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Full Length Research Paper

Cloning and sequence analysis of benzo-a-pyrene-inducible cytochrome P450 1A in Nile tilapia (*Oreochromis niloticus*)

Abeer A. I. Hassanin[1,2], Yoshino Kaminishi[1]*, Mohamed M. M. Osman[2], Zamzam H. Abdel-Wahad[3], Mohamed A. H. El-Kady[1,4] and Takao Itakura[1]

[1]Laboratory of Marine Biotechnology, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan.
[2]Department of Animal Wealth Development, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.
[3]Department of Animal Hygiene, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.
[4]The National Institute of Oceanography and Fisheries, Alexandria, Egypt.

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Polycyclic aromatic hydrocarbons (PAHs), dioxins, dibenzofurans and polychlorinated biphenyls (PCBs) present in polluted environment induce cytochrome P4501A (CYP1A) isozyme in fish which in turn results in a marked increased production of carcinogenic metabolites. The induction of hepatic CYP1A in fish by certain classes of chemicals has been suggested as an early warning system, a "most sensitive biological response" for assessing environmental contamination conditions. This has implications for human fish consumption as well as for the health status of aquatic organisms. Considering the importance of *Oreochromis niloticus* fish as a laboratory animal, the common CYP1A sequence was determined from cDNA and genomic DNA after intraperitoneal injection with benzo-a-pyrene (BaP). The full-length cDNA was 2530 bp long and contained an open reading frame of 1566 bp encoding a protein of 521 amino acids and a stop codon. The sequence exhibited 5' and 3' noncoding regions of 134 and 830 bp, respectively. The deduced amino acid sequence of *O. niloticus* CYP1A shows similarities of 80.5, 79.3, 79.1, 77.8, 77.6, 74.3, 72.4, 77.2, 71.8, 70.7 and 50.8% with European flounder CYP1A, scup CYP1A, killifish CYP1A, butterfly fish CYP1A, European sea bass CYP1A, rainbow trout CYP1A, Japanese eel CYP1A, toad fish CYP1A, European eel CYP1A, red sea bream CYP1A and common carp CYP1A, respectively. The phylogenetic tree based on the amino acid sequences clearly shows tilapia CYP1A and killifish CYP1A to be more closely related to each other than to the other CYP1A subfamilies. Sequence analysis of 3727 bp of genomic DNA showed that the clone obtained was the structural gene of CYP1A which consists of seven exons and six introns, the initiation codon was not found in the first exon but in the second one as was reported for the CYP1A genes of fish and mammals.

Key words: *Oreochromis niloticus*, benzo-a-pyrene, CYP1A gene, sequence analysis, phylogenetic tree.

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are widely distributed in both fresh water and coastal marine ecosystems where they have been found to bioaccumulate in several aquatic species. They also represent one of the most significant classes of organic pollution due to their carcinogenic and mutagenic potentials (Holladay et al., 1998; Barra et al., 2001).

PAH's easily metabolized in fish, and in general their half-life is relatively short (Neff, 1979). In several fish species BaP half-life was found to be less than a day (Niimi and Palazzo, 1986; LeMaire et al., 1992). Therefore it is generally thought that toxicity of BaP is not caused by the parent compound itself, but by the meta-

*Corresponding author. E-mail: kaminisi@fish.kagoshima-u.ac.jp. Tel: 81-99-286-4221. Fax: 81-99-286-4015.

bolites which are formed during biotransformation (Gelboin and Tso, 1978). Toxicity of the parent compound will thus be dependent on the activity of the metabolizing pathways.

Acute toxicity data of BaP are seldom available due to its lipophilic properties (Diekmann and Nagel, 2005). Zheng et al. (2005) found that exposure of *Sebastiscus marmoratus* fish to levels of BaP as high as 200 mg/kg BW for up to 7 days post-exposure had no effect upon the fish survival and no noticeable effects (compared to the vehicle control) upon behavior. This observation coincides with data obtained in fish studies that demonstrated BaP not to be acutely toxic (Carlson et al., 2002).

The fish, as a bioindicator species, plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fish indicates heavy pollution; the effects of exposure to sub lethal levels of pollutants can be measured in terms of biochemical, physiological or histological responses of the fish organism (Mondon et al., 2001).

Biochemical markers are measurable responses to the exposure of an organism to xenobiotics. One of the most intensively studied biomarkers, in both laboratory and field conditions, is cytochrome P450 (Stegeman, 2000).

For detection of pollution in aquatic environments, the CYP1 family members have been so far proved to be the most sensitive indicators (Machala et al., 1995; Machala et al., 1997; Anzenbacherova and Anzenbacher, 1999; Machala et al., 2000; Schlenk and Di Giulio, 2002). They respond to water contamination at levels too low to be detected by other laboratory methods or at the time when the contaminant is no longer dissolved in water but persists in the living matter, such as residues of biocidal agents.

The induction of CYP1A, particularly in fish, by PAHs has been used as a biomarker of exposure since the mid 1970s (Payne and Penrose, 1975). A number of studies have correlated CYP1A induction with biliary PAH metabolites, DNA adducts, immune suppression and tumor formation in wild fish and following laboratory exposures (Collier et al., 1992; Willett et al., 1995; Wirgin and Waldman, 1998; Carlson et al., 2004). More recently a refractory CYP1A phenotype has been noted in fish living in highly PAH- or HAH-contaminated environments (Bello et al., 2001; Meyer et al., 2002; Nacci et al., 2002).

Several complementary DNA sequences have been reported for fish CYP1A (Heilmann et al., 1988; Leaver et al., 1993; Mizukami et al., 1994; Morrison et al., 1995; Vrolijk and Chen, 1995), but genomic DNA sequences are available for only a few fish species (Berndtson and Chen, 1994; Roy et al., 1995).

Subsequently, in this study, cDNA and genomic DNA of the CYP1A gene were isolated from the liver after intraperitoneal injection with benzo-a-pyrene and sequenced. Phylogenetic analysis was also performed to assess the relationship of this newly identified CYP1A gene with other CYP1A family members.

MATERIALS AND METHODS

Treatment of fish

Nile tilapia (*Oreochromis niloticus*) with a mean weight of 500 g were obtained from a local fish farm and were treated with a single intraperitoneal injection of benzo-a-pyrene (100 mg/kg body weight) suspended in corn oil . Simultaneously with the treated fish, control fish of similar mean weight was intraperitoneally injected with an equivalent volume of the vehicle (corn oil). The treated and control fish were killed 24 h after the injection and samples of liver, kidney, gills and intestine were collected, immediately frozen in liquid nitrogen and stored at -80°C.

RNA isolation

Total RNA was isolated from 2 gm of each of the samples of frozen liver, kidney, gills and intestine according to the Standard Acid Guanidinium Thiocyante Phenol Choloroform (AGPC) extraction method (Chomczynski and Sacchi, 1978). Total RNA concentration and purity were determined spectrophotometrically as described by (Sambrook and Russel, 2001) and A_{260}/A_{280} ratios were between 1.7 and 1.9. Poly $(A)^+$ RNA was purified using an Oligotex-dt30 <super> mRNA purification kit (Takara, Japan).

Reverse transcriptase-assisted polymerase chain reaction

Reverse transcription of mRNA was performed with Superscript II reverse transcriptase (Gibco BRL, USA) to generate 5' and 3'-RACE-Ready first strand cDNA using a SMART™ RACE cDNA amplification kit (Clontech, USA) according to the manufacture's protocol.

3' and 5' RACE PCRs for full length cDNA

We designed one sense (F) primer and an antisense (R) primer specific to Tilapia CYP1A for 3' and 5' RACE PCRs respectively. The primers sequences are shown in Table 1. The sense and antisense gene specific primers were used in combination with the universal primer mix included in a RACE PCR kit to generate RACE PCR products.

The cycle conditions for RACE PCR were as follows: denaturation for 2 min and 30 s at 94°C, 60 cycles of denaturation for 30 s at 94°C, annealing for 1 min at 65°C, extension for 3 min at 72°C and a final extension step for 5 min at 72°C. After purification using GFX PCR DNA and a gel band purification kit (GE Health Care, UK), the PCR products obtained were subjected to restriction mapping with various enzymes and subcloned into PT7BlueT- vector (Novagen, USA). Purified plasmids were directly sequenced by dye terminator cycle sequencing using an ABI PRISM dye terminator cycle sequencing kit (PE Biosystemes, USA) and an applied Biosystems 3130 xl DNA sequencer.

Phylogenetic analysis

DNA sequences with the following Genbank accession numbers were retrieved from the database and used in the phylogenetic analysis: U19855 (butterfly fish CYP1A), AB048939 (common carp CYP1A), AB015638 (Japanese eel CYP1A), AF420257 (European eel CYP1A), AJ310693 (European flounder CYP1A), AF026800 (killifish CYP1A), AF015660 (rainbow trout CYP1A), EU107275 (red

Table 1. Oligonucleotide primers used in PCR amplification of tilapia CYP1A cDNA fragments.

Primer	Nucleotide sequence	Nucleotide location
F	5'- TTGGCGAGGTCATTGCACGAAATG	1525 – 1548
R	5'- CCGATCAGAGAAAACAGGCATACGA	1211 – 1235

Table 2. Oligonucleotide primers used in PCR amplification of tilapia genomic CYP1A.

Primer	Nucleotide sequence	Nucleotide location
F1	5'-AGACTTCATCCCCCTTCTTCAGTA	1249 - 1272
R1	5'-TTCTCTCCCTCTACCTTGTTGACC	2693 - 2716
F2	5'-GTGTGAACTCAAAACAACTCTTCCA	13 - 37
R2	5'-CCTGTCGAACTGTTTCATTACCAC	807 - 830

sea bream CYP1A), U14162 (scup CYP1A), U78316 (European sea bass CYP1A), U14161 (toad fish CYP1A). In order to determine homology among CYP1A family cDNAs or deduced amino acid sequences from various species, sequence alignment was performed by the CLUSTAL W method using Laser gene Megalign program (Ver 5.52,2003, DNASTAR Inc).

Isolation of genomic DNA

Genomic DNA was isolated from 5 mg of the liver sample by the Fuji film Quick gene automated nucleic acid system (QuickGene-810) using Fuji film QuickGene DNA tissue kit S (Life Science, Tokyo, Japan) according to the manufacture's protocol. DNA concentration and purity were determined spectrophotometrically as described before.

Ampification of genomic DNA using PCR

We designed two sense (F) and two antisense (R) primers specific for tilapia CYP1A genomic DNA (Table 2) using Tilapia CYP1A cDNA sequence (accession no. FJ389918).The cycle conditions was as follow: denaturation for 2 min and 30 s at 94°C, 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 65°C, extension for 2 min at 72°C. Purification using GFX PCR DNA and a gel band purification kit (GE Health Care, UK) and sequencing were done as previously described. The sequence results identify two genomic clones of approximately 1467 and 817 bp, respectively. The orientation of these genomic DNA fragments was determined by comparing them with tilapia CYP1A cDNA sequence to obtain the complete tilapia CYP1A genomic DNA sequence.

RESULTS

Nucleotide sequence analysis of CYP1A cDNA

The nucleotide sequence (Figure 1) contained a 5' noncoding region of 134 bp, an open reading frame of 1566 bp coding for 521 amino acids and a stop codon, and a 3' noncoding region of 830 bp. The predicted molecular weight was 59.26 KDa. The sequence had one polyadenylation signal (AATAAA) and a poly A tail of 30 nucleotides. This sequence was aligned with the previously mentioned sequences by Clustal W (Thompson et al., 1994) using Lasergene Megalign program, version 5.52, 2003 (DNASTAR Inc) and has been deposited in the GenBank/NCBI data bank with an accession number FJ389918.

Comparison of amino acid sequences

Table 3 shows the percent identities of deduced amino acid sequences of *O. niloticus* CYP1A with the other fish CYP1A genes. The highest identity was 80.5% with European flounder CYP1A, followed by 79.3% with scup CYP1A, 79.1% with killifish CYP1A, 77.8% with butterfly fish CYP1A, 77.6% with European sea bass CYP1A, 74.3% with rainbow trout CYP1A, 72.4% with Japanese eel CYP1A, 77.2% with toad fish CYP1A, 71.8% with European eel CYP1A then ended by 70.7% with red sea bream CYP1A and 50.8% with common carp CYP1A.

Phylogenetic analysis

The phylogenetic tree based on the amino acid sequences were used to assess the relationship of CYP1A of *O. niloticus* with those of other fish species. Figure 2 clearly shows tilapia CYP1A and killifish CYP1A to be more closely related to each other than to the other CYP1A subfamilies.

Nucleotide sequence analysis of genomic CYP1A

Sequence analysis showed that the clone obtained contained the CYP1A structural gene of approximately 3727 bp in length (Figure 3). The gene has been deposited in

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                                                                                                  GGAACATCAAGAGT
GTGAACTCAAAACAACTCTTCCAAACACTTTACATGCACTTTGGAAAAACTACTCCAAGGATCACAGTGCCGTTCCTCCTGCATCAGTTCAACAAAGGTTGAAGCCAGAAAAACGTCATC      -134
ATGGCACTAATGATACTGCCATTCATTGGAGCACTGTCAGTATCACATGTTTTGGTGGCTGTGACAACAGCGTGTCTGGTCTACCTGATTATTAAGAATGCACAAAACAAGATTCCCGAA       120
 M  A  L  M  I  L  P  F  I  G  A  L  S  V  S  H  V  L  V  A  V  T  T  A  C  L  V  Y  L  I  I  K  N  A  Q  N  K  I  P  E       40
GGGCTTCAGCAACTCCCTGGCCCAAAGCCCCTCCCTATCATTGGGAATTTGTTAGAGCTGGGAAAAAGACCCTACCTGAGTCTCACTTCTATGAGCAAACGCTACGGTGACGTCTTCCAG       240
 G  L  Q  Q  L  P  G  P  K  P  L  P  I  I  G  N  L  L  E  L  G  K  R  P  Y  L  S  L  T  S  M  S  K  R  Y  G  D  V  F  Q        80
ATCCAGATTGGCATGCGTCCTGTGGTTGTTTTAAGTGGTAATGAAACAGTTCGACAGGCTCTCATCAAACAAGGGGATGAGTTTGCAGGCAGACCTGACCTGTACAGCTTTCGCTACATC       360
 I  Q  I  G  M  R  P  V  V  V  L  S  G  N  E  T  V  R  Q  A  L  I  K  Q  G  D  E  F  A  G  R  P  D  L  Y  S  F  R  Y  I       120
AATGATGGCAAGAGTCTGTCTTTCAGTACAGACCAAGCTGGCATTTGGCGTGCCCGCAGAAAGCTGGCCTACAGTGCTCTGCGCTCTTTTTCCAACTTAGAGGGCACAACCCCAGAGTAC       480
 N  D  G  K  S  L  S  F  S  T  D  Q  A  G  I  W  R  A  R  R  K  L  A  Y  S  A  L  R  S  F  S  N  L  E  G  T  T  P  E  Y       160
TCATGTGCCCTGGAGGAACATATCAGCAAAGAGGCTGAGTATCTGATCAAAGAGCTCAACACTGTCATGAAGACCAAAGGCAGCTTTGACCCCTTCCGCTACGTCGTTGTCTCTGTTGCC       600
 S  C  A  L  E  E  H  I  S  K  E  A  E  Y  L  I  K  E  L  N  T  V  M  K  T  K  G  S  F  D  P  F  R  Y  V  V  V  S  V  A       200
AATGTCATCTGTGGCACGTGCTTTGGCCGGCGCTATGACCACCACGACGATGAGCTGGTTAGCTTAGTGAACCTCAGTGATGATTTTGTCAAGGTTGTGGGCAGTGGCAACCCAGCAGAC       720
 N  V  I  C  G  T  C  F  G  R  R  Y  D  H  H  D  D  E  L  V  S  L  V  N  L  S  D  D  F  V  K  V  V  G  S  G  N  P  A  D       240
TTCATCCCCCTTCTTCAGTACCTGCCCAGCACAAAAATGAAAAAATTTGTGAGCCTCAATGCTCGCTTCAGCAAGTTTGTTCAAAAGCTTGTCACCGAGCACTATGCCACCTTTGACAAG       840
 F  I  P  L  L  Q  Y  L  P  S  T  K  M  K  K  F  V  S  L  N  A  R  F  S  K  F  V  Q  K  L  V  T  E  H  Y  A  T  F  D  K       280
GACAACATCCGTGACATCACAGACTCCCTCATAGATCACTGCGAGGACAGAAAGCTGGATGAGAATGCAAATATCCAGATGTCAGATGAGAAGATTGTTGGAATCGTCAATGATCTCTTT       960
 D  N  I  R  D  I  T  D  S  L  I  D  H  C  E  D  R  K  L  D  E  N  A  N  I  Q  M  S  D  E  K  I  V  G  I  V  N  D  L  F       320
 GGAGCTGGTTTTGACATCATCTCCACTGCTCTGTCATGGTCACTGATGTACTTTGTGGCTTACCCAGAGATCCAGAACAGGCTTTTTGAAGAAATGAAGGAAAAAGTAGGTCTCGATCGT      1080
 G  A  G  F  D  I  I  S  T  A  L  S  W  S  L  M  Y  F  V  A  Y  P  E  I  Q  N  R  L  F  E  E  M  K  E  K  V  G  L  D  R       360
ATGCCTGTTTTCTCTGATCGGAACAACTTGCCCCTTCTTGAAGCCTACATCCTGGAACTCTTTCGCCATTCTTCATACTTGCCCTTCACAATCCCGCACTGCACCACAAAAGACACATCA      1200
 M  P  V  F  S  D  R  N  N  L  P  L  L  E  A  Y  I  L  E  L  F  R  H  S  S  Y  L  P  F  T  I  P  H  C  T  T  K  D  T  S       400
CTGAATGGCTACTTCATCCCCAAAGACACCTGTGTCTTCATCAATCAGTGGCAGATCAATCATGACCCGGAGCTGTGGGAAGATCCATTTTCCTTCAAGCCAGGACGTTTCCTGAATGCT      1320
 L  N  G  Y  F  I  P  K  D  T  C  V  F  I  N  Q  W  Q  I  N  H  D  P  E  L  W  E  D  P  F  S  F  K  P  G  R  F  L  N  A       440
GATGGCACTGAGGTCAACAAGGTAGAGGGAGAGAAGGTGATGACTTTTGGCTTGGGAAAGCGACGCTGCATTGGCGAGGTCATTGCACGAAATGAACTCTTCCTCTTCTTGGCTATTCTC      1440
 D  G  T  E  V  N  K  V  E  G  E  K  V  M  T  F  G  L  G  K  R  R  C  I  G  E  V  I  A  R  N  E  L  F  L  F  L  A  I  L       480
ATTCAAAAACTAAACTTTCAAGCTTTGCCTGGAGACCAGCTAGACCTGACCCCAGAGTATGGTCTAACAATGAAGCACAAACGCTACCATCTGAGAGCCACAATGCGAGTTAAGAATGAG      1560
 I  Q  K  L  N  F  Q  A  L  P  G  D  Q  L  D  L  T  P  E  Y  G  L  T  M  K  H  K  R  Y  H  L  R  A  T  M  R  V  K  N  E       520
CAGTGAAGTTCCTTATAACGTACAATTTGTAACTTAGTAGGTGATATAAGCTGAAGCTGTAAGGATCAAAGTTAGAATGTAAGACACTGGATGTCAAATTTAGCTTATAGAGCTAATGGC      1680
 Q  *                                                                                                                           521
ATTAAAGCAAATAAGCAGAGTTTGCTTATTGGAGATTCTAAGTGATGTCTAGGTCTGTGTCTCTTGTTCTGTTTTTGGTTAGCTAAGAGATACTTCTGCAAATGGCTGCTGCTTGTCAGT      1800
GAAGTTTAAGGAGTTAGTTTGTTGCTCCTGCTGTATTGTTAGCAGGAATCTAAACTTCTCCCATTCAGAAATGAAAACAAAACAACTGAACTTTGGGACAAACAGTATGTCTTACTGGTT      1920
GGATAGTTTAGGATGCTGCAACCAAACATTTTCTTTGATATGGGACTCTGAAGTGAATAGATTTTACCTTGTGATAACATTTACACATGTAAGCTAATTATTATATTATATTGCATTCCA      2040
TTATGCTGTGGATGTAAGACACACTTAAGCTATATCTGTATCCCAAAATGTGATTTTGAGTGTACACCAAGACTTTTGTATTTCATAATGACATGTTGTCTTTGTTTGTTTGTTTGTTTG      2160
TTTTTGTTTGTTTTTTTTAAGATCATAGCATTTATATTTGTATTAGGGGTTAAAATTGTTTTGCTGTGCAATGATATGACTTATCAAGACAGCATTGCGATCGATTGATTTTGGAATATA      2280
TGTAACACACTTTTATATTTCAGTTGGTTATGTACAAAATGTAAGGGCATGAACCCTTGATACACA<b>AATAAA</b>AATGTATTTTAATGAAAAAAAAAAAAAAAAAAAAAAAAAAAA      2396
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Figure 1. Nucleotide sequence (2530 bp) of cytochrome CYP1A cDNA and its deduced amino acids (521) residues. Consensus sequence for polyadenylation signal is in bold. The stop codon, TGA, is marked with an asterisk.

the GenBank/NCBI data bank with an accession number FJ664151. Comparison of genomic and cDNA sequences for CYP1A identified seven exons and six introns, no difference were found between the tilapia CYP1A cDNA and exons in the tilapia CYP1A genomic DNA. The nucleotide size of the seven exons were 111, 863, 127, 90, 124, 87 and 1098 bp, respectively; while for the six introns were 398, 91, 100, 214, 170 and 254 bp, respectively (Table 4). All the introns begin with the sequence GT and end with AG. The coding region of the CYP1A gene starts in the second exon and ends within the last exon. Comparison of tilapia CYP1A intronic and exonic sequences with those of Japanese eel (AB015744), human (EF094025) and mouse (FJ392393) revealed conservation of intron number, but little similarity in their size and nucleotide sequence, all four species share six introns; however their size differs dramatically (Table 4). Regarding the exonic sequences, the relative length of each of the exons is very similar in all the four species, the length of the first exon (86 - 111 bp) and second one (851 - 904 bp) while the

Table 3. Percent identities of deduced amino acid sequences of fish CYP1A gene subfamilies.

	European flounder	scup	killifish	Butterfly fish	European sea bass	Rainbow trout	Japanese eel	Toad fish	European eel	red sea bream	common carp
O. niloticus	80.5	79.3	79.1	77.8	77.6	74.3	72.4	77.2	71.8	70.7	50.8
European flounder		85.6	79.3	84.1	83.5	79.7	76.4	82.0	76.2	74.9	51.7
scup			78.7	83.9	83.5	82.6	78.7	80.5	78.0	80.8	52.9
killifish				77.8	79.5	75.5	72.6	76.1	72.2	69.0	49.8
butterfly fish					82.2	77.6	76.4	81.0	75.7	73.6	51.0
European sea bass						79.8	76.4	80.4	75.0	72.7	52.2
rainbow trout							79.2	76.7	78.0	72.3	53.5
Japanese eel								74.6	98.1	68.3	51.2
toad fish									73.8	70.3	50.6
European eel										67.2	50.8
red sea bream											45.9

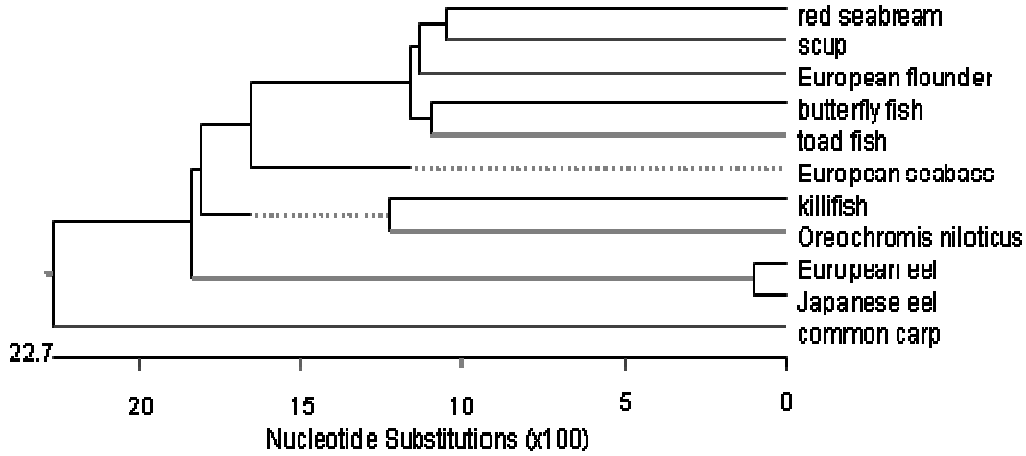

Figure 2. Phylogenetic tree of CYP1A genes using the amino acid sequences of fishes.

length of exons 3 through 6 are almost identical in all the four species (127, 90, 124 and 87 bp, respectively) (Table 4).

DISCUSSION

We cloned cDNA for CYP1A from Bap-treated liver of *O. niloticus* fish; the nucleotide sequence contained a 5' noncoding region of 134 bp, an open reading frame of 1566 bp coding for 521

GGAACATCAAGAGTGTGAACTCAAAACAACTCTTCCAAACACTTTACATGCACTTTGGGAAAACTACTCCAAGGATCACAGTGCCGTTCCTCCTGCATCAGTTCAACAAAGgtaagctta 120

aaacatgcatatacaaaatggcaagaatgcagcagactgatttatagacttactgacttattagctggaagaaaagagaagtgtaaaatggagattatttctgctagaataactttgatg 240

cattacttagtatcttcacctgaatcatagaatgtagtaattaacttatacctatacacataggccactactttgcatagactgcctgtaataagtgctggtgtgcttttaaaaataaaa 360

agccaaatagatacacgcaaatacatgtcacagttctcagttacttcaaactttatagtgaatgttttctacatgttgcgtaacacttatttctctctctctctctcattgtctacactg 480

ctctaatgcacctgctatttaatttacagGTTGAAGCCAGAAAAACGTCATC**ATG**GCACTAATGATACTGCCATTCATTGGAGCACTGTCAGTATCACATGTTTTGGTGGCTGTGACAAC 600

AGCGTGTCTGGTCTACCTGATTATTAAGAATGCACAAAACAAGATTCCCGAAGGGCTTCAGCAACTCCCTGGCCCAAAGCCCCTCCCTATCATTGGGAATTTGTTAGAGCTGGGAAAAAG 720

ACCCTACCTGAGTCTCACTTCTATGAGCAAACGCTACGGTGACGTCTTCCAGATCCAGATTGGCATGCGTCCTGTGGTTGTTTTAAGTGGTAATGAAACAGTTCGACAGGCTCTCATCAA 840

ACAAGGGGATGAGTTTGCAGGCAGACCTGACCTGTACAGCTTTCGCTACATCAATGATGGCAAGAGTCTGTCTTTCAGTACAGACCAAGCTGGCATTTGGCGTGCCCGCAGAAAGCTGGC 960

CTACAGTGCTCTGCGCTCTTTTTCCAACTTAGAGGGCACAACCCCAGAGTACTCATGTGCCCTGGAGGAACATATCAGCAAAGAGGCTGAGTATCTGATCAAAGAGCTCAACACTGTCAT 1080

GAAGACCAAAGGCAGCTTTGACCCCTTCCGCTACGTCGTTGTCTCTGTTGCCAATGTCATCTGTGGCACGTGCTTTGGCCGGCGCTATGACCACCACGACGATGAGCTGGTTAGCTTAGT 1200

GAACCTCAGTGATGATTTTGTCAAGGTTGTGGGCAGTGGCAACCCAGCAGACTTCATCCCCCTTCTTCAGTACCTGCCCAGCACAAAAATGAAAAAATTTGTGAGCCTCAATGCTCGCTT 1320

CAGCAAGTTTGTTCAAAAGCTTGTCACCGAGCACTATGCCACCTTTGACAAGgtacgcctcacactatacaatattatttagctcattgtactttgcagaaattacagacattaatttag 1440

atggtcctttaatctacttttagGACAACATCCGTGACATCACAGACTCCCTCATAGATCACTGCGAGGACAGAAAGCTGGATGAGAATGCAAATATCCAGATGTCAAATGAGAAGATTG 1560

TTGGAATCGTCAATGATCTCTTTGGAGCTGgtaggagaatttttttctttgtgttaagggatataatcataaagcaacataacttgggggggaaaaaagtattctgacagaacttttgtg 1680

tctctcctagGTTTTGACACCATCTCCACTGCTCTGTCATGGTCACTGATGTACTTTGTGGCTTACCCAGAGATCCAGAACAGGCTTTTTGAAGAAATGAgtgcgtatagtttcttttgg 1800

atgtattgtatttctcttgcattatagatttttaataaaagaacaactaaatgataagaaagtgcaactgatcttgttgctctcaaagatgaatggttaactgtaattcggtaatcactg 1920

acagagtcagtaggcacagtgtccatcttcatcttctcatgcgctaatattttgtgatctcttttcattttcagAGGAAAAAGTAGGTCTCGATCGTATGCCTGTTTTCTCTGATCGGAA 2040

CAACTTGCCCCTTCTTGAAGCCTACATCCTGGAACTCTTTCGCCATTCTTCATACTTGCCCTTCACAATCCCGCACTGgtgagattcattcattttgtagtaagaaagtacaaggatata 2160

ggtgttaatgacctgctaagtatcacagaacaacaaaaagatggacacaaaacaaggcttttacagtcataaaaataaatgtaaaaacctgtcattatgcaatgaattgacactttgttg 2280

ttttttagCACCACAAAAGACACATCACTGAATGGCTACTTCATCCCCAAAGACACCTGTGTCTTCATCAATCAGTGGCAGATCAATCATGACCCgtaggtttcctttgctacattttta 2400

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aatagtagtcttcatttccttccccaaatgcatccctgcctagagatttgtaagctttcaagcctgttagtgcaatatttttttattcctttatttttctgaaccttttcagGGAGCTGTGGG 2640

AAGATCCATTTTCCTTCAAGCCAGAACGTTTCCTGAATGCTGATGGCACTGAGGTCAACAAGGTAGAGGGAGAGAAGGTGATGACTTTTGGCTTGGGAAAGCGACGCTGCATTGGCGAGG 2760

TCATTGCACGAAATGAACTCTTCCTCTTCTTGGCTATTCTCATTCAAAAACTAAACTTTCAAGCTTTGCCTGGAGACCAGCTAGACCTGACCCCAGAGTATGGTCTAACAATGAAGCACA 2880

AACGCTACCATCTGAGAGCCACAATGCGAGTTAAGAATGAGCAGTGAAGTTCCTTATAACGTACAATTTGTAACTTAGTAGGTGATATAAGCTGAAGCTGTAAGGATCAAAGTTAGAATG 3000

TAAGACACTGGATGTCAAATTTAGCTTATAGAGCTAATGGCATTAAAGCAAATAAGCAGAGTTTGCTTATTGGAGATTCTAAGTGATGTCTAGGTCTGTGTCTCTTGTTCTGTTTTTGGT 3120

TAGCTAAGAGATACTTCTGCAAATGGCTGCTGCTTGTCAGTGAAGTTTAAGGAGTTAGTTTGTTGCTCCTGCTGTATTGTTAGCAGGAATCTAAACTTCTCCCATTCAGAAATGAAAACA 3240

AAACAACTGAACTTTGGGACAAACAGTATGTCTTACTGGTTGGATAGTTTAGGATGCTGCAACCAAACATTTTCTTTGATATGGGACTCTGAAGTGAATAGATTTTACCTTGTGATAACA 3360

TTTACACATGTAAGCTAATTATTATATTATATTGCATTCCATTATGCTGTGGATGTAAGACACACTTAAGCTATATCTGTATCCCAAAATGTGATTTTGAGTGTACACCAAGACTTTTGT 3480

ATTTCATAATGACATGTTGTCTTTGTTTGTTTGTTTGTTTGTTTTTGTTTGTTTTTTTTAAGATCATAGCATTTATATTTGTATTAGGGGTTAAAATTGTTTTGCTGTGCAATGATATGA 3600

CTTATCAAGACAGCATTGCGATCGATTGATTTTGGAATATATGTAACACACTTTTATATTTCAGTTGGTTATGTACAAAATGTAAGGGCATGAACCCTTGATACACAAATAAAAATGTAT 3720

TTTAATG 3727

Figure 3. Nucleotide sequences (3727 bp) of tilapia genomic cytochrome CYP1A, nucleotides in the exons are represented in upper case letters while nucleotides in the introns are represented in lowercase letters. Initiation codon is boxed.

Table 4. Comparison of CYP1A exons and introns length (bp).

CYP1A exons	Tilapia CYP1A	J.eel CYP1A	Human CYP1A1	Mouse CYP1A1	CYP1A introns	Tilapia CYP1A	J.eel CYP1A	Human CYP1A1	Mouse CYP1A1
Exon 1	111	123	90	86	Intron 1	398	837	2,317	2,380
Exon 2	863	904	851	851	Intron 2	91	108	555	748
Exon 3	127	127	127	127	Intron 3	100	204	87	97
Exon 4	90	90	90	90	Intron 4	214	659	91	83
Exon 5	124	124	124	124	Intron 5	170	308	145	150
Exon 6	87	87	87	87	Intron 6	254	129	192	137
Exon 7	1098	2,020	1,179	1,225					

amino acids, a stop codon and a 3' noncoding region of 830 bp. Itakura et al. (2002) also found that the nucleotide sequence of medaka (*Oryzias latipes*) contained an open reading frame of 1566 bp coding for 521 amino acids and a stop codon while the 5' noncoding region was 141 bp and the 3' noncoding region was 680 bp long. Oh et al. (2008) cloned and sequenced the cytochrome P450 1A (CYP1A) gene from goldfish (*Carassius auratus*); a total of 2537 bp were sequenced and contained a 63 bp 5' untranslated region, a 1578 bp open reading frame (encoding a 526 amino acid protein) and an 893 bp 3' untranslated region. Also Mitsuo et al. (1999) cloned Cytochrome P450 1A (CYP1A) cDNA from eel (*Anguilla japonica*) liver and found that The cDNA contained a 5′ untranslated region of 163 bp, an open reading frame of 1560 bp coding for 519 amino acids, a stop codon, and a 3′ untranslated region of 1730 bp.

Liver CYP1A induction in fish by certain classes of chemicals has been applied extensively as a biomarker in field studies. The fish cytochrome P450 1A (CYP1A) gene has been cloned and sequenced from many organisms for use in assessing contamination in the aquatic environment (Williams et al., 1998; Meyer et al., 2002; Fent, 2003; Moore et al., 2003).

The phylogenetic tree based on the amino acid sequences clearly shows tilapia CYP1A and killifish CYP1A to be more closely related to each other than to other CYP1A subfamilies. Gonzalez (1989) mentioned that two genes (CYP1A1 and CYP1A2) in mammals characterize the CYP1A subfamily. CYP1A1 can activate PAHs such as benzo-a-pyrene to mutagenic compounds, thus its increased synthesis may ultimately result in carcinogenicity. In fish, CYP1A seems to exist as a hybrid protein coded by a gene ancestral to both mammalian CYP1A1 and CYP1A2 forms, and the use of the name CYP1A rather than CYP1A1 has been suggested (Stegeman, 1995).

Tilapia CYP1A structural gene (3727 bp) contained seven exons and six introns. All the introns begin with the sequence GT and end with AG, consistent with the GT/AG rule of exon-intron junction sequences (Padgett et al., 1986). The coding region of the CYP1A gene starts in the second exon and ends within the last exon. The first exon is untranslated as reported for the other members of the CYP1A subfamily (Hines et al., 1985; Kawajiri et al., 1986; Berndtson and Chen, 1994; Sagami et al., 1994; Roy et al., 1995). The conservation of the untranslated first exon observed in fish and mammals may suggest that first exon has an important role in the gene expression of CYP1A. These results agreed with Aoki et al. (1999) who cloned CYP1A gene from Japanese eel and mentioned that it consists of seven exons and six introns in a region approximately 5800 bp in length as well as a 5' upstream region of about 2300 bp. Also, Kim et al., (2004) cloned the cytochrome P450 1A (CYP1A) gene from *Rivulus marmoratus* and the Japanese medaka (*Oryzias latipes*) after screening of both genomic DNA libraries, and sequenced 11,863 and 7,243 bp including all the exons and introns with promoter regions, respectively. The *Rivulus* and the medaka CYP1A gene consisted of seven exons (including non-coding exons) and the accepting and donor sequences of exon/intron boundary were according to the GT/AG rule.

In conclusion, we cloned and sequenced CYP1A cDNA and CYP1A gene from Nile tilapia (*O. niloticus*) after the intraperioteal injection of Bap and found that no difference was observed between the tilapia CYP1A cDNA and exons in the tilapia CYP1A genomic DNA. Although Nile tilapia has been used as sentinel species of aquatic biomonitoring, this is the first report of cloning and sequencing of tilapia Bap-related gene. Therefore, this result offers basic information for further research related to biomarker use of CYP1A in Nile tilapia.

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