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Molecular characterization and organs expression of cytochrome P450 1B1 from Japanese eel *(Anguilla japonica)*

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ABSTRACT

The CYP1 family, one of the gene families of the CYP superfamily, has four subfamilies deposited in the GenBank/EMBL so far; *CYP1A, CYP1B, CYP1C*, and the newly identified *CYP1D*. The metabolic activation and elimination of polyaromatic hydrocarbons, polychlorinated biphenyls, and aryl amines from fish body is largely mediated by the CYP enzymes. A new cDNA of the *CYP1B* subfamily encoding CYP1B1 was isolated from Japanese eel liver after a single intraperitoneal injection of β-naphthoflavone (BNF). The full-length cDNA obtained was 2985 bp and contained a 5' noncoding region of 294 bp, an open reading frame of 1626 bp coding for 541 amino acids and a stop codon and a 3' noncoding region of 1065 bp. The predicted molecular weight of the protein was approximately 61.27 kDa. The deduced amino acid sequence of Japanese eel *CYP1B1* showed 62% similarity to three-spined stickleback *CYP1B1* and zebrafish *CYP1B1***.** It exhibited similarities of 66% with that of killifish, Indian medaka and our previously reported carp *CYP1B1* and *-1B2* while the higher similarities (67 and 69%) of the deduced amino acids was observed with that of Nile tilapia *CYP1B1* and rainbow trout respectively. The percent identities of Japanese eel *CYP1B1* cDNA showed similarities with those of the reported *CYP1Bs* of mammals of 57, 57, and 56% for human, rat, and mouse *CYP1B1*, respectively. Japanese eel *CYP1B1* was aligned with the *CYP1* sequences and has been deposited in the GenBank/EMBL data bank with the accession number AY518340. The phylogenetic tree constructed using the previously reported CYP1B sequences of mammals and fish suggested the closer relationship of the newly identified Japanese eel *CYP1B1* to rainbow trout *CYP1B1*. QRT-PCR analysis of liver, kidney, gills and intestine revealed a distinct induced expression in liver, kidney and gills (71.93, 3.87 and 539.56 respectively) while the constitutive expression (0.062) was observed in intestine.

Keywords*:* *Anguilla japonica*, β-naphthoflavone, cytochrome P450, *CYP1B1*.

INTRODUCTION

In aquatic animals that inhabit environments often contaminated with high levels of organic pollutants, the induction of CYP genes as well as the catalytic activity of their products can serve as a sensitive biomarker for environmental contamination by dioxin and other chemical compounds (Payne 1976; Goksoyr 1995; Monostory *et al.,* 1996)**.** Cytochrome P450 enzymes are intermediates to the metabolic activation of polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and aryl amines. The cytochrome P450 enzymes catalyze the metabolic reactions of a variety of substrates from endogenous compounds such as steroids, fatty acids, vitamins, prostaglandins, and retinoids to numerous exogenous chemicals such as drugs,

carcinogens, mutagens, and other chemical pollutants often resulting from combustion process (Nebert & Gonzalez, 1987; Guengerich, 1990; Porter & Coon, 1991; Coon *et al.,* 1996; Nelson *et al.,* 1996). Compared with humans that possess three CYP1 genes named *CYP1A1*, *CYP1A2* and *CYP1B1* (Nebert & Russell, 2002), fish such as medaka, zebrafish and killifish possess five genes: CYP1A, CYP1B1, CYP1C1, CYP1C2 and CYP1D1 (Goldstone *et al.,* 2007, 2009). Until the recent identification and characterization of the *CYP1B1* gene in mammals (Savas *et al.,* 1994, Bhattacharyya *et al.,* 1995, Walker *et al.,* 1995, Tang *et al.,* 1996) and fish (Leaver & George, 2000, Godard *et al.,* 2000, El-Kady *et al.,* 2004a,b, Willett *et al.,* 2006, Hou-Chu *et al.,* 2008, Zanette *et al.,* 2009; 2010), *CYP1A1* and *CYP1A2* were the main *CYP1* family members held responsible for the oxidation and activation of PAHs, PCBs and aryl amines. Some laboratory chemicals like β-naphthoflavone (BNF) and 3-methylcholanthrene (3-MC) are regarded as prototypical PAHs-type inducers of *CYP* genes. These chemicals exert their effects on mammalian *CYP1A* and *CYP1B* through the interaction of the ligand-bound aromatic hydrocarbon receptor (AHR) and its specific binding sequences located upstream of the respective target genes (Hakinson, 1995; Zhang *et al.,* 1998; Buters *et al.,* 1999). The isolated five CYP1s from fish present a markedly distinct organ-specific distribution in the gene expression levels suggesting that different physiological roles could exist (Jönsson *et al.,* 2007b; Goldstone *et al.,* 2009). Up to date, only a single isoform of CYP1B has been identified from all fish species, whereas both *CYP1B1* and *CYP1B2* genes have been cloned in carp (*Cyprinus carpio*).

Recent studies on metabolism and carcinogenesis have shown mammalian *CYP1B1* to be a critical and in some cases, necessary enzyme in the activation of several pollutants, notably the PAH 7,12-dimethylbenzene[a]anthrathene (DMBA) (Buters *et al.,* 1999; Chun and Kim, 2003). They reported that *CYP1B1* is located exclusively at extrahepatic sites and mediates the carcinogenesis of DMBA. Also, Shimada *et al.,* 1996; 2002) reported that *CYP1B1* participates with *CYP1A1* and *CYP1A2* in the activation of 2,3,7,8-tetrachlorodibenzo-p-dioxin, benzo[a]pyrene and related carcinogens causing initiation of cancers in human and mice. *CYP1B1* is also potentially playing a role in endocrine regulation and estrogen-induced carcinogenesis (Hayes *et al.,* 1996). Human *CYP1B1* has been shown to metabolize 17 β-estradiol to a 4-hydroxylated product, a chemical considered to cause breast cancer in women (Spink *et al.,* 1998).

In contrast to *CYP1As* which have been identified in most vertebrate groups (mammals, birds, fish), *CYP1Bs* have been identified in a few mammal and fish species. The Genbank survey on fish *CYP1Bs* revealed only nine sequences; our previously reported carp *CYP1B1* and *-1B2*, Nile tilapia sequence from our laboratory, and *CYP1B1* sequences of plaice, catfish, Indian medaka, killifish, rainbow trout and zebrafish.

In this study, a cDNA of the *CYP1B1* gene was isolated from the liver of BNF-treated Japanese eel and sequenced. Phylogenetic analysis was also performed to assess the relationship of this newly identified *CYP1B1* gene with the other *CYP1B* subfamily members. Also the expression pattern of *CYP1B1* mRNA was determined in liver, kidney, intestine and gills using QRT- PCR.

MATERIALS AND METHODS

Fish treatment and RNA preparation

Japanese eel *(Anguilla japonica)* with a mean weight of 400 g were obtained from a commercial source and were treated with a single intraperitoneal injection of BNF (100 mg/kg) suspended in corn oil. Similar control fish were intraperitoneally injected at the same time with an equivalent volume of the vehicle (corn oil). Three days under fasting conditions, the treated and the control fish were killed and samples of the liver, kidney, intestine and gills were collected, immediately frozen in liquid nitrogen, and stored at–80 °C. Total RNA was isolated from 2 g of each of the samples of frozen liver, kidney, intestine and gills according to the Standard Acid Guanidinium Thiocyante Phenol Choloroform (AGPC) extraction method (Chomczynski & Sacchi, 1978). Total RNA concentration and purity were determined spectrophotometrically as described by Sambrook & Russel (2001), and A260/A280 ratios were between 1.7 and 1.9. Poly (A)+ RNA was purified using an Oligotex-dt30 <super> mRNA purification kit (Takara, Japan).

Reverse transcription for full-length cDNA generation

Reverse transcription of mRNA was performed with Superscript II reverse transcriptase (Gibco BRL, USA) to generate 5'-RACE-Ready and 3'-RACE-Ready first-strand cDNAs using the SMART^{TM} RACE (rapid amplification of cDNA ends) cDNA amplification kit (Clontech, USA). According to the manufacturer's protocol, 3'- and 5'-RACE Ready cDNA to be used as a template in the 3' and 5' RACE PCR respectively.

3' and 5'-RACE PCRs for full-length cDNA

We designed one sense (F) and an antisense (R) primer specific to Japanese eel *CYP1B1* for 3' and 5' RACE PCRs, respectively. The primer sequences were designed from short sequence obtained at our laboratory and is given in Table 1. The sense and antisense gene specific primers were used in combination with the universal primer mix (UPM) of the RACE PCR kit to generate the RACE PCR products. The RACE PCR reactions with the reverse transcription (RT) products obtained were conducted according to the principles of touch down PCR in order to avoid spurious amplifications (Don *et al.,* 1991).

Table 1: Oligonucleotide primers used in the PCR amplification of Japanese eel CYP1B1 cDNA fragments.

Primer	Nucleotide sequence	Nucleotide location
F	5' GTTTATCGATGCAACGCTCAGGC	2111 to 2133
R	5'-GCCGATCACAGGTCATGCTTAGTA	597 to 574
UPM (long)	5'- CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	
UPM (short)	5'-CTAATACGACTCACTATAGGGC	

The cycle conditions for RACE PCR were as follows: 5 cycles of denaturation for 5 s at 94°C and annealing for 3 min at 72 °C; 5 cycles of 94 °C for 5 s, 70 °C for 10 s, and 72 °C for 3 min; and 35 cycles of 94 °C for 5 s, 68 °C for 10 s, and 72 °C for 3 min. The DNA band of expected size was excised with a scalpel, purified using a GFX PCR DNA and a gel band purification kit (Amersham Biosciences, USA). The PCR products were subjected to restriction mapping with various enzymes and were cloned into PT7BlueT-vector (Novagen, USA). Purified plasmids were directly sequenced by dye terminator cycle sequencing using the ABI PRISM dye terminator cycle sequencing kit (PE Biosystemes, USA) and an Applied Biosystems 3100 DNA Sequencer.

Phylogenetic analysis

DNA sequences with the following GenBank accession numbers were retrieved from the database and used in the phylogenetic analysis: DQ088663 (channel catfish *CYP1B1*), FJ786959 (killifish *CYP1B1*), HQ202282 (three-spined stickleback fish *CYP1B1*), NM_001174149 (rainbow trout *CYP1B1*), JX4546651 (Indian medaka *CYP1B1*), AY727864 (zebrafish *CYP1B1*), AY437774 (carp *CYP1B1*), AY437775 (carp *CYP1B2*), HQ829968 (Nile tilapia *CYP1B1*), U03688 (human *CYP1B1*), U09540 (rat *CYP1B1*) and X78445 (mouse *CYP1B1*). In order to determine homology among CYP1B subfamily cDNAs or deduced amino acid sequences of various species, sequence alignments were performed by the CLUSTAL W multiple sequence alignment method (online alignment site constructed by Kyoto University, Japan, http://www.genome.jp/tools/clustalw/). The phylogenetic tree was constructed by unweighted pair group method (UPGM) using the amino acid sequences of the previously reported CYP1B subfamily sequences of teleosts and mammals.

CYP1B1 expression in different organs of Japanese eel using QRT- PCR Reverse transcription

Reverse transcription of the RNA samples isolated from liver, kidney, intestine and gills was performed using Primescript^{TM} RT reagent kit (Takara, Japan) according to the manufacturer's instructions. Reactions were incubated for 15 min at 37°C then 5 s at 85°C to inactivate the reverse transcriptase. RT products were stored at 4°C for further PCRs.

Primer design for QRT- PCR reaction

Primers for Japanese eel CYP1B1 cDNA were designed using Laser gene primer select program (Ver. 5.52, 2003, DNASTAR Inc), with melting temperatures (Tm) ranging from 58 to 60°C, and amplicon lengths of 50 to 150 bp. The primer sequences are given in Table (2).

Table 2: Oligonucleotide primers used in the QRT- PCR amplification of Japanese eel probe.

Primer	Nucleotide sequence	Nucleotide location	Product length
F	5'- GTTTATCGATGCAACGCTCAGGC	2111 to 2133	92bp
R	5'- TGAGGACAACAACAACAACAACTA	783 to 806	

Quantitative Real-time PCR conditions and analysis

Each PCR reaction consisted of 10 µl of SYBR^{R} Premix Ex Taq^{TM} II (2X), 10 µM of each primer, 2 µl of cDNA template and double distilled water to a final volume of 20 µl. All standard plasmid DNA dilutions, template controls and induced samples were run in triplicates. Reactions were then analyzed on an ABI 7300 Real-Time PCR system under the following conditions: 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 3 min. The relative quantification relates the PCR signal of the target transcript in a treatment group to that of another sample such as an untreated control (calibrator). For quantification of induced Japanese eel CYP1B1 normalized to an untreated control, standard curves were prepared for both the induced and the untreated reference. Each of the normalized induced Japanese eel CYP1B1 values was divided by the untreated control normalized value to generate the relative expression levels. Accordingly, CYP1B1 mRNA levels were reported as fold change in abundance relative to the average calibrator response.

Statistical analysis

The statistical differences between the groups were determined, and the data expressed as mean ± standard deviation. Excel (Microsoft, NY) were used to analyze the data, and Student's t test was used for the comparisons. A P-value <0.05 was considered significant. At least three determinations were carried out for each data point.

RESULTS

Nucleotide sequence analysis

Figure, 1 shows the full-length cDNA sequence (2985 bp) of Japanese eel cytochrome P450, *CYP1B1*. The obtained nucleotide sequence of Japanese eel *CYP1B1* contained a 5' noncoding region of 294 bp, an open reading frame (ORF) of 1626 bp coding for 541 amino acids and a stop codon, and a 3' noncoding region of 1065 bp. The predicted molecular weight of the protein was approximately 61.27 kDa. The sequence has one polyadenylation signals (AATAAA) and a poly A tail of 30 nucleotides. This sequence was aligned with the *CYP1* sequences and has been deposited in the GenBank/EMBL data bank with the accession number AY518340.

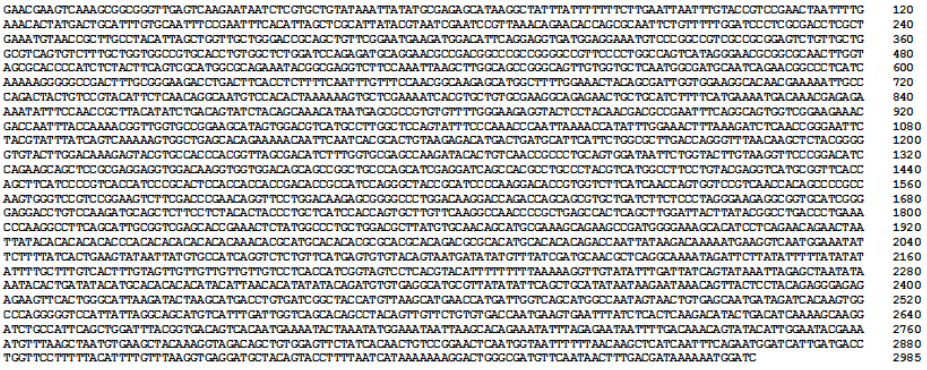

Fig. 1: Nucleotide sequence (2985 bp) of Japanese eel cytochrome P450 CYP1B1 cDNA. Consensus sequence for polyadenylation signal is bolded.

Comparison of amino acid sequences

The open reading frame and its deduced amino acid residues of Japanese eel cytochrome P450, *CYP1B1* cDNA is shown in Figure 2. Table 3 shows the similarity percentage of the deduced amino acid sequences of Japanese eel *CYP1B1* with those of the previously reported other *CYP1B1* members. The deduced amino acid sequence of Japanese eel *CYP1B1* showed 62% similarity to three-spined stickleback *CYP1B1* and zebrafish *CYP1B1*. It exhibited similarities of 66% with that of killifish, Indian medaka and our previously reported carp *CYP1B1* and *-1B2* while the higher similarities (67 and 69%) of the deduced amino acids was observed with that of Nile tilapia *CYP1B1* and rainbow trout respectively. The percent identities of Japanese eel *CYP1B1* cDNA showed similarities with those of the reported *CYP1Bs* of mammals of 57, 57, and 56% for human, rat, and mouse *CYP1B1*, respectively.

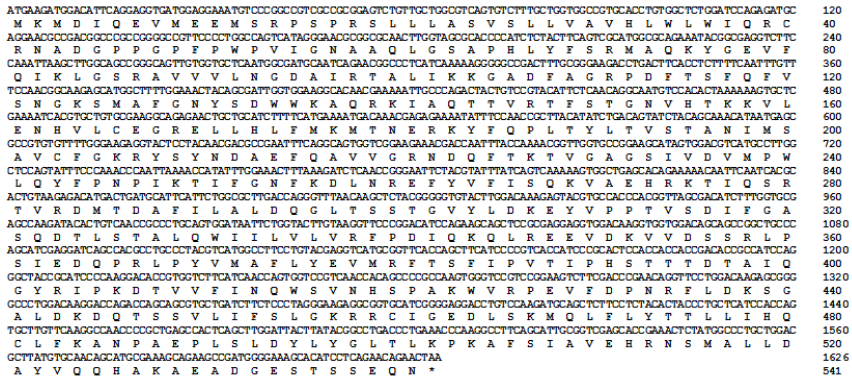

Fig. 2: The open reading frame (ORF) and its deduced amino acid residues of Japanese eel cytochrome P450 CYP1B1 cDNA.

Table 3: Percent identities of deduced amino acid sequences of CYP1B subfamily genes

	carp 1B1	carp 1B2	Indian medaka 1B1	zebrafish 1B1	three-spined 1B1	rainbow trout 1B1	Nile tilapia 1B1	killifish 1B1	human 1B1	mouse 1B1	rat 1B1
Japanese eel 1B1	66	66	66	62	62	69	67	66	57	56	57
carp 1B1		91	63	84	61	63	60	61	55	54	54
carp 1B2			63	84	61	64	60	62	56	54	55
Indian medaka 1B1				60	74	67	76	74	58	57	57
zebrafish 1B1					68	61	63	65	57	55	55
three-spined 1B1						68	78	76	60	58	59
rainbow trout 1B1							70	65	54	53	52
Nile tilapia 1B1								73	56	56	56
killifish 1B1									59	59	60
human 1B1										81	82
mouse 1B1											92

Phylogenetic analysis

The phylogenetic tree based on the amino acid sequences of *CYP1Bs* of teleost and mammalian species is shown in Figure, 3. The higher similarity (69%) of the deduced amino acid sequences between Japanese eel *CYP1B1* and that of rainbow *CYP1B1* resulted in the clustering of the two genes in one branch apart from other *CYP1Bs*.

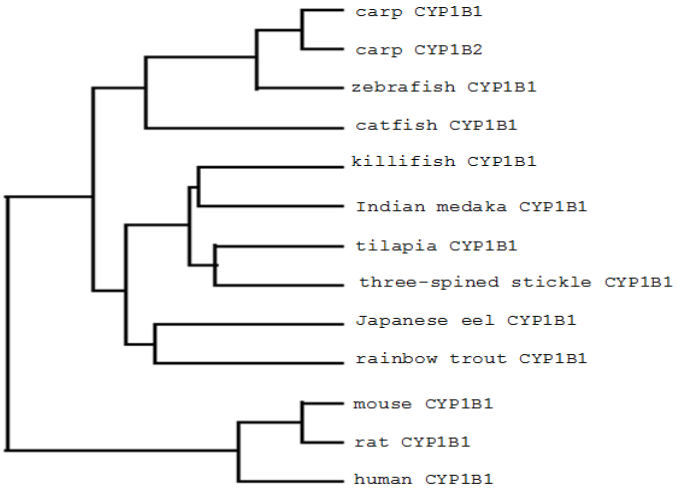

Fig. 3: Phylogenetic tree of CYP1B subfamily genes constructed by pair group unweighted method (UPGM) using the amino acid sequences of teleosts and mammals.

CYP1B1 mRNA level in different tissues of BNF treated Japanese eel

QRT-RCR results revealed that there was a large increase in CYP1B1 mRNA in gills (539.56 fold), followed by liver (71.93), and kidney (3.78) while the constitutive expression was observed with the induced intestine (0.062) (Table, 4) (Figure 4).

Table 4: QRT-PCR results for Japanese eel CYP1B1 mRNA

Sample	Mean Qty	Std Dev	Fold	t-value	Pr
L.cont	0.1309	0.05		8.29	<0.005
L.ind	9.4163	1.94	71.93		***
K.cont	5.0478	0.45		3.12	<0.05
K.ind	19.0766	7.77	3.78		*
G.cont	0.0025	0.00		8.42	<0.005
G.ind	1.3489	0.28	539.56		***
I.cont	0.48	0.10		-7.45	<0.005
I.ind	0.0296	0.01	0.06		***

L.cont = Liver control; L.ind = liver induced; K.cont = kidney control; K.ind = kidney induced; G.cont = gill control; G.ind = gill induced; I.cont = intestine control; I.ind = intestine induced.

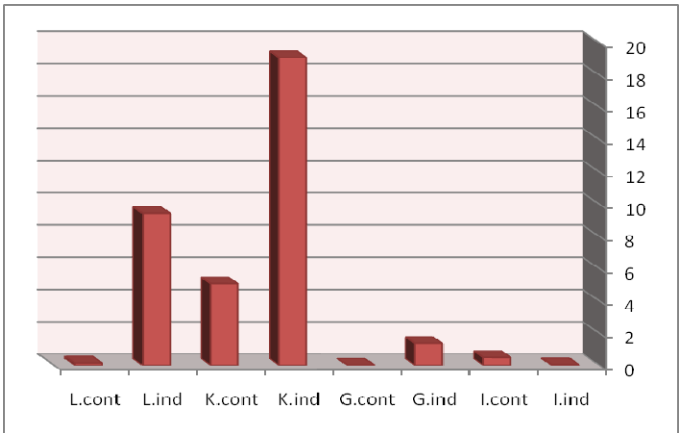

Fig. 4: The expression pattern of CYP1B1 in different organs of BNF-treated Japanese eel compared to the control; L. cont = Liver control; L. ind = liver induced; K. cont = kidney control; K. ind = kidney induced; G. cont = gill control; G. ind = gill induced; I. cont = intestine control; I. ind = intestine induced.

DISCUSSION

The obtained nucleotide sequence of Japanese eel *CYP1B1* contained an open reading frame of 1626 bp coding for 541 amino acids. Carp *CYP1B1* and *-1B2* ORF were found to code for 530 amino acids (El-Kady *et al.,* 2004a, b) while the ORF of tilapia was found to code for only 531 amino acids (Hassanain *et al.,* 2013). The ORF of the other CYP1B1 subfamily members coding for 514, 525, 526, 536, 537 and 541 for Indian medaka, three-spined stickleback fish, zebrafish, catfish, killifish and rainbow trout CYP1B1 respectively.

The phylogenetic tree based on the amino acid sequences of CYP1B of teleost and mammalian species showing the clustering of the newly identified Japanese eel *CYP1B1* sequence with that of rainbow trout sequence in one branch showing a closer relationship to each other apart from other *CYP1Bs*.

In the present study, QRT-PCR results revealed that there was a large increase in CYP1B1 mRNA in gills (539.56 fold), followed by liver (71.93), and kidney (3.78) while the constitutive expression was observed with the induced intestine (0.062). Concerning *CYP1B1* expression levels in other fish species, studies on plaice *CYP1B1* expression reported more restricted tissue expression profile, only being detectable in gill tissue suggesting a role in gas and fluid regulation (Leaver & George, 2000). Carp exposed to 3-MC had *CYP1B1* messenger RNA expression in liver, intestine, and gill, while *CYP1B2* was only induced in the gills (El-Kady *et al.,* 2004a, b). Similarly, BaP-exposed catfish had significant *CYP1B1* mRNA induction in blood, liver, and gonad tissues while the high constitutive expression was detected in gill (Willett *et al.,* 2006). The Constitutive expression of *CYP1B1* was restricted to gills of *Trematomus bernacchii*, an Antarctic fish where it was also induced by BNF (Di Bello *et al.,* 2007). Dorrington *et al.,* (2012) stated that the induction of Brazilian guppy with 3-MC revealed the strong induced expression of *CYP1B1* in the gill. Also the same result was observed with the expression of mRNA of three-spined stickleback fish *(Gasterosteus aculeatus)* that revealed the induction of *CYP1B1* and *CYP1C1* in gills at all concentrations of effluent from drug manufacturing while effects on these genes in liver and brain were weak or absent (Beijer *et al.,* 2013). On the other hand, Hassanain *et al.,* 2013 reported that after intracoelomic injection with BaP, the

expression of *CYP1B1* in Nile tilapia revealed a large increase in *CYP1B1* mRNA in liver (22.8), intestine (2.0) and muscles (1.3). In adult zebrafish, the eye, the brain and the heart are the organs with the highest basal expression of *CYP1B1* (Jönsson *et al.,* 2007b). There is also a high basal expression of *CYP1B1* in zebrafish embryos, possibly related to the development of the eye, brain or heart (Jonsson *et al.,* 2007a).

Studies on human *CYP1B1* revealed its expression constitutively in extrahepatic organs, including fetal tissue samples and is highly inducible in various organs by 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds in experimental animal models (Shimada *et al.,* 1996). *CYP1B1* is also expressed in the eye in mammals, and is essential for normal eye development (Choudhary *et al.,* 2006).

CONCLUSION

This study provides the first description of the molecular characterization of Japanese eel CYP1B1 and the relationship of this newly identified sequence with that of the previously reported CYP1B subfamily members of fish and mammals. Also the higher induction of Japanese eel CYP1B1 mRNA in gills; the tissue in direct contact with the environment, followed by liver and kidney, may have important implication for the possible endogenous functions in these organs.

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ARABIC SUMMARY

التوصيف الجزيئي وقياس التعبير الجينى للسيتوكروم P450 1B1 فى أعضاء ثعبان السمك اليابانى (*Anguilla japonica*)

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تعتبر عائلة السيتوكروم CYP1 ، إحدى العائلات الجينية للفصيلة CYP ، ولها حتى الآن أربع تحت عائلات مودعة في بنك الجينات / EMBL هى: CYP1A، CYP1B، CYP1C ، و CYP1D التي تم التعرف عليها حديثا. تقوم الإنزيمات الناتجة من هذه الجينات بتفعيل التمثيل الغذائى داخل جسم السمك وتخلصه بدرجة كبيرة من الملوثات الكيميائية المختلفة مثل: الهيدروكربونات العطرية متعددة الحلقة، ومركبات ثنائي الفينيل متعدد الكلور، وأريل الأمينات.

لقد تم عزل الحمض النووي التكميلى الجديد لفصيلة CYP1B وسجل تحت إسم CYP1B1 وذلك من كبد ثعبان السمك اليابانى بعد الحقن داخل الغشاء البريتونى بمادة β - Naphthoflavone. كان طول هذا الحمض ٢٩٨٥ قاعدة نيتروجينية، منها ١٦٢٦ قاعدة تمثل منطقة "إيطار القراءة المفتوح" (ORF) والتى تبدأ هنا بكودون البدء ATG وتشفر لـ ٥٤١ حمضا أمينيا وتنتهى بكودون الوقف TAA، وتترجم هذه الأحماض إلى بروتين وزنه الجزيئ ٦١،٢٧ كيلو دالتون. أظهر تسلسل الأحماض الأمينية لبروتين CYP1B1 المستخلص من ثعبان السمك اليابانية ٦٢٪ تشابه مع CYP1B1 لأسماك three-spined stickle back والزرد. كما أظهرت نسبة تشابه بلغت ٦٦٪ مع كل من أسماك الكيلي فيش، الميداكا الهندية وأسماك المبروك الشائع CYP1B1 و1B2 بينما لوحظ أعلى نسبة تشابه للأحماض الأمينية (٦٧ و ٦٩٪) مع أسماك البلطي النيلي CYP1B1 وتراوت قوس قزح على التوالي. كما أظهر هذا الجين المعزول من ثعبان السمك اليابانى CYP1B1 نسبة تشابه مع الجينات المعزولة من الثدييات CYP1Bs كانت ٥٧، 57، 56 ٪ للإنسان، الجرذان والفئران المنزلية على التوالي .

كان تتابع الجين CYP1B1 المعزول من ثعبان السمك اليابانى يتماشى مع جينات عائلة CYP1 وتم إيداعه في بيانات بنك الجينات EMBL تحت رقم AY518340 .أظهرت شجرة النشوء والتطور باستخدام جينات تحت عائلة CYP1B المذكورة سابقا من الثدييات و الأسماك وتبين أن الجين الجديد قريب إلى حد كبير لأسماك تراوت قوس قزح CYP1B1 . وبإجراء تحليلات تفاعل البلمرة المتسلسل الكمى (QRT-PCR) لعينات الكبد والكلى والخياشيم والأمعاء أظهرت الأعضاء الثلاثة الأولى تعبيرا جينيا مستحثا ومتميزا بلغت قيمته ٧١،٩٣ ، ٣،٨٧ و ٥٣٩،٥٦ على التوالي إلا أن الأمعاء لم تتأثر.