by two β -sheets in the N'-end and ended with four β -sheets next to α -helix in the C'-end of this CYP (Sirim et al. 2010; Zheng et al. 2013).

The sequence alignment analysis that the characteristic conserved domain of CYP4 and heme-binding domain had high similarity among different species (Fig. 3). CYP4V82 exhibited 53.17%–58.19% similarity to the CYPs from other Annelida, such as *Sternaspis scutata* and *Thalassodrilides sp.*, and showed 53.94%–56.17% homology to CYP4V from mollusks and fishes.

Phylogenetic analysis

Fig. 4 inferred that all CYP4V genes clustered in a distinct clade, which included CYP4V vertebrates and invertebrates. The CYP4V from *S. scutata* first clustered with CYP4V from *Crassostrea* and then with the sequence of *P. aibuhitensis*. The invertebrates formed a clade and then clustered with the vertebrates. The phylogenetic analysis also showed that the two CYP4 obtained in *P. aibuhitensis* belonged to two different CYP subfamilies due to their separate division in two different clades. The gene obtained by Chen et al. (2012) was strongly clustered with *A. virens* CYP4B and then with CYP4F.

Expression patterns of two CYP4s under various concentrations of B(a)P

The gene expressions of *CYP4V82* and *CYP4BB4* under B(a)P exposure were detected to compare the function of different CYP4 in *P. aibuhitensis* (Fig. 5). No significant difference is found between acetone solvent and control groups, which illustrates the absence of acetone effect on *CYP4* expression. The gene expression of *CYP4V82* in *P. aibuhitensis* was upregulated with prolonged time (Fig. 5a). On day 7, the gene expression of *CYP4V82* in all B(a)P concentrations increased with the value of 1.15, 1.41, 2.18, and 2.96. The expression of *CYP4V82* in 4 and 8 $\mu\text{g/L}$ groups was significantly higher than the control group ($P < 0.05$). On day 14, the gene expression of *CYP4V82* was rapidly upregulated compared with that on day 7 with transcripts level in each group as 3.82, 7.64, 4.91, and 3.64. Thus, a significant difference is observed between the control and B(a)P exposure groups ($P < 0.01$).

Different from the *CYP4V82* gene expression, the gene change in *CYP4BB4* in *P. aibuhitensis* was dramatically induced by B(a)P exposure on day 7, and the *CYP4BB4* expression was downregulated with exposure time (Fig. 5b). On day 7, the *CYP4BB4* was significantly upregulated with the value of 16.78, 21.86, 12.73, and 9.21 ($P < 0.01$). The gene expression of *CYP4BB4* in each concentration group decreased on day 14 compared with that on day 7. The transcript level of each group was 4.97, 13.17, 7.14, and 3.53, demonstrating a significant difference from the control group ($P < 0.01$). The gene expression in the 2 $\mu\text{g/L}$ B(a)P group was the highest, and gene expression decreased with the increase in B(a)P concentration. Compared with *CYP4V82*, the change in the *CYP4BB4* gene appeared remarkably early, and the gene expression in *CYP4BB4* was substantially higher than that in *CYP4V82* at the same B(a)P concentration.

Gene expression of *CYP4V82* and *CYP4BB4* under different PAHs

The gene expression pattern of CYP4V82 under B(a)P, Flu, and Phe exposure is shown in Fig. 6a. The highest gene expression appeared in the B(a)P group followed by the Phe group, and the Flu group exhibited the lowest gene expression. The CYP4V82 expression in all three PAH groups increased with the prolonged exposure time. On day 7, the CYP4V82 mRNA transcripts in the B(a)P, Flu, and Phe groups were 2.18, 1.60, and 1.89, respectively, and no difference was observed among the different treatments ($P > 0.05$). On day 14, the CYP4V82 mRNA transcripts in all groups increased with the value of 4.91, 2.03, and 2.55, and the CYP4V82 gene expression in the B(a)P group was significantly higher than that in the two other groups ($P < 0.05$).

The gene expression of CYP4BB4 dramatically changed with the higher expression level compared with CYP4V82. The mRNA transcript level from the highest to the lowest was Phe > B(a)P > Flu (Fig. 6b). The gene expression of CYP4BB4 in each group first increased and then decreased with prolonged time. On day 7, the gene expression levels of CYP4BB4 in B(a)P, Flu, and Phe groups were 12.73, 7.68, and 17.83, respectively, demonstrating significant differences between the Phe and the two other groups ($P < 0.05$). The gene expression level of CYP4BB4 in each group was downregulated at day 14, and the mRNA transcript levels in the B(a)P, Flu, and Phe groups were 7.14, 3.92, and 8.38, respectively. A significant difference was observed between the Flu and Phe groups ($P < 0.05$).

Discussion

The cytochrome P450 superfamily is a large group of enzymes involved in catalyzing a range of endogenous and foreign compounds (Zheng et al. 2013; Dejong and Wilson 2014). A novel full-length cDNA of the CYP gene was identified in the current study to investigate the CYP response to xenobiotics in *P. aibuhitensis*. The novel *P. aibuhitensis* CYP sequence contained 1709 bp encoding 509 amino acids. The conserved motifs of CYP, including the typical heme-binding domains, oxygen-binding domain (EVDTFMFEGHDTT) in the I-helix, hydrogen-binding domain ExxR in the I-helix and WxxxR motif were found in this deduced sequence. These highly conserved regions in the CYP indicate the essential functions of these regions for the CYP enzyme. Moreover, the glutamic acid residue E³¹⁶ within the heme-binding domain, which is unique to the CYP4 family and recognized to ligate with heme macrocycle, is retained in this sequence (Colas and Ortiz de Montellano 2003; Miao et al. 2011). CYP4 is possibly involved in xenobiotic biotransformation in marine invertebrates (Rewitz et al. 2006; Ito et al. 2016). The cysteine residue in the heme-binding domain is essential for monooxygenase activity (Jørgensen et al. 2005). Cysteine in the conserved domain in this sequence also inferred its xenobiotic biotransformation activity. The conservation of consensus sequence indicates that this new CYP in *P. aibuhitensis* is most homologous to CYP4 enzymes. Multiple sequence alignment showed that this deduced amino acid had more than 55% similarity to CYP4V in some aquatic vertebrates and invertebrates. Nelson et al. (1996) reported that CYPs sharing 40% primary amino acid sequence identity are assigned to the same family, and those with 55% sequence identity are classified into the same subfamily. Therefore, this novel CYP is highly homologous to CYP4V and was annotated as CYP4V82. Phylogenetic analysis also suggested

that CYP4V of invertebrates (such as bivalves and polychaetes) and vertebrates (such as fishes) showed high identity and clustering. The CYP4V subfamily is the only subfamily shared in vertebrates and invertebrates. The conservation across phylogenetically distant species suggests the possibility that the CYP4V subfamily emerged from a common ancestral gene early in eukaryotic evolution and also inferred the functional importance of CYP4V in the animal kingdom (Fujita et al. 2004). The phylogenetic analysis also suggested that the two CYP4s in *P. aibuhitensis* were separated into different clades. The separation of these CYP4 sequences may be due to the high diversity of the CYP4s in polychaetes, and the two sequences may represent different paralogs of CYP.

CYP4 enzymes in vertebrates can catalyze the hydroxylation of fatty acid and is also responsible for many drug substrates. The CYP4 in insects has been proven to respond to the detoxification of exogenous compounds (Rewitz et al. 2006; Jørgensen et al. 2008). The CYP4 and CYP3 have been recently reported to response to xenobiotic biotransformation in phyla Annelida. Ito et al. (2016) cloned *CYP4V30* in oligochaete *Thalassodrilides* sp. and found its inducibility in the worm exposed to PAHs. The gene expression in *N. virens*, *P. nuntia*, *C. capitata* sp. I, and some other marine polychaetes was induced by PAHs (Li et al. 2004; Rewitz et al. 2004; Jørgensen et al. 2005; Won et al. 2013; Zheng et al. 2013). One *CYP4BB4* gene in *P. aibuhitensis* was cloned in a previous study, and this gene showed its inducibility by petroleum hydrocarbon in a concentration-dependent manner (Chen et al. 2012). The novel *CYP4V82* and *CYP4BB4* from *P. aibuhitensis* were induced by different kinds of PAHs in the present study, suggesting the possible involvement of the CYP4 subfamily in *P. aibuhitensis* in detoxification of PAHs.

Except for *CYP4V82* on day 7, the expression pattern of the two CYP4 under different B(a)P exposure was similar, and the expression level of *CYP4BB4* and *CYP4V82* in *P. aibuhitensis* decreased in a dose-response manner after B(a)P exposure. The decreasing trend might be related to the production of reactive oxygen species (ROS). PAHs could be metabolized through redox cycling (Livingstone 2003), which enhances the production of ROS. The high-concentration B(a)P exposure could induce additional ROS, which might cause cell damage. Recording the ROS concentration and analyzing the correlation is suggested in future studies. The expression level of *CYP4BB4* induced by B(a)P, Flu, and Phe was higher than that of *CYP4V82*, and the highest expression of *CYP4BB4* appeared earlier than that of *CYP4V82*. The different responses in CYP to xenobiotics were also observed in other polychaetes. Li et al. (2004) inferred that *CYP331A1* in *C. capitella* sp. I was upregulated by B(a)P and FLA, but the expression of *CYP4AT1* was not induced. Rewitz et al. (2004) found that *CYP342A1* in *N. virens* was induced by crude oil and B(a)A, whereas the *CYP4BB1* gene expression was not induced. The reasons may be related to the different affinities of CYP genes to substrates. Moreover, the different expression patterns in the current study may be caused by metabolic specificities of CYP4 subfamilies. Seven CYP4 subfamilies were found in vertebrates (Nelson 2003), and the different subfamily enzymes show preferences for different substrates. The CYP4B subfamily specializes in the hydroxylation of short-chain fatty acids and metabolism of exogenous compounds and several aromatic amines, but the CYPV preferentially metabolizes medium fatty acids (Baer and Rettie 2006; Nakano et al. 2009; Kirischian and Wilson 2012). The preference of the CYP4B subfamily to aromatic amines might be the reason for the high inductivity

to PAHs, which have similar benzene rings to aromatic amines. Thus, CYP4BB4 in *P. aibuhitensis* might play an active role in the detoxification of PAHs.

The molecular weight, water solubility, and lipophilicity of a compound would affect its bioaccumulation and degradation. The transcript responses of *CYP4BB4* and *CYP4V82* to PAHs with different benzene rings were type-specific in the present study. The lowest transcript level of the two genes appeared in the Flu group, indicating the difficulty of removing Flu by *P. aibuhitensis*. The highest expression level of *CYP4BB4* appeared in Phe group, and the expression level of *CYP4V82* in Phe group was higher than that of the Flu group. PAHs with less than three rings are generally more susceptible to degradation than those with more than three rings (Hong et al. 2008). Phe, Flu, and B(a)P have different benzene rings, and Phe has the lowest octanol–water partition coefficient (Log Kow) among Flu and B(a)P (Miller et al. 1985; Sverdrup et al. 2002; Mackay et al. 2008). The relatively high gene transcript in the Phe group inferred the remarkable bioavailability of Phe.

Conclusion

This study presents the full-length cDNA of a novel CYP4 gene from *P. aibuhitensis* named CYP4V82. The phylogenetic and sequence analyses suggest that the structure of the CYP4V82 gene is highly conserved and homologous to the CYP4V subfamily. The gene expression of two *CYP4* in *P. aibuhitensis* was both responsive to B(a)P, Phe, and Flu exposure. However, the CYP4BB4 was more sensitive to PAH exposure than CYP4V82. The induction response of the two CYP4 was also different between various PAHs. These inducible CYPs may play an important role in the phase I detoxification of PAHs in polychaetes.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The origin data in this study are available from corresponding author upon request.

Competing interests

The authors declare that they have no known competing interests.

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Authors contributions

Xiutang Yuan: Conceptualization, Funding acquisition, Writing- Original draft preparation

Huan Zhao: Data curation, Funding acquisition, Writing- Original draft preparation

Lili Wang: Funding acquisition, Methodology

Yan Lei: Methodology, Formal analysis

Yinan Wang: Investigation, Validation

Dazuo Yang and Yibing Zhou: Writing- Reviewing and Editing

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Figures

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1  AAAAACCATGGTCACGTGACCTATGTCAACAACCTCGAGTGATAAAAACGCCGCCGCCGGGGGATCCCCAGCTTAGTGATGATCAGTGGAG
91  GGAAAATTTGTCTGTGGTGAGCAAACTCATCTGAGATGTCAGTGGGTCTTTTGATCACGGTCTGCTCATTCTTTTGCCTACTTTCTT
1   M A V G L L I T V C S F L F A Y F L
181  TGAAGACACTAAATTCACGCGATAGAAAATGGCTCTCCAAGGTCTCTGGTCTCCAACATTGCCTGTGTTTGGAAATGCACACATGCTC
19   W K T L N S R D R K W L S K V S G P P T L P V F G N A H M L
271  AAAACTGGAGGATCAGATTTCTTTGACCAGGTTCTTGAATACAGCACTCTTTATGAATCCCAAACAATGTTCAAAATCCTGCTAGGAAGC
49   K T G G S D F F D Q V L E Y S T L Y E S Q T M F K I L L G S
361  CATGCAATTGTTGTGCTCATTAAAGCTGAATCTGTGGAGAAATTGTTGAATTCTAGCAGCCATATTACAAAATCAGTTGAATACAGGTTCT
79   H A I V V L I K A E S V E K L L N S S S H I T K S V E Y R F
451  CTTGAACCATGGCTTGGCACTGGGTTATTGACAAGTACTGGTGCAAAGTGGCATTCAAGAAGGAAATTGCTGACTCCAACATTTCACTTC
109  L E P W L G T G L L T S T G A K W H S R R K L L T P T F H F
541  CGCATTTTGCAAGATTTCTTGAAGTGTTTAATGAACAATCACAAATCATGGTAGAAAATCTGAAGAAAAAGGTTGGTGAAATAAGTTT
139  R I L Q D F L E V F N E Q S Q I M V E N L K K K V G G N K F
631  GACATATTTCCATACATAACTCATTGTGCACTGGATATTATTTGTGACACTGCCATGGGAGTCAATGTTGATGCTCAAAATGATAGTGAC
169  D I F P Y I T H C A L D I I C D T A M G V N V D A Q N D S D
721  ACTCAGTATGTGAAGGATGTTTATAAAATCAGTGAGCTTGTGCACAGGCGGCAAAAAGCACCTTGGTTGTGGCCTGATTTTTTATATAGC
199  T Q Y V K D V Y K I S E L V H R R Q K A P W L W P D F L Y S
811  TTGTTACCTGCCGGACGACAGACCAAGAATGCCTGAGAAATCTACATAGTTTCACACAATCGGTCATTAAAGAGCGTCAGAGAGACCTT
229  L L P A G R Q T K E C L R N L H S F T Q S V I K E R Q R D L
901  CAAGCCAGTTTGGAAAATGAGGAAACATTAAGTTCAACATCAAGCATTGAAGACTTTCTAAGGATCACCAAACGAAAGAGAGTTGCATTC
259  Q A S F E N E E T L S S T S S I E D F L R I T K R K R V A F
991  TTAGACATGCTTTTACTTTACCAAAGGAATTCCAATTTATCTGACGAGGACATTCGGAAGAAGTTGACACATTCATGTTTGAGGGTCAT
289  L D M L L L Y Q R N S N L S D E D I R E E V D T F M F E G H
1081  GATACAACAGCAGCAGCAGCAAAATTTGGGCAATGCATCTTATTGGGTCCCATCCCAACGTGCAAGAAAAAATTCACAAAGAACTGGATGAA
319  D T T A A A A N W A M H L I G S H P N V Q E K I H K E L D E
1171  GTATTTGACGGGAGTAATCGCGCTATCACCGATGAAGATCTTAAAAAGATGAAATATCTGGAGTGTGTTATCAAGGAAACTTTGCGCTTA
349  V F D G S N R A I T D E D L K K M K Y L E C V I K E T L R L
1261  TTCCCTCAGTACCAATGTATGGAAGAGAATTGAAAGAAGACTTCAATATCGATGGTGTAGATATACCGAAAACGACTACCCTGCTTGT
379  F P S V P M Y G R E L K E D F N I D G V D I P K T T T L L V
1351  CTCACATCTGCATTGCATAGAGATCCACGTTATTTCCAGATCCCGAAAAATTTGATCCCGATCGCTTCACACTAGAAAAACTGTAGGA
409  L T S A L H R D P R Y F P D P E K F D P D R F T L E N T V G
1441  AGGCACCCCTATGCCCTTTGTACCATTCTCTGCTGGTCAAAGGAATTGCATCGGTGAGAAATTTGCAATGAATGAAGAGAAAGTCTTACTG
439  R H P Y A F V P F S A G Q R N C I G Q K F A M N E E K V L L
1531  GCATCAATCTTACGCAACTTCACGGTCAAGGCTCACCAGAAATATTGTGATCTTCGACCAATGGGAGAGCTTATTTTGCACCCGAAAAAC
469  A S I L R N F T V K A H Q K Y C D L R P M G E L I L R P E N
1621  GGCATATGGGTTAGCCTGCAGAATCGGGATGTTTAACTCATCCCAGATTTGTCTGGGAGAATTTATGAGAGTGGACGAAAAA
499  G I W V S L Q N R D V *

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Figure 1

Nucleotide and deduced amino acid sequence of CYP4V82 from *Paibuhitensis*. The initial codon and stop codon were underlined with red box. The conserved domains in CYP4 family were highlighted with

grey (including heme-binding domain (FxxGxxxCxG), characteristic conserved domain of CYP4 EVDTFMFEGHDTT, motif ExxR within the K-helix, GxxT)

Figure 2

The protein structure homology modeling image of the deduced polypeptides of the novel CYP4V82 from *Paibuhitensis*. The conserved K-Helix domain, I-helix was colored with orange and heme-binding domains was colored with red.

Perinereis_aibuhitensis	-MAVGLLITVCSFLFAY-----FLWKLTNSRDRKWLKSVSGPP-TLPVFGNAHMLKTGGSDFFDVLEYSTLYESQ	69
Sternaspis_scutata	MAGAGLVIAFVVAVISF-----LVWVFQPTTKRMLNQIPGPP-ALPLIGNAHMLKPGGSEFYQILEYTMFDKD	70
Lingula_anatina	MAEQNFVSYTSASLPLIGITFIFATLVAYALHYFKTKRHFDKMAGPP-ALPLVGNTHQFAVGGADEFYQIMKYSKDYEEH	79
Oncorhynchus_tshawytscha	MVIMSGTYTLGIICLVLLVLLACIMYHPLKKYFQDWNLDLRPIPGVDGAYPIIGNALLFSTNAGDFENCIIEGTKEFRHL	80
Thalassodrilides_sp._MI-2015	MAVTGILLTCLAALLIY----LAVRLYSKYPPSSKVRALVEKIPGPP-ARPIVGHAFIFKRG-AEPFVQLHTLCKEYGHK	73
Crassostrea_gigas	MAAAIIVILAVAVLFT-----TWLLKTELARKKVNTIPGEV-AVPLIGNAHQLRNTSRREFDCMESFLKLYADR	69
Perinereis_aibuhitensis	TMFRIILGG-SHAIIVLIKAESVEKLNS--SSRITKSVEYRFLBPLWLTG--LLTSTGAKWHSRRRLLTPTTFHRIQLQDF	144
Sternaspis_scutata	GIDHLRVG-LQPSVALYKPEVEVLHT--KSHITKSQBYRFLBPLWLTG--LLTSTGDKWKMMRRRLTPTTFHRIILYDF	145
Lingula_anatina	GIIRVWLG-ESPRVILFHADTVEELFNS--TKHSEKSSDYRFLBPLWLTG--LLTSTGKEKWHTRRRMLTPTAFHRIKLNPF	154
Oncorhynchus_tshawytscha	PLLRVWVG-PLPLVVLFAETVEGILHS--SKHIDKAYYRFMOPLWLTG--LLTSTGDKWRGRRRLTPTTFHRIILAEF	155
Thalassodrilides_sp._MI-2015	GIIRAWLG-YHCLVATNANAAEVLLKS--STHMTKSPLYDFIRBPLWLTG--LLTSTGSKWHSRRRLTPTTFHRIILQDF	150
Crassostrea_gigas	GIFRLWLG-TTLVLIFKAEYAEVILQS--SKHLDKAEYKFLBPLWLTG--LLTSTGSKWHSRRRLTPTTFHRIILNDF	144
Perinereis_aibuhitensis	LEVFNESQAI-MVENLKKKVGGN-KDIFPPIYTHCALDIICDTAMGVNVDAQNDSDTQ-VVKDVYKISELVHRRQKAPAWW	222
Sternaspis_scutata	LCVFNEQSAIMVQRLEKQVDKG-EFDMFPFITLCALDIICDTAMGKHVNQAQSDSDK-YVKAVYKMSLVHRRQKSPFWW	223
Lingula_anatina	LNVMCBQADIMVNKMAKHTGGS-APDVFPPIYTHCALDIICDTAMGQTVNAQQSDSDS-VYKAVYRLCYLVSRQKQFPWW	232
Oncorhynchus_tshawytscha	LEVFNESQAEVLTQLEKQAGD-PFNCFSYITLCALDIICDTAMGKNIYAQSNSESE-YVRSVYKMSDIIGRRQKAPAWW	233
Thalassodrilides_sp._MI-2015	LEVFNESQATVMTKHLKMAKSGKGDVIFPFIYTHCALDIICDTAMGRHVNQAQSDSE-YVQAIYRISDLVHRRQKAPAWW	229
Crassostrea_gigas	VGVFHDAQSIMVKKLSQVADGK-EFNFNFIYTHCALDIICDTAMGRHVNQAQSNSESE-YVKAVYKMSLVHRRQKAPAWW	222
Perinereis_aibuhitensis	P-DFLYSLPAGRQTKCELRNLHSTFQSVIKERQORDLOASFENEET--LSSTSSIEDFLRITKRKRVAFDMLLYLQ-R	297
Sternaspis_scutata	P-DVIYNSLPQKQKQCEQLILHGFTKAVIREQSVIRKKIAEEG---PTEPAKIDLYAEGGKKRMFLDMLIYA-AE	296
Lingula_anatina	P-SLIYDIFGDGKETKECLRLVHNFTNKVIAERKEQFQKSLIQDRKKTLEQYSEGGEHLSIGGRKKLALLDMLLHS-SE	310
Oncorhynchus_tshawytscha	P-DFIYSLWEGRDHNDRLRLHSTFQSVIKERAESMENA-----GSDSESDHGKIK---RRRLAFDMLLKATDE	299
Thalassodrilides_sp._MI-2015	P-DGIYRLPAGKEHERLLTVLHSTFNEVIRERTAEWRHLASD---DKDSATTEKPSLIGGRKKLAFDMLLHA-SA	303
Crassostrea_gigas	P-DFLYNTIGYGRHEDRCILKILHYFTLKVIKERMDAVSDASGNSMK---EMLNQHSIEDVYRGKGQKAFDMLLCT-AD	297
Perinereis_aibuhitensis	N-SNLSDEDIREEVDTFMFEGHDTTAAANWAMHLIGSHFNVDKRIHKEDEVFDG-SNRRAITDEDLKKMKYLECVIKET	375
Sternaspis_scutata	GGANMTDEDIREEVDTFMFEGHDTTAAANWAVHLIGANPEIQKQVQDELDLDFRG-SDRPAITMEDLADMKYLECCIKEA	375
Lingula_anatina	TETPLTDEDIREEVDTFMFEGHDTTAAAGVTWASYFIFGRYVEVQKLEHEEMDSIFGD-SDRPAITPQDLAQMKYLECVIKEA	389
Oncorhynchus_tshawytscha	EGNYLSHSDIQEEVDTFMFEGHDTTAAAMNWLHLGSEYVEVQTKVQBELQVVFSG-SNRSVTVDDLKRLKYLECVIKET	378
Thalassodrilides_sp._MI-2015	KHQ-LTVEDIREEVDTFMFEGHDTTAAISWATHLIGANPEVQKQVHDELDQIFGQ-SDRLVTMDDEKMKYLECVVKEF	381
Crassostrea_gigas	DGSHLSMEGIREEVDTFMFEGHDTTAAAVNWAHLIGANPEVQKQVHDEMDQLFGG-SDRPAITMNDLKEMKYLECCIKEA	376
Perinereis_aibuhitensis	LRLFPSPVPMYGRLEKEDFNIDGVDTEKTTTLVLTSAHLRDPRIYF-PDPEKEDFDRFLENTVGRHPPAFVFFSAGQRNC	454
Sternaspis_scutata	LRLFPSPVPMFARTISEDCVIGGYDVEEGTTAVVTAALHRDPDHF-PDPEKEDFDRMLPENCASRHPCFIPFSAGLRNC	454
Lingula_anatina	LRIYPSVPFHARTFTEDCEIKGFKEIKGTGEGILVLTALHRDAKYF-PDPEKEDFDRFLENTVGRHPPAFVFFSAGMRNC	468
Oncorhynchus_tshawytscha	LRLFPSPVPMFARTVSDCHINGFKIKGVNALIIPFALHRDPRIYF-PDPEKEDFDRFLENTVGRHPPAFVFFSAGPRNC	457
Thalassodrilides_sp._MI-2015	LRLFPSPVPMMAIEAEDCTINGFFVPGSTGVQTSVIRHPDHYF-PEPEAFKPERETNENSVGRHPPAFVFFSAGLRNC	460
Crassostrea_gigas	LRLFPSPVPGFGRKLTECKFGYEYSIEKNTTVIITPPDLHRDKRYF-PDPEKEDENRRLPENSLEKRRHPPAFVFFSAGPRNC	455
Perinereis_aibuhitensis	IGQKFAMNFEKVLASILRNFTVKAHQKYCDLRPMGELILRPENGIWVSLQNRDV----	509
Sternaspis_scutata	IGQKFAMKEEKVVSSILRRFNKSMQTRADLLPMGELILRPQNGIRVKLTRRQ----	509
Lingula_anatina	IGQKFAIMEEKVMSAMLRKFNMTSTKREELRFVSEIILRPEHGIWIKLTPRNEE----	524
Oncorhynchus_tshawytscha	IGQKFAMMEKVLSSVLRHFSVQACQSRRELRLPLGLDLILRPEKGIWILEKRQC----	512
Thalassodrilides_sp._MI-2015	IGQKFAMIEEKVLLSILRHFQIASLQKRGDLNPSGEIILRSQNGINIKLEKR-----	513
Crassostrea_gigas	IGQKFAILEEKVMSNIFRNFTVTSKQSRRELRLPLGLDLILRPEHGIWILEKRPTT-----	510

Figure 3

The alignment of CYP4V82 with other amino acid sequences. Amino acid residues that are conserved in at least of 50% sequence are shaded in dark. The homology and the Genbank accession numbers were as follows: Sternaspis scutata (AIT 16229.1) 58.19%, Lingula anatina (XP 013386571.1) 53.17%, Oncorhynchus tshawytscha (XP 024284637.1) 56.17%, Thalassodrilides sp. MI-2015 (BAV 38819.1) 53.59%, Crassostrea gigas (XP 011420768.2) 53.94%.

Figure 4

The neighbor-joining phylogenetic tree of those two CYP4 in *P. aibuhitensis*.

Figure 5

The expression pattern of CYP4V82 and CYP4BB4 mRNA in *P. aibuhitensis* under different concentration B(a)P exposure. a represents the expression pattern of CYP4V82, b represents the expression pattern of CYP4BB4; data (expressed as mean $\pm$ S.D., n=3) with different letters in the same exposure time indicates significant differences.

Figure 6

Effects of B(a)P, Flu and Phe treatments on CYP4V82 and CYP4BB4 mRNA expression in *P. aibuhitensis*. a represents the expression pattern of CYP4V82, b represents the expression pattern of CYP4BB4; data (expressed as mean $\pm$ S.D., n=3) with different letters in the same exposure time indicates significant differences.