Biochimica et Biophysica Acta 1840 (2014) 2813–2828

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

The cytochrome P450 genes of channel catfish: Their involvement in disease defense responses as revealed by meta-analysis of RNA-Seq data sets

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ARTICLE INFO

Article history:
Received 4 December 2013
Received in revised form 18 April 2014
Accepted 22 April 2014
Available online 27 April 2014

Keywords:
Cytochrome P450
CYP
Catfish
Genome
Immunity
Bacterial infection

ABSTRACT

Background: Cytochrome P450s (*CYP*s) encode one of the most diverse enzyme superfamily in nature. They catalyze oxidative reactions of endogenous molecules and exogenous chemicals.
Methods: We identified *CYP*s genes through *in silico* analysis using EST, RNA-Seq and genome databases of channel catfish. Phylogenetic analyses and conserved syntenic analyses were conducted to determine their identities and orthologies. Meta-analysis of RNA-Seq databases was conducted to analyze expression profile of *CYP* genes following bacterial infection.
Results: A full set of 61 *CYP* genes was identified and characterized in channel catfish. Phylogenetic tree and conserved synteny provided strong evidence of their identities and orthology. Lineage-specific gene duplication was evident in a number of clans in channel catfish. *CYP46A1* is missing in the catfish genome as observed with syntenic analysis and RT-PCR analysis. Thirty *CYP*s were found up- or down-regulated in liver, while seven and eight *CYP*s were observed regulated in intestine and gill following bacterial infection.
Conclusion: We systematically identified and characterized a full set of 61 *CYP* genes in channel catfish and studied their expression profiles after bacterial infection. While bacterial challenge altered the expression of large numbers of *CYP* genes, the mechanisms and significance of these changes are not known.
General significance: This work provides an example to systematically study CYP genes in non-model species. Moreover, it provides a basis for further toxicological and physiological studies in channel catfish.

1. Introduction

Cytochrome P450s (*CYP*s), named for their characteristic spectral property of Soret absorption peak at 450 nm when binding with carbon monoxide, constitute a widespread and highly diverse heme-thiolate enzyme superfamily [1–4]. These enzymes catalyze the oxidative reactions, which are not only involved in synthesizing or metabolizing of endogenous molecules, such as arachidonic acid, eicosanoid, retinoic acid, steroid, vitamin D3, bile acid, biogenic amine, prostaglandin and cholesterol, but also engaged in the detoxification processes of exogenous substrates, including pharmaceuticals, and foreign chemicals [5–10]. Moreover, it has been demonstrated that the expression level of many *CYP*s is regulated during the host defense responses in liver, as well as in extrahepatic tissues such as kidney and brain in mice or rats [11–21].

With the application of the next-generation sequencing, rapid progress has been made for expanding *CYP* family from model species to non-model species. At present, thousands of *CYPs* have been identified from all domains of life including Animalia, Plantae, Fungi, Protista, Archaea, Bacteria and even virus [22–33]. All these *CYP*s are classified into clans, families and subfamilies based on sequence similarities (40% amino acid sequence identity rule for membership in a family and 55% amino acid sequence identity rule for membership in a subfamily), phylogenetic relationships and syntenic relationships [23,34–36]. According to these rules, vertebrate *CYP*s could be clustered into 19 families within 10 clans — including *CYP* clan 2 (includes *CYP1*, *CYP2*, *CYP17* and *CYP21* family), *CYP* clan 3 (includes *CYP3* and *CYP5* family), *CYP* clan 4 (includes *CYP4* family), *CYP* clan 7 (includes *CYP7*, *CYP8* and *CYP39* family), *CYP* clan 19 (includes *CYP19* family), *CYP* clan 20 (includes *CYP20* family), *CYP* clan 26 (includes *CYP16* and *CYP26* family), *CYP*

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http://dx.doi.org/10.1016/j.bbagen.2014.04.016
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clan 46 (includes *CYP46* family), *CYP* clan 51 (includes *CYP51* family) and mitochondrial clan (includes *CYP11*, *CYP24* and *CYP27* family) [22,23,35]. Generally, families among *CYP5-51* are involved in endogenous metabolism, whereas *CYP1-3* families and several subfamilies of *CYP4* play an important role in detoxification processes.

Currently, all 19 vertebrate *CYP* families have been identified in teleost fish, including rainbow trout (*Oncorhynchus mykiss*) [37–44], Japanese pufferfish (*Fugu rubripes*) [45,46], zebrafish (*Danio rario*) [47–56], Atlantic salmon (*Salmo salar*) [57–60], European seabass (*Dicentrarchus labrax*) [61–63], largemouth bass (*Micropterus salmoides*) [64], medaka (*Oryzias latipes*) [65–71], common carp (*Cyprinus carpio*) [72–74], mummichog (*Fundulus heteroclitus*) [75–78], three-spined stickleback (*Gasterosteus aculeatus*) [70], gilthead seabream (*Sparus aurata*) [79], fathead minnow (*Pimephales promelas*) [80,81], and half-smooth tongue sole (*Cynoglossus semilaevis*) [82,83]. By far, with the accomplishment of whole genome sequence [84,85], *CYP* genes in Japanese pufferfish [45] and zebrafish [55] have been analyzed at the whole genome level, revealing 54 *CYP*s (update to 61 *CYP*s, Table 3) and 94 *CYP*s are presented in the Japanese pufferfish and zebrafish, respectively.

Several channel catfish *CYP*s have been characterized previously including *CYP1B1*, *CYP2X1*, *CYP11A1*, *CYP17A1*, *CYP19A1* and *CYP19A2* [86–89]. In recent years, following the development of genomic resources of channel catfish [90], particularly the ESTs [91,92], transcriptome sequences generated by RNA-Seq [93–96] and the draft whole genome sequence, the systematic analysis of *CYP* genes in channel catfish genome becomes feasible. Channel catfish (*Ictalurus punctatus*) is the leading aquaculture species in the United States. Its sustainable production is threatened by the widespread ESC and columnaris disease outbreaks caused by the two pathogens: *Edwardsiella ictaluri* and *Flavobacterium columnare*. Here we report the identification of a full set of *CYP* genes, their phylogenetic analysis and syntenic analysis in the channel catfish, and their involvement in response to bacterial infections with *E. ictaluri* and *F. columnare*.

2. Materials and methods

2.1. CYP homologous genes collection and database mining

In order to identify the complete set of *CYP* genes in channel catfish, we collected all the fish *CYP*s (*Anguilla japonica*, *Carassius auratus*, *Carassius carassius*, *C. carpio*, *C. semilaevis*, *D. rario*, *D. labrax*, *F. rubripes*, *F. heteroclitus*, *G. aculeatus*, *Gobiocypris rarus*, *M. salmoides*, *O. mykiss*, *Oreochromis niloticus*, *O. latipes*, *P. promelas*, *Salmo sala*, *S. aurata*, *Squalus acanthias* and *Stenotomus chrysops*) from the database of NCBI (http://www.ncbi.nlm.nih.gov), Ensembl (http://www.ensembl.org) and *CYP* homepage (http://drnelson.uthsc.edu/CytochromeP450.html) [22]. In addition, other *CYP* homologous genes from human, mouse, chicken and frog (*Xenopus laevis* and *Xenopus tropicalis*) were also collected.

More than 1000 collected *CYP*s were first used as query sequences to search against channel catfish EST and RNA-Seq databases. In order to pull all potential *CYP* genes, the cutoff value was set at the level of $1e-5$ such that conserved transcripts are captured initially for further analysis. The retrieved sequences were then translated using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Further, the predicted ORFs were verified by BLASTP against NCBI non-redundant (Nr) protein sequence database. All identified channel catfish *CYP* transcripts and all other query sequences were utilized to search channel catfish draft genome sequence using TBLASTN program. The following steps are the same with above with the exception of applying FGENESH from SoftBerry (http://linux1.softberry.com/berry.phtml) to predict exons and amino acid sequences using genomic sequences.

2.2. Phylogenetic and syntenic analyses

In order to identify channel catfish *CYP*s, all the amino acids from channel catfish and other species were used to conduct phylogenetic analyses. We constructed separate phylogenetic trees for different *CYP* clans since the number of sequences under study was too many to fit a page. Sequence alignment was performed using MUSCLE (multiple sequence comparison by log-expectation) [97]. JTT (Jones–Taylor–Thornton) and gamma distributed rate with invariant sites (G + I) model was proposed by ProtTest3 [98]. Maximum likelihood phylogenetic trees were built with MEGA 5.10 with 1000 bootstrap replications [99].

Shared synteny was searched by examining the conserved co-localization of neighboring genes on scaffold (unpublished data) of channel catfish and zebrafish. Generally, in order to obtain the location information of these channel catfish *CYP*s on scaffolds, all identified *CYP* transcripts together with query sequences from other species were used as queries to blast against channel catfish draft genome sequence. Neighboring genes of the channel catfish *CYP*s were predicted by FGENESH (http://linux1.softberry.com/berry.phtml?topic=fgenesh&group=programs&subgroup=gfind). Neighboring genes of zebrafish were identified from Zv9 database in ENSEMBL.

2.3. Degenerate primers design and touchdown RT-PCR for CYP46

*CYP*46 sequences from human, mouse, rat, pig, cow, frog, Japanese pufferfish, green spotted pufferfish, and zebrafish were first aligned using software MUSCLE. The degenerate primers were designed by CODEHOP (consensus–degenerate hybrid oligonucleotide primers) [100] using the alignment file generated as the input file. Parameters were selected as maximum core degeneracy: 64, target clamp temperature: 60 °C, genetic code: standard, and codon usage: *I. punctatus*. Two sets of degenerate primers were chosen to conduct the reaction with the estimated size of production around 400 bp and 200 bp, respectively (Table 1).

One healthy channel catfish (Marion strain) was sacrificed in this study. Following manufacturer's protocol, total RNA (RNeasy Mini Kit, Qiagen, USA) was extracted from brain. The extracted RNA was quantified using UV-spectrophotometer and then reverse-transcribed using iScript™ cDNA Synthesis Kit (Bio-Rad, USA). Degenerated primers were applied to amplify brain cDNA. PCR amplification was carried out using KAPA HiFi PCR kit (Kapa Biosystems, USA). The 25 μl PCR reaction mixture contained 5.0 μl of 5 × KAPA HiFi Fidelity Buffer, 1 μl of KAPA dNTP Mix (10 mM), 0.5 μl of KAPA HiFi DNA Polymerase, 1 μl (10 pmol/μl) of each primers, 1 μl of template, and 15.5 μl PCR-grade water. Touchdown PCR was carried out on a Bio-Rad PCR system using the cycling conditions: denaturation, 95 °C/4 min, 10 cycles of 95 °C/30 s, 65 °C/30 s (each cycle decrease 1 °C), and 72 °C/30 s, 20 cycles of 95 °C/30 s, 55 °C/30 s and 72 °C/30 s, then, 72 °C 10 min. PCR products were resolved on 1.5% agarose gel.

2.4. Meta-analyses of RNA-Seq data sets

Illumina-based RNA-Seq reads were retrieved from three RNA-Seq data sets: liver samples from catfish experimentally challenged with *E. ictaluri* (3 day and 14 day post infection) (SRA accession number SRP028159) [93], intestine samples from catfish challenged with *E. ictaluri* (3 h, 24 h and 3 day post infection) (SRA accession number SRP009069) [94] and gill samples from catfish challenged with *F. columnare* (4 h, 24 h and 48 h post infection) (SRA accession number SRP012586) [95]. Trimmed high quality RNA-seq reads were mapped onto the deduced channel catfish *CYP*s reference assembly using CLC Genomics Workbench software (version 5.5.2; CLC bio,

Table 1
Degenerate primers used for *CYP*46A1 amplification.

Primer name	Forward primer	Reverse primer
Primer 1	agagggcccagacggarrtngayga	cggggtcccagggagaanggrwarta
Primer 2	gaccttcttcatcgccggncangarac	agccgcagggtctccttnarnacytg

Aarhus, Denmark). Mapping parameters were set as ≥95% of the reads in perfect alignment and ≤2 mismatches. After the total mapped reads number for each transcript was determined, normalization was conducted in order to determine RPKM (reads per kilobase of exon per million mapped reads). The proportions-based Kal's test was performed to identify the differently expressed genes comparing with control sample and fold changes were calculated. Transcripts with absolute fold change value ≥ 2, p-value ≤ 0.05 and total read number ≥ 5 were included in the analyses as differently expressed genes.

3. Results

3.1. Identification of CYP genes in channel catfish

CYP genes collected from various species (as detailed in Materials and Methods) were used as queries to conduct BLAST searches against the channel catfish transcriptome and genome databases. All sequences with significant hits were assembled into unique sequences of 61 *CYP* genes in channel catfish (Table 2). Among all these genes, 40 sequences were identified in both databases with full-length or nearly full-length

Table 2
Cytochrome P450 genes of channel catfish.

Catfish *CYPs*	Transcripts or genome	Sequence integrity	Accession number of NCBI	Linkage group	Reference
*CYP*1A1	Both	Complete	JT412024.1	Not available	This study
*CYP*1B1	Both	Complete	AAY90143.1	6	[92]
*CYP*1C1	Both	Complete	JT408041.1	16	This study
*CYP*2K8	Both	Complete	JT406642.1	3	This study
*CYP*2K17	Both	Complete	JT415766.1	3	This study
*CYP*2M1	Both	Complete	JT414187.1	22	This study
*CYP*2R1	Both	Complete	JT416283.1	21	This study
*CYP*2U1	Both	Complete	JT416818.1	21	This study
*CYP*2X1	Both	Complete	AAG30296.1	9	[93]
*CYP*2X20	Genome	Partial	KF531904	9	This study
*CYP*2X21	Genome	Complete	KF531901	9	This study
*CYP*2X22	Genome	Partial	KF531905	14	This study
*CYP*2Y7	Genome	Partial	KF531905	3	This study
*CYP*2Y8	Genome	Partial	KF531907	3	This study
*CYP*2AA14	Both	Complete	JT411919.1	11	This study
*CYP*2AD6	Genome	Partial	KF531902	28	This study
*CYP*2AD8	Genome	Partial	KF531903	28	This study
*CYP*2AD9	Both	Complete	JT418663.1	28	This study
*CYP*2AD10	Genome	Partial	KF531908	28	This study
*CYP*3A125	Genome	Partial	KF531909	20	This study
*CYP*3A126	Both	Complete	JT418825.1	20	This study
*CYP*3A127	Genome	Partial	KF531910	20	This study
*CYP*3A128	Genome	Partial	KF531911	20	This study
*CYP*3C5	Both	Complete	JT408739.1	28	This study
*CYP*4F60	Both	Complete	JT479785.1	5	This study
*CYP*4F66	Genome	Partial	KF531912	5	This study
*CYP*4T16	Genome	Partial	KF531913	19	This study
*CYP*4T17	Both	Partial	JT320237.1	19	This study
*CYP*4V7	Both	Complete	JT408109.1	9	This study
*CYP*4V8	Both	Complete	JT414161.1	29	This study
*CYP*5A1	Both	Complete	JT316268.1	4	This study
*CYP*7A1	Both	Complete	JT408390.1	1	This study
*CYP*7C1	Both	Complete	JT411121.1	11	This study
*CYP*7D1	Both	Complete	JT415644.1	18	This study
*CYP*8A1a	Both	Complete	JT411628.1	3	This study
*CYP*8A1b	Both	Partial	JT470032.1	7	This study
*CYP*8B5	Both	Complete	JT323293.1	28	This study
*CYP*8B6	Genome	Partial	KF531914	8	This study
*CYP*8B7	Genome	Partial	KF531915	20	This study
*CYP*11A1	Both	Complete	NP_001187241.1	14	Unpublished
*CYP*11C1	Both	Complete	JT399700.1	Not available	This study
*CYP*17A1	Both	Complete	NP_001187242.1	6	Unpublished
*CYP*17A2	Both	Partial	JT364637.1	11	This study
*CYP*19A1	Both	Complete	Q92111.1	6	[94]
*CYP*19A2	Both	Complete	AF417239.1	18	[95]
*CYP*20A1	Both	Complete	JT411689.1	13	This study
*CYP*21A1	Both	Complete	JT407837.1	1	This study
*CYP*24A1	Genome	Partial	KF531916	7	This study
*CYP*26A1	Both	Complete	JT413111.1	5	This study
*CYP*26B1	Both	Complete	JT416269.1	23	This study
*CYP*26C1	Both	Complete	JT411012.1	7	This study
*CYP*27A2	Both	Complete	JT244274.1	13	This study
*CYP*27A3	Genome	Partial	KF531917	Not available	This study
*CYP*27A14	Both	Complete	JT406399.1	17	This study
*CYP*27A15	Genome	Partial	KF531918	17	This study
*CYP*27A16	Genome	Partial	KF531919	17	This study
*CYP*27A17	Genome	Partial	KF531920	17	This study
*CYP*27A18	Genome	Partial	KF531921	17	This study
*CYP*27B1	Both	Complete	JT483829.1	28	This study
*CYP*27C1	Both	Complete	JT408218.1	13	This study
*CYP*51A1	Both	Complete	JT418451.1	28	This study

CYPs, while the remaining 21 genes were identified in the genome database with partial sequences of transcripts in the transcriptome database. All these sequences have been deposited to GenBank with their accession numbers being summarized in Table 2.

Based on the rules described previously for *CYP* genes nomenclature [35], the 61 *CYP* genes can be classified into 9 *CYP* clans (Table 2) including *CYP* clans 2, 3, 4, 7, 19, 20, 26, 51 and mitochondrial clan. These represented all vertebrate CYP clans but clan 46 (*CYP*46) which is absent in channel catfish.

The 61 channel catfish *CYP*s fall into 16 families (Table 2) including *CYP1*, *CYP2*, *CYP3*, *CYP4*, *CYP5*, *CYP7*, *CYP8*, *CYP11*, *CYP17*, *CYP19*, *CYP20*, *CYP21*, *CYP24*, *CYP26*, *CYP27* and *CYP51* families. Three families, *CYP16*, *CYP39* and *CYP46*, of the vertebrate *CYP*s are missing from channel catfish. Of the 16 CYP families in channel catfish, *CYP2*, *CYP3*, *CYP4* and *CYP27* are the four biggest families contributed more than half (32 *CYP*s) of the *CYP* genes in channel catfish.

3.2. Location of CYPs on linkage groups

To better understand the genome distribution of channel catfish *CYP*s, we examined their locations on channel catfish linkage map [101]. Of the 61 channel catfish *CYP*s, genomic locations of 58 *CYP*s can be determined, while genomic locations for three *CYP*s: *CYP1A1*, *CYP11C1*, and *CYP27A3*, cannot yet be determined. The 58 channel catfish *CYP*s were located on 21 linkage groups of the channel catfish genome (Table 2). Among which, linkage group 28 had the largest number of *CYP*s including five family of eight different genes, followed by LG17 and LG20 with five *CYP*s each.

3.3. Specific CYPs and comparative studies

A comparison of *CYP* gene numbers in various species is shown in Table 3. Mammals (human and mouse) had eight specific subfamilies: *CYP2A*, *CYP2B*, *CYP2E*, *CYP2S*, *CYP2T*, *CYP4A*, *CYP4X* and *CYP4Z*; chickens had one specific subfamily, *CYP2H* that is absent from other taxa analyzed. Amphibians have eight specific subfamilies: *CYP2Q*, *CYP2AM*, *CYP2AN*, *CYP2AP*, *CYP2AQ*, *CYP2AR*, *CYP2AS* and *CYP2AT*. A large number of 18 *CYP* subfamilies appeared to be teleost-specific: *CYP2K*, *CYP2M*, *CYP2N*, *CYP2P*, *CYP2V*, *CYP2X*, *CYP2Y*, *CYP2Z*, *CYP2AA*, *CYP2AD*, *CYP2AE*, *CYP3B*, *CYP3C*, *CYP3D*, *CYP7C*, *CYP7D*, *CYP11C*, and *CYP16A* (Table 3). It is noteworthy that these teleost-specific *CYPs* belong to five families, and the vast majority [11] of these belongs to *CYP2* family. Of the 18 teleost-specific subfamilies, 10 were identified from channel catfish (Table 3).

Within the 37 *CYP* subfamilies identified in channel catfish, 13 (*CYP2K*, *CYP2X*, *CYP2Y*, *CYP2AD*, *CYP3A*, *CYP4B*, *CYP4F*, *CYP4T*, *CYP8A*, *CYP8B*, *CYP17A*, *CYP19A* and *CYP27A*) possess multiple copies of *CYP* genes while 24 possess a single copy for each *CYP* gene (Table 3). Among these subfamilies, *CYP27A* subfamily obtained the largest number of *CYP* gene copies of seven (Table 3).

Compared to zebrafish, channel catfish genome appeared to have lost several subfamilies including *CYP2N*, *CYP2P*, *CYP2V*, *CYP2AE*, *CYP39A*, and *CYP46A*. In addition, channel catfish had much fewer copies for *CYP2K*, *CYP2AA*. Zebrafish had 11 copies of *CYP2K* while channel catfish had only 2 copies; similarly, zebrafish had 12 copies of *CYP2AA*, while channel catfish had just a single copy of *CYP2AA* (Table 3).

3.4. Phylogenetic analysis and lineage-specific gene duplication

A total of 61 channel catfish *CYP* genes have been phylogenetically analyzed. First, the 61 genes were categorized into clans by initial phylogenetic analysis. Each clade (clan) then was subsequently analyzed separately due to the enormous size of the phylogenetic tree. As shown in Figs. 1–9, phylogenetic analyses provided clear evidence for the identities of most channel catfish *CYP* genes. Among these, *CYP1A1*, *CYP1B1*, *CYP1C1*, *CYP2K8*, *CYP2K17*, *CYP2M1*, *CYP2R1*, *CYP2U1*, *CYP2AD6*, *CYP4V7*, *CYP4V8*, *CYP5A1*, *CYP7A1*, *CYP7C1*, *CYP7D1*, *CYP11A1*, *CYP11C1*, *CYP17A1*, *CYP17A2*, *CYP19A1*, *CYP19A2*, *CYP20A1*, *CYP21A1*, *CYP24A1*, *CYP26A1*, *CYP26B1*, *CYP26C1*, *CYP27A2*, *CYP27A3*, *CYP27B1*, *CYP27C1* and *CYP51A1* were placed into their corresponding clades containing other teleost equivalents, respectively, with strong bootstrap support.

Lineage-specific gene duplication was evident in a number of clans (clan 2, clan 3, clan 4, clan 7 and mitochondrial clan). For instance, within clan 2, two *CYP2Y* genes (*CYP2Y7* and *CYP2Y8*), four *CYP2X* genes (*CYP2X1*, *CYP2X20*, *CYP2X21* and *CYP2X22*), one *CYP2AA* gene (*CYP2AA14*), and three *CYP2AD* genes (*CYP2AD8*, *CYP2AD9* and *CYP2AD10*) were clustered together; similarly, four *CYP3A* genes (*CYP3A125*, *CYP3A126*, *CYP3A127* and *CYP3A128*), one *CYP3C* gene (*CYP3C5*), two *CYP4F* genes (*CYP4F60* and *CYP4F66*), two *CYP4T* genes (*CYP4T16* and *CYP4T17*), two *CYP8A* genes (*CYP8A1a* and *CYP8A1b*), three copies of *CYP8B* genes (*CYP8B5*, *CYP8B6* and *CYP8B7*) and five *CYP27A* genes (*CYP27A14*, *CYP27A15*, *CYP27A16*, *CYP27A17* and *CYP27A18*) clustered together to form their own clade adjacent to zebrafish counterparts in teleost branch, respectively, suggesting channel catfish lineage-specific gene duplication. Similar lineage-specific gene duplication may have also occurred as many of zebrafish genes were clustered together themselves as well (Figs. 1–4, 9).

3.5. Syntenic analysis and tandem duplication of channel catfish CYPs

Though phylogenetic relationships provide strong support for the identities of most *CYP* genes, syntenic analyses were required to provide additional evidence for orthologies or otherwise the paralogies. Syntenic analyses for a group of *CYP* genes were conducted, including subfamily 2K, subfamily 2X, subfamily 2Y, subfamily 2AA, subfamily 2AD, subfamily 3A, subfamily 3C, subfamily 4F, subfamily 4T, subfamily 8A, subfamily 27A, subfamily 27B and subfamily 27C.

Positions of these channel catfish *CYP*s and their neighbor genes were identified from the draft genome scaffolds. And the genes were also identified from the zebrafish genome. As shown in Figs. 10–14, conserved syntenic blocks were characterized in these subfamilies between channel catfish and zebrafish counterparts. On the one hand, the conserved syntenies provide the strongest evidence for the orthologies, and on the other hand, a number of *CYP* genes were found to be located together in tandem, apparently as a result of tandem gene duplications. For instance, channel catfish *CYP2AA14* shared the conserved neighbor genes with a cluster of eight zebrafish *CYP2AA* genes on chromosome 23; channel catfish *CYP3C5* shared the conserved neighboring genes with three *CYP*3C genes on chromosome 3; channel catfish *CYP27B1* shared the conserved neighboring genes with zebrafish *CYP27B1* on chromosome 11; and channel catfish *CYP27C1* shared the conserved neighboring genes with zebrafish *CYP27C1* on chromosome 6. Tandem *CYP* genes were found within the conserved syntenies, suggesting both lineage-specific gene duplication in channel catfish and zebrafish, and the paralogies among the channel catfish genes and among the zebrafish genes within the conserved syntenic blocks. For instance, four channel catfish *CYP2AD* genes (*CYP2AD6*, *CYP2AD8*, *CYP2AD9* and *CYP2AD10*) were present in tandem in the channel catfish genome within the conserved syntenic region in zebrafish genome that included 11 *CYP*2 genes arranged in tandem arrays (*CYP2J*, five *CYP2P*s, one *CYP2V*, three *CYP2AD*s and one *CYP2N*). Similarly, two channel catfish *CYP2K* genes (*CYP2K8* and *CYP2K17*) corresponded six *CYP2K* genes, all present in tandem arrays in both species; three channel catfish *CYP2X* genes (*CYP2X1*, *CYP2X20* and *CYP2X21*), two channel catfish *CYP2Y* genes (*CYP2Y7* and *CYP2Y8*), four channel catfish *CYP3A* genes (*CYP3A125*, *CYP3A126*, *CYP3A127* and *CYP3A128*), two channel catfish *CYP4F* genes (*CYP4F60* and *CYP4F66*), two channel catfish *CYP4T* genes (*CYP4T16* and *CYP4T17*) and three channel catfish *CYP27* genes (*CYP27A14*, *CYP27A15* and *CYP27A16*) were all present in tandem in the channel catfish genome, corresponding the conserved syntenies of zebrafish

Table 3
Copy number of *CYP*s among human, chicken, frogs, Japanese pufferfish, zebrafish and catfish. Shading indicates lineage-specific *CYP* subfamilies.

CYP450 subfamily	Copy # human	Copy # mouse	Copy # chicken	Copy # western clawed frog	Copy # african clawed frog	Copy # japanese pufferfish	Copy # zebrafish	Copy # catfish
1A	2	2	2	1	2	1	1	1
1B	1	1	1	1	1	1	1	1
1C			1	1	1	2	2	1
1D				1	1		1	
2A	3	4						
2B	1	5						
2C	4	15	1					
2D	1	9	1	7	5			
2E	1	1						
2F	1	1				1		
2G		1[a]						
2H			2					
2I								
2J	1	7	5				1	
2K						3	11	2
2M								1
2N						3	1	
2P						1	6	
2Q				8	5			
2R	1	1	1	1	1	1	1	1
2S	1	1						
2T		1						
2U	1	1	1	1	1	1	1	1
2V							1	
2W	1	1	2					
2X						2	7	4
2Y						2	2	2
2Z						2		
2AA							12	1
2AB		1	5	2	3			
2AC			3	12	6			
2AD						1	3	4
2AE							2	
2AM				6	4			
2AN				5	5			
2AP				1				
2AQ				2	2			
2AR				1				
2AS				1				
2AT				1	1			
3A	4	8	2	6	4	3	1	4
3B						2		
3C							4	1
3D						1		
4A	2	7						
4B	1	1	1					
4F	6	9	1	3	2	1	1	2
4T				4	10	1	1	2
4V	1	1	1	2	2	1	2	2
4X	1	1						
4Z	1							
5A	1	1	1	1	2	1	1	1
7A	1	1	1	1		1	1	1
7B	1	1	1	1				1
7C						1	1	1
7D							1	1
8A	1	1		1		2	1	2
8B	1	1	1	1	1	2	1	3
11A	1	1	1	1	1	1	2	1
11B	2	2		1	1			
11C						1	1	1
16A						1		
17A	1	1	1	1	1	4	2	2
19A	1	1	1	1	1	2	2	2
20A	1	1	1	1	1	1	1	1
21A	1	1	1	1	1	1	1	1
24A	1	1	1			1	1	1
26A	1	1	1	1	2	1	1	1
26B	1	1	1	1	1	1	1	1
26C	1	1	1	1	1	1	1	1
27A	1	1	1	4	3	5	5	7
27B	1	1		1		1	1	1
27C	1		1	1	2	1	1	1
39A	1	1	1	1	1		1	
46A	1	1	1	6	3	1	4	
51A	1	1	1	1	2	1	1	1

[a] CYP2G subfamily is not mammal specific, because it is also found in lizard (belong to tetrapod).

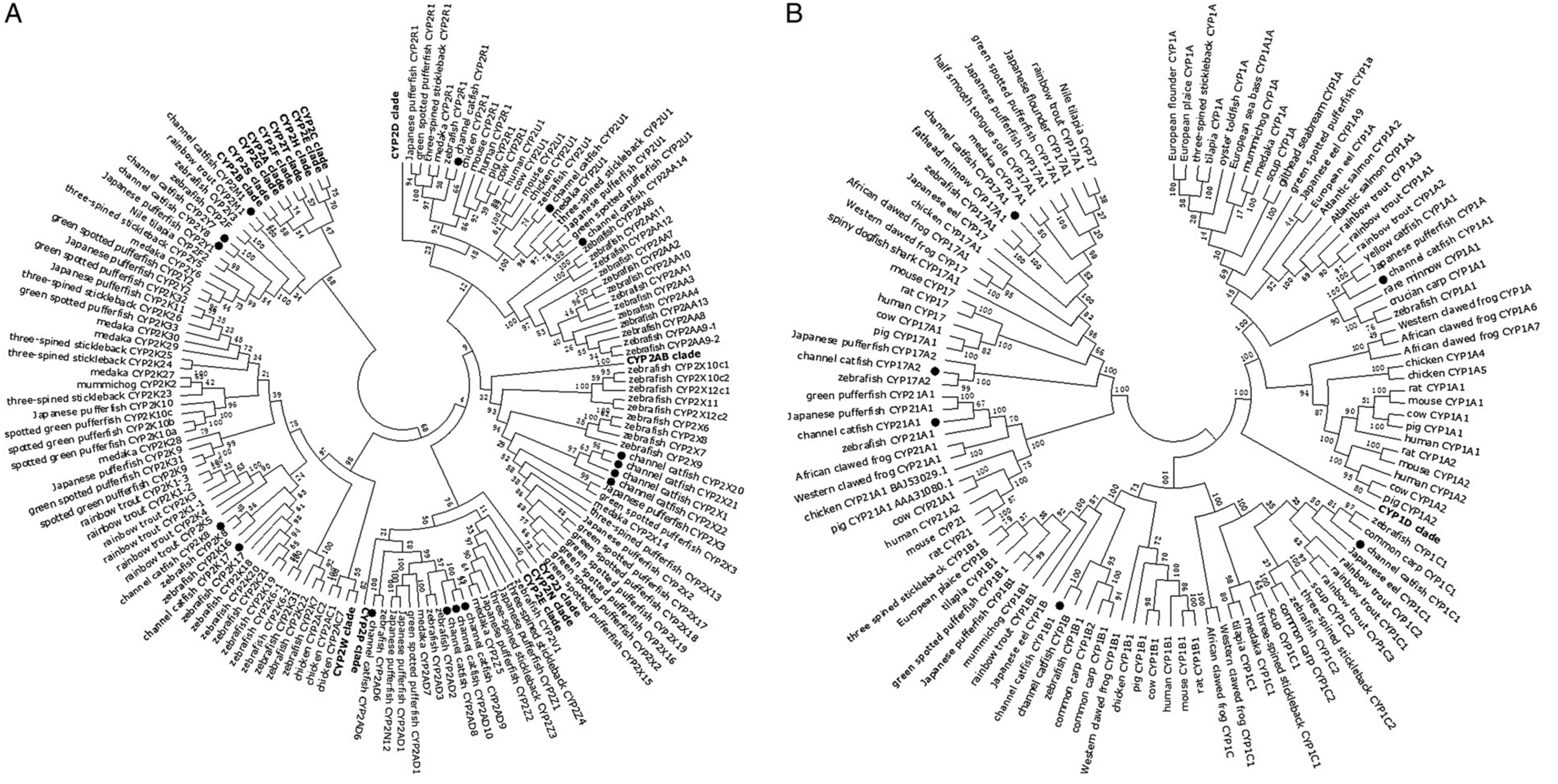

Fig. 1. Phylogenetic relationship of channel catfish *CYP* genes in clan 2. (A) Phylogenetic relationship of channel catfish *CYP*2 subfamily. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values was given next to the branches. (B) Phylogenetic relationship of channel catfish *CYP*1, *CYP*7 and *CYP*21 subfamilies. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values was given next to the branches.

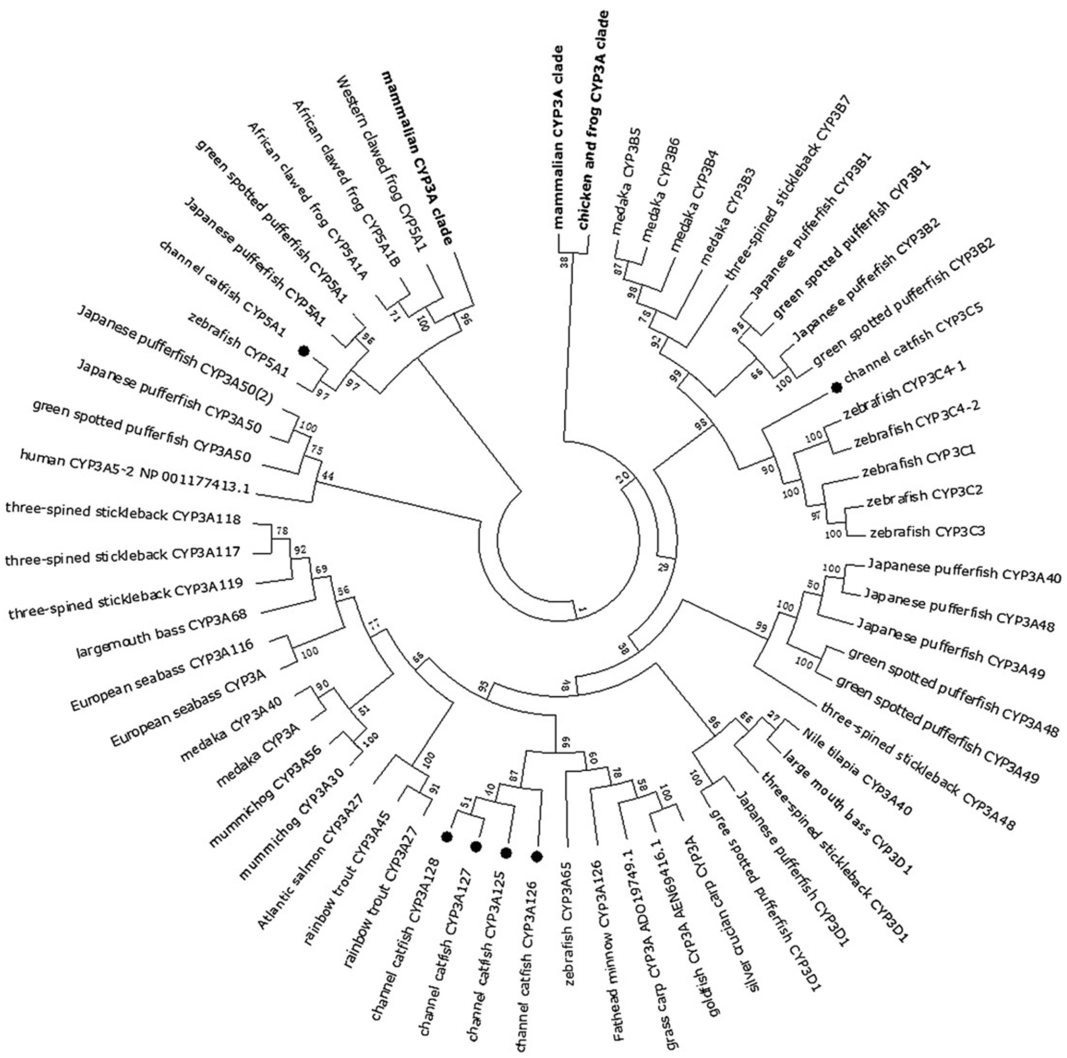

Fig. 2. Phylogenetic relationship of channel catfish *CYP* genes in clan 3. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values was given next to the branches.

genomic regions containing four *CYP2X* genes, two *CYP2Y* genes, *CYP3A65*, *CYP4F3*, *CYP4T8* and four *CYP27A* genes of zebrafish, respectively. In addition, two *CYP8A* genes were identified in the channel catfish genome that had only one counterpart in the zebrafish genome, the *CYP8A1* gene. We annotated the one with identical neighboring genes in channel catfish as *CYP8A1a* gene, and its paralog as *CYP8A1b* in channel catfish because this paralog shared only KCNB1 neighboring gene with zebrafish *CYP*8A1 gene, following the nomenclature of duplicated genes in zebrafish.

3.6. Meta-analyses of CYP expression after bacterial infection using RNA-Seq data sets

Three RNA-Seq data sets, RNA-Seq data from the liver of catfish infected with *E. ictaluri* [93], RNA-Seq data from the intestine of catfish infected with *E. ictaluri* [94], and RNA-Seq data from the gill of catfish infected with *F. columnare* [95], were used in this study. These data sets were analyzed to determine expression profile of all *CYP* genes after bacterial infection, using the cut-off of 2-fold change, p-value < 0.05, and reads number per gene ≥ 5. There were a total of 36 channel catfish *CYP*s which showed significant differential expression for at least one time point post infection in the three tissues compared with control (Table 4).

Several channel catfish *CYP*s exhibited drastic induction or suppression after bacterial infection. As shown in Table 4, 3 days after *E. ictaluri* infection in the liver, drastic up-regulation was found in *CYP8A1b*, *CYP8A1a* and *CYP1C1*. *CYP8A1b* was up-regulated over 30-fold; *CYP8A1a* was up-regulated over 27-fold, while *CYP1C1* was up-regulated 17-fold. In contrast, dramatic down-regulation was observed with *CYP2X22*, *CYP4V7*, *CYP2X21*, *CYP2X20* and *CYP7A1* with 52-fold, 32-fold, 31-fold, 14-fold and 11-fold reduction in expression after bacterial infection, respectively.

The involvement of *CYP* genes in the disease response appeared to be at early stages after infection. As shown in Table 4, numerous *CYP* genes exhibited differential expression 3 days after infection, but at the time point of 14 days after *E. ictaluri* infection in the liver, only two *CYP* genes, *CYP4F60* and *CYP8B7*, were highly up-regulated or down-regulated, with eight-fold up and six-fold down for *CYP4F60* and *CYP8B7*, respectively.

Many *CYP* genes were regulated by bacterial infection in the liver, but only few were regulated in the intestine and the gill (Table 4). Compared with the liver where 30 *CYP* genes were significantly regulated by bacterial infection compared with control, only 7 *CYP* genes were regulated in the intestine, and 8 *CYP* genes were regulated in the gill. Not only the number of *CYP* genes under regulation was much fewer in the intestine and gill, the level of induction and suppression was also much less dramatic. The most highly down-regulated *CYP* gene in the intestine is *CYP*2X1 (down 5.6X), and the most highly down-regulated *CYP* gene in the gill is *CYP*26A1 (down 5.9 X). Apparently, much of this difference was caused by the expression patterns of *CYP* genes, which

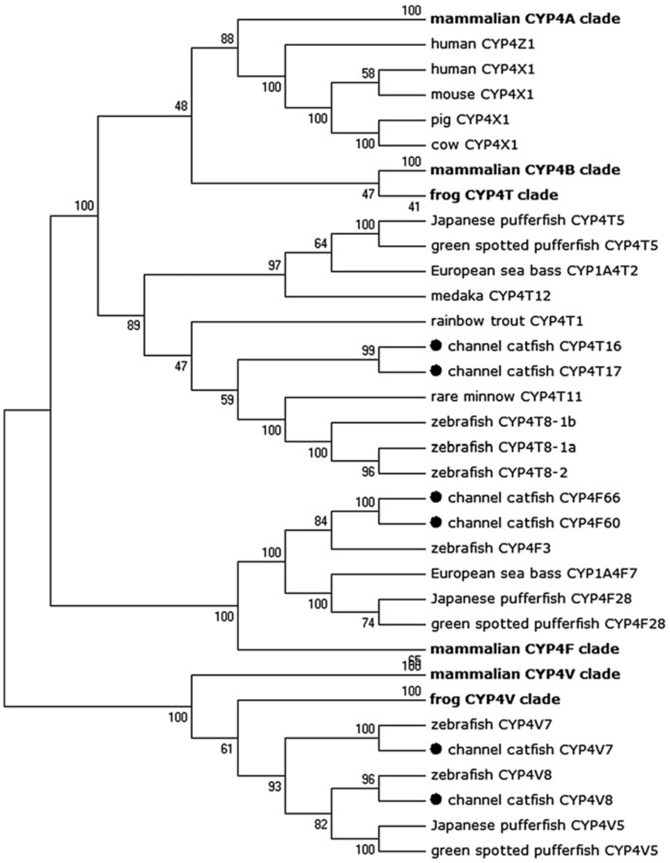

Fig. 3. Phylogenetic relationship of channel catfish *CYP* genes in clan 4. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values was given next to the branches.

are naturally expressed most abundantly in the liver (Table 4). Nonetheless, the use of the high throughput next generation sequencing RNA-Seq allowed detection of *CYP* gene expression in tissues other than the liver. A total of seven *CYP* genes were detected in the intestine: *CYP2K17*, *CYP2R1*, *CYP2X1*, *CYP2AA14*, *CYP4T16*, *CYP26A1* and *CYP51A1*; and a total of eight *CYP* genes were detected in the gill: *CYP2X20*, *CYP2AD6*, *CYP4T16*, *CYP17A1*, *CYP19A1*, *CYP26A1*, *CYP27A17*, and *CYP51A1*. Three of these genes, *CYP4T16*, *CYP26A1*, and *CYP51A1*, were expressed in all three tested tissues.

4. Discussion

CYP genes play key roles in many crucial biological processes including oxidative transformation of xenobiotics and metabolism of endogenous substrates. They belong to one of the most widespread and diverse gene families that consist more than 18,500 members [24] among various species. In spite of their importance, only a few *CYP* genes were characterized from channel catfish [86–89]. Systematic analysis of channel catfish *CYP* genes has been lacking. In this study, we identified 61 catfish *CYP* genes, which may represent the vast majority, if not all, *CYP* genes in the channel catfish genome. This assessment is based on the resources we used for the identification of these genes: several hundred thousands of ESTs [91,101–105], RNA-Seq [93–95], and the draft genome sequences that are yet not published, but represent over 200X genome coverage (unpublished data). This repertoire of genomic resources allowed thorough identification of *CYPs* in channel catfish. However, it is possible that additional *CYP* genes are yet to be discovered. At any rate, we believe that the vast majority of channel catfish *CYPs* in channel catfish have been discovered in this study.

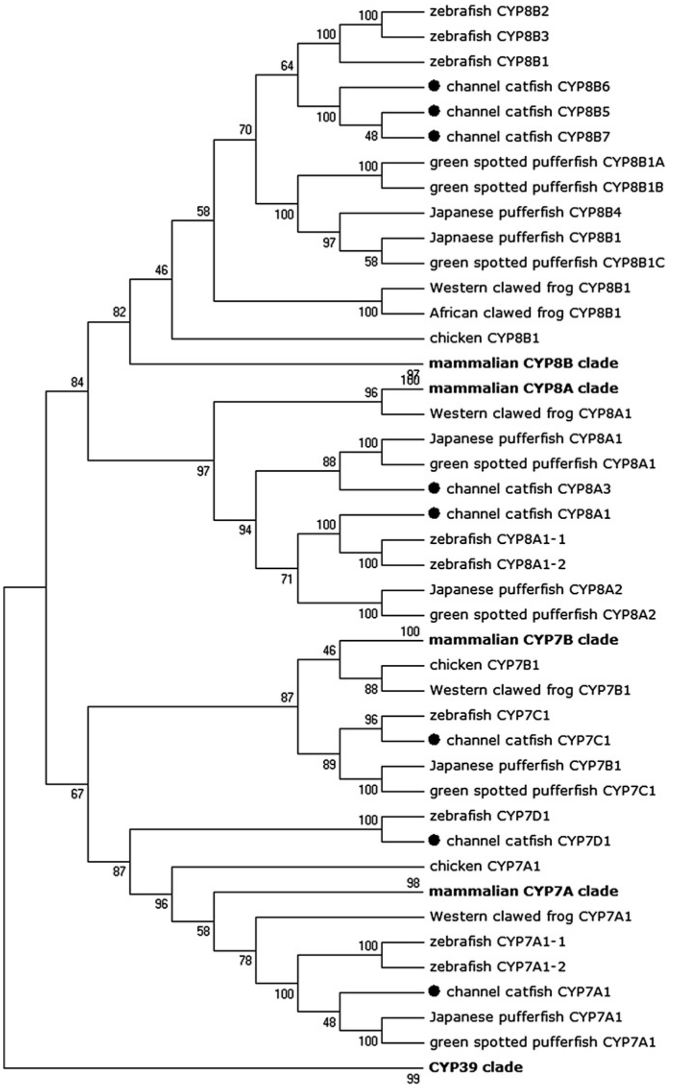

Fig. 4. Phylogenetic relationship of channel catfish *CYP* genes in clan 7. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values was given next to the branches.

Phylogenetic and syntenic analyses allowed annotation of these genes and revealed that many of the *CYP* genes had gone through lineage-specific gene duplications, leading to the presence of a large number of paralogs within a species. Meta-analysis was conducted for the analysis of *CYP* gene expression. To our surprise, a large number of *CYP* genes, 36 in total, were up- or down-regulated after bacterial infection, suggesting their involvement in disease responses. The transcripts of all 61 P450 genes in the channel catfish not only were detected from the liver, where they are believed to be expressed; a significant number of *CYP* genes were expressed in tissues other than the liver, 7 in the intestine and 8 in the gill.

4.1. CYP families 1–4

The significance of *CYP* genes in detoxification of a variety of environmental pollutants, food additives, organic compounds and even drugs in aquatic species has been well established in previous studies [32,55]. This feature is particularly important for channel catfish that inhabits at the bottom of water column. Among all the *CYPs*, the genes in families of *CYP*1, *CYP*2, *CYP*3 and *CYP*4 were reported to be highly involved in metabolism of xenobiotics, drugs and fatty

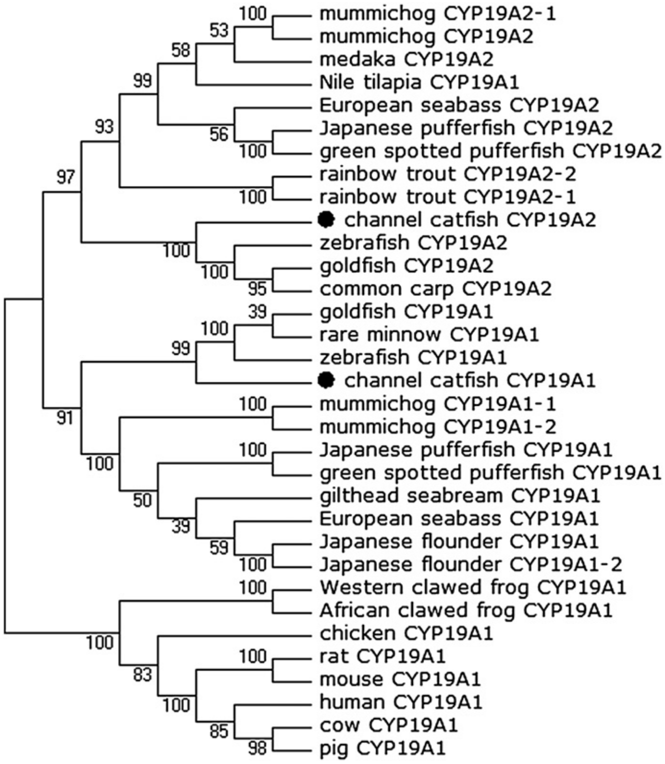

Fig. 5. Phylogenetic relationship of channel catfish *CYP* genes in clan 19. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values was given next to the branches.

acids [55]. Identification of *CYP*s in these four families is critical for understanding of the detoxification mechanism in channel catfish.

In the present study, we identified 30 *CYP* genes belonging to *CYP* families 1, 2, 3 and 4 in channel catfish (Table 2). In contrast to those *CYP*s primarily involved in endogenous metabolism, *CYP* genes among families 1–4 exhibited a larger degree of divergence across species (Table 3). This is strikingly significant in some fish-specific subfamilies belong to *CYP*2 family and *CYP*3 family. For example, gene number differences were observed in *CYP2K*, *CYP2N*, *CYP2P*, *CYP2X*, *CYP2AA*, *CYP2AD*, *CYP3A* and *CYP3C* subfamilies among species (Table 3). This observation indicated that these *CYP* subfamilies which exhibited high level of divergence could be result of individual gene duplication or gene loss, or remnants of the genome duplication

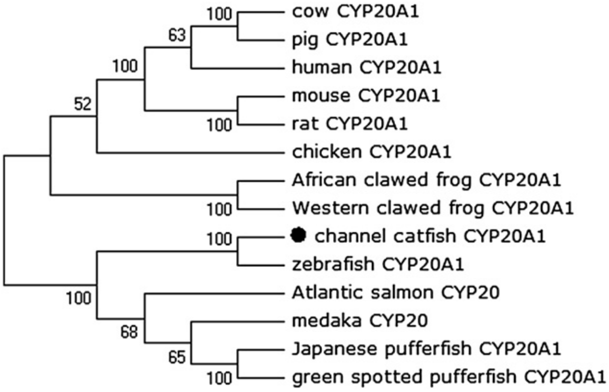

Fig. 6. Phylogenetic relationship of channel catfish *CYP* genes in clan 20. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values was given next to the branches.

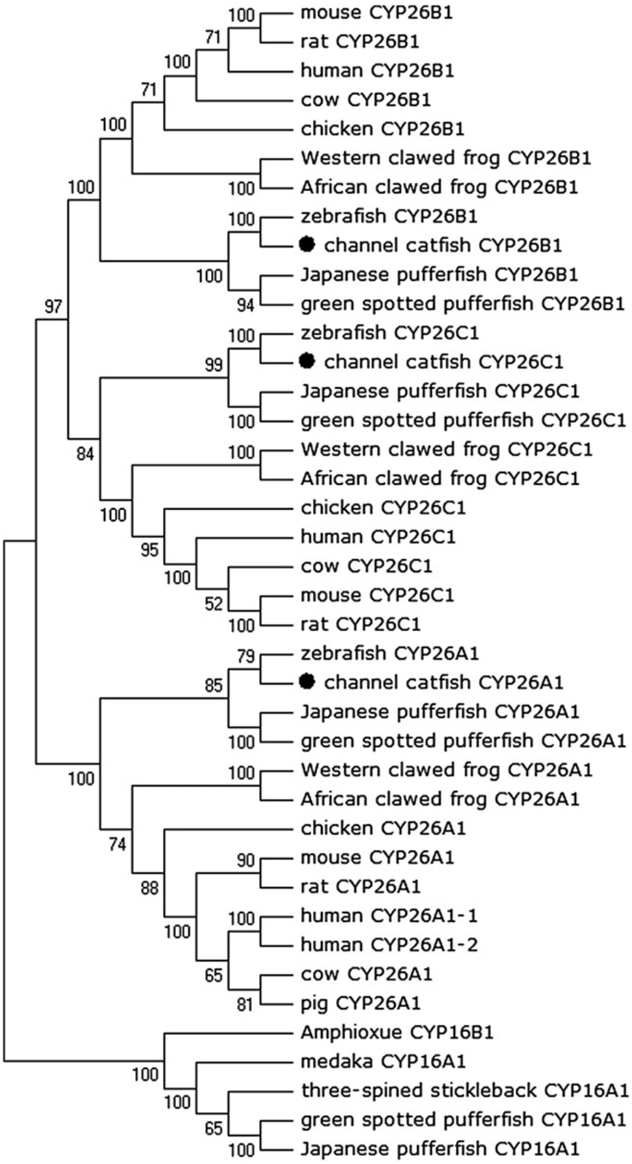

Fig. 7. Phylogenetic relationship of channel catfish *CYP* genes in clan 26. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values was given next to the branches.

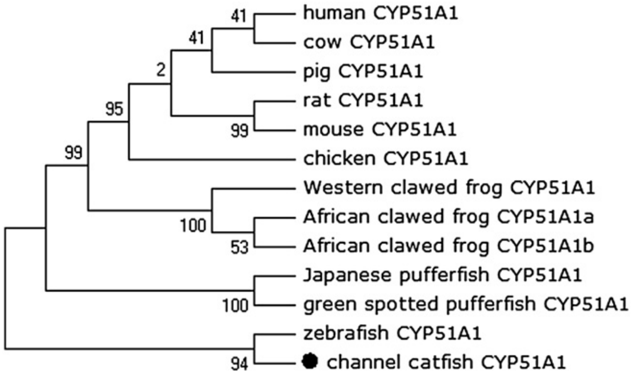

Fig. 8. Phylogenetic relationship of channel catfish *CYP* genes in clan 51. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values were given next to the branches.

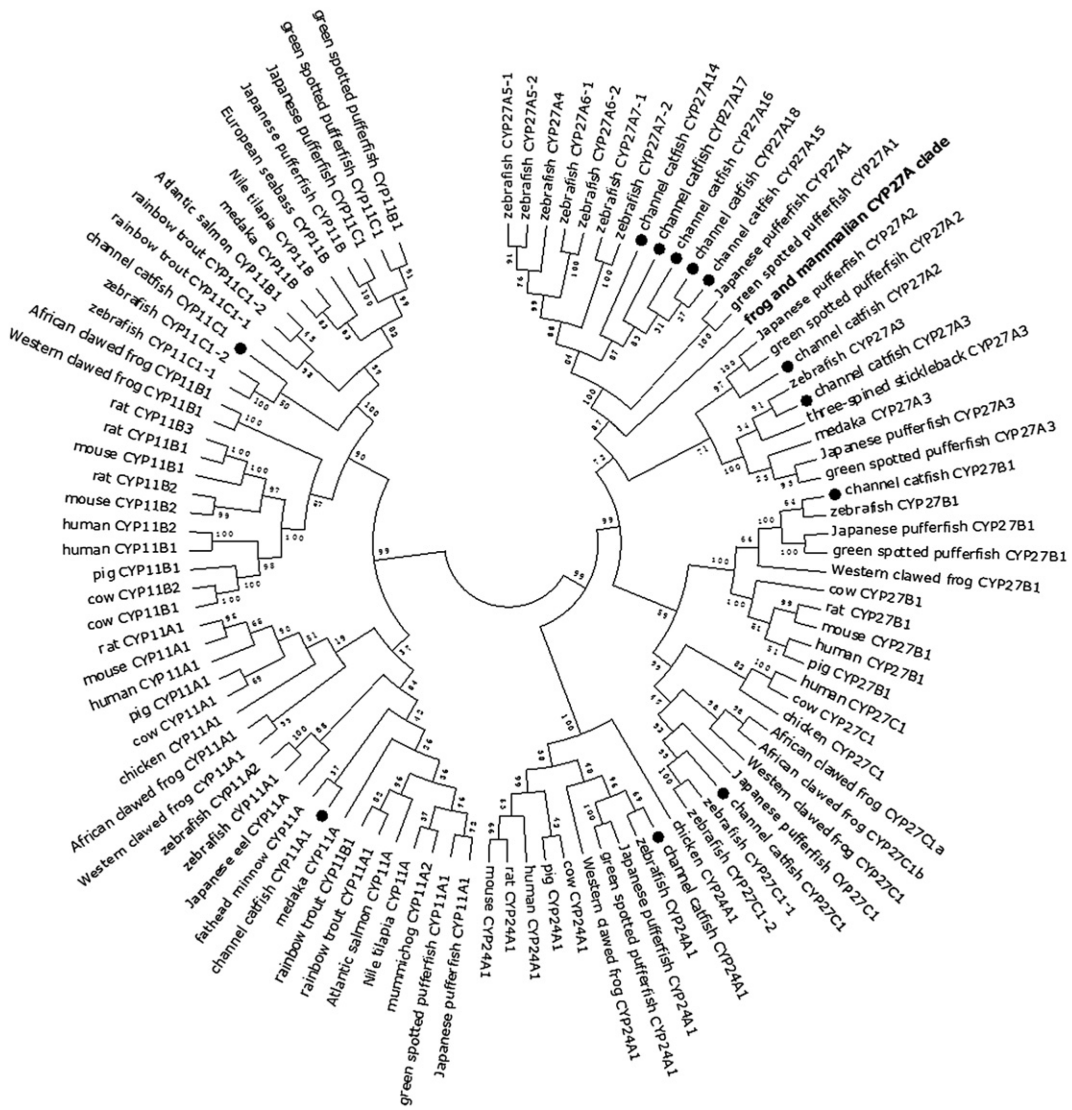

Fig. 9. Phylogenetic relationship of channel catfish *CYP* genes in mitochondria clan. MUSCLE alignments of all the amino acid sequences were used to generate a phylogenetic tree using maximum likelihood method. The tree was shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values was given next to the branches.

[55]. Among these, *CYP2N* and *CYP2P* genes were commonly found in teleost species [45,55,64,107,108] but absent in catfish. In killifish, these two genes have been shown to catalyze benzphetamine N-demethylation and metabolize arachidonic acid [107,108]. In catfish, however, their function could be replaced by other *CYP* genes during evolution. For example, CYP2X1 in channel catfish has been proved to possess benzphetamine demethylase activity [109]. In addition, only subfamilies *CYP*1A, *CYP*1B, *CYP*2U and *CYP*2R appear to be evolutionarily conserved across species. Of these four subfamilies, *CYP*2R1 and *CYP*2U1 catalyze modifications on the endogenous substrates vitamin D and arachidonic acid [101–111], while *CYP*1A and *CYP*1B are induced by a variety of drugs or contaminants [32,112–114].

Phylogenetic analysis and syntenic analysis provided strong support for the identity of the majority of *CYPs* in channel catfish. However, for subfamily *CYP2Y*, *CYP2X*, *CYP2AA*, *CYP2AD*, *CYP3A*, *CYP3C*, *CYP4F* and *CYP4T*, phylogenetic analysis did not yield informative conclusion concerning their identities because members in these subfamilies formed their own clades in the phylogenetic tree, respectively. Consistent with phylogeny, syntenic analyses showed that genes in these *CYP* subfamilies existed as tandem duplication arrays which shared synteny with corresponding gene clusters in zebrafish, indicating that members of these subfamilies could be derived from recent lineage-specific gene duplication events. As such, these tandem arranged *CYP* genes are paralogous to one another within a conserved syntenic block. The high level of lineage-specific multiplication of these *CYP* genes may suggest that the involved organisms were under evolutionary selection for the rapid expansion of such *CYP* genes, perhaps in the face of heavy environmental pollution.

4.2. CYP families 5–51

CYP genes in families 5–51, including *CYP5A1*, *CYP7A1*, *CYP7C1*, *CYP7D1*, *CYP11A1*, *CYP11C1*, *CYP17A1*, *CYP17A2*, *CYP19A1*, *CYP19A2*, *CYP20A1*, *CYP21A1*, *CYP24A1*, *CYP26A1*, *CYP26B1*, *CYP26C1*, *CYP27A2*, *CYP27A3*, *CYP27B1*, *CYP27C1* and *CYP51A1*, are mainly involved in the metabolism of endogenous substrates [32,36]. In contrast to *CYP* gene families 1–4, all *CYP* subfamilies in families 5–51 have single copy gene in channel catfish with the exception of *CYP8A*, *CYP8B*, *CYP17A*, *CYP19A* and *CYP27A*, in which 2, 3, 2, 2 and 7 copies are presented in each of them. Phylogenetic analysis provides clear evidence for majority of *CYP* genes in these families, where they exhibit 1:1 correspondence with teleost counterparts, indicating conservation of enzyme activities and physiological functions.

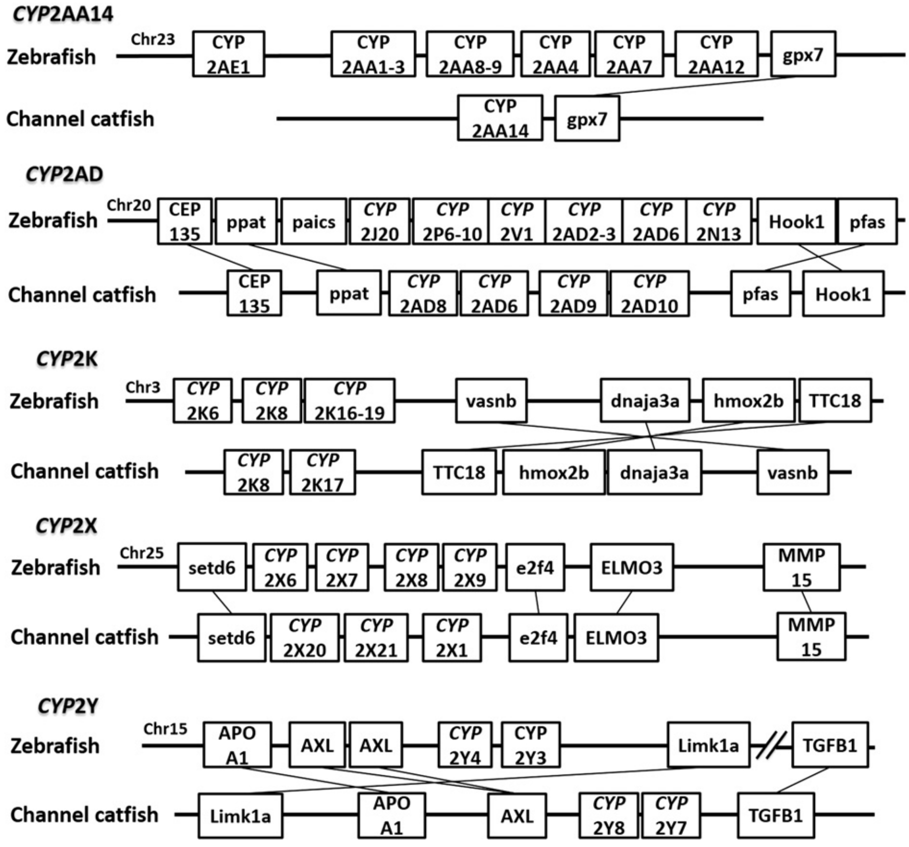

Fig. 10. Syntenic analysis of subfamilies *CYP*2AA, *CYP*2AD, *CYP*2K, *CYP*2X and *CYP*2Y.

Syntenic analysis of *CYP8A* genes indicated that there were two conserved syntenic blocks in channel catfish in comparison with just one in zebrafish. One of them contains five conserved genes and the other one possesses only two conserved genes in the block. We simply named the first one as *CYP8A1*a and the second one as *CYP8A1*b, following the nomenclature rule of zebrafish duplicated genes. Apparently, segmental duplication in the catfish genome accounted for the observed additional copy of the *CYP8A1* gene. It is likely that several genes in the conserved syntenic block of the catfish genome were lost after segmental duplication.

For *CYP*27A subfamily, syntenic analyses were only available for *CYP*27A14, *CYP*27A15 and *CYP*27A16. These three genes showed a conserved syntenic block with zebrafish *CYP*27A cluster. Syntenic analysis of *CYP*8B genes, *CYP*27A17 gene and *CYP*27A18 gene was not available at this stage because of missing information of their neighboring genes. Gap filling in the genomic sequence is required to provide a better resource for syntenic analysis in the future.

CYP46A1 gene in mammals is a cholesterol 24-hydroxylase enzyme and only present in the brain. It has been widely identified among species including teleost fish and plays an essential role in the majority of cholesterol turnover in vertebrate central nervous system [115]. Though the function of *CYP46* gene has not been studied in teleost, mutation of *CYP46A1* gene could lead to serious neurodegenerative disease including multiple sclerosis, Alzheimer and Huntington diseases in human [116]. Four *CYP46A* genes have been identified in zebrafish [55]. However, in channel catfish, no *CYP*46 homolog was found in any databases (EST, RNA-seq and genome sequence) in this study. Syntenic analyses with zebrafish indicated a potential gene loss in the conserved block between ak7a gene and SPTLC2 gene (Fig. 15). We had also tried to amplify this gene in the catfish brain tissue using degenerate primers, in

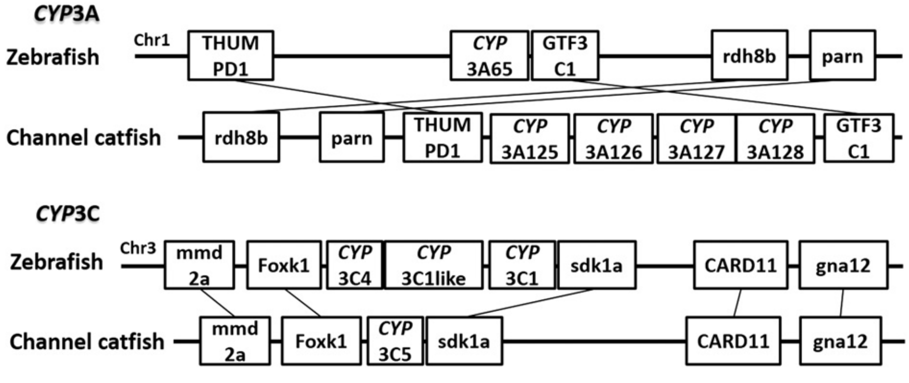

Fig. 11. Syntenic analysis of subfamilies *CYP*3A and *CYP*3C.

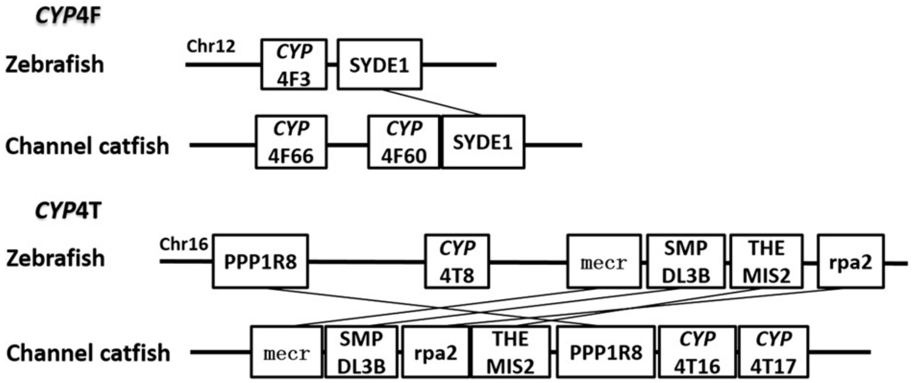

Fig. 12. Syntenic analysis of subfamilies *CYP*4F and *CYP*4T.

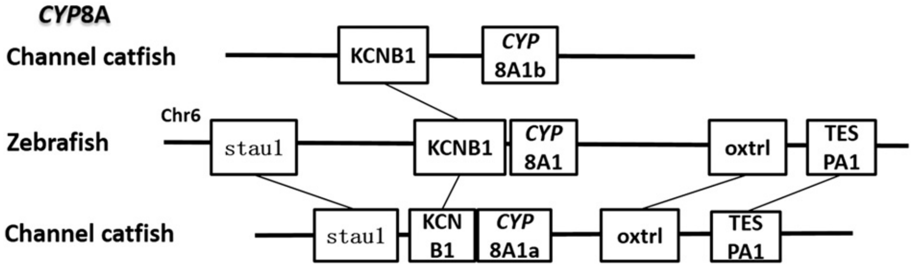

Fig. 13. Syntenic analysis of subfamily *CYP*8A.

case that the gene may have existed, but had not been found. We were unable to amplify the *CYP*46 gene transcripts from the brain (data not shown). Although the negative PCR results still do not exclude the possibility of *CYP*46 presence in channel catfish, it is highly possible that channel catfish has lost the *CYP*46 gene set in its genome. Further analyses are required to validate this speculation. In addition to *CYP*46, the catfish appeared not to possess *CYP*16 and *CYP*39. *CYP*16 was also lost in the zebrafish genome [55], and *CYP*39 was lost in the fugu genome [45].

4.3. CYP gene expression in disease defense response

RNAseq-based expression analysis has become a robust method to assess transcriptional profile to different challenge experiment [117]. As described in our recent studies [93–95], using the 100 bp paired-end reads, we successfully captured comprehensive transcriptomes from catfish intestine and gill after *E. ictaluri* and *Columnare* infection, respectively. The expression patterns of differentially expressed genes from these two studies were validated by quantitative real-time RT-

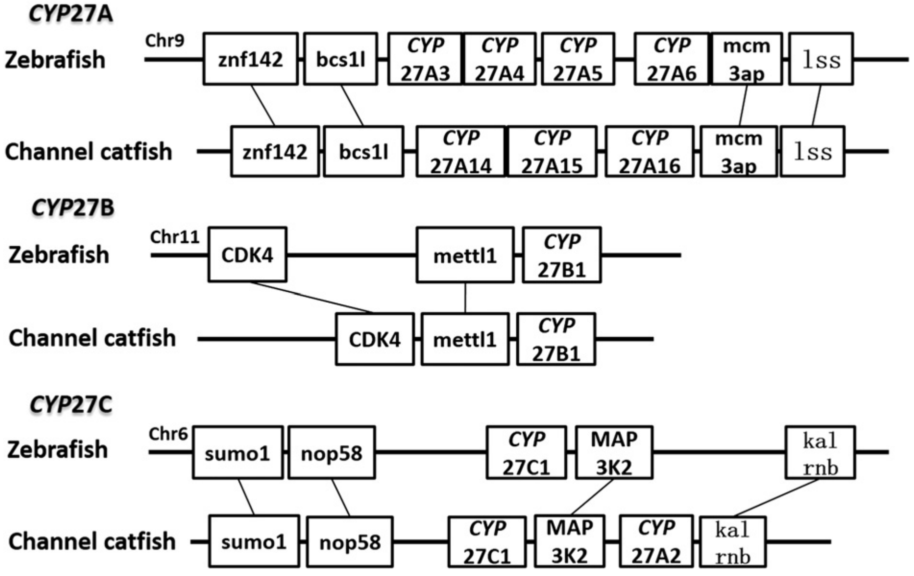

Fig. 14. Syntenic analysis of subfamilies *CYP*27A, *CYP*27B and *CYP*27C.

Table 4
Differentially expressed *CYP*s in channel catfish following *Edwardsiella ictaluri* and *Flavobacterium columnare* infection. Bold values indicate time-points where *CYP* was significantly changed relative to the control and absolute fold change was larger than two. Dash indicates all values of time-points in the experiment were out of threshold (threshold: *p*-value < 0.05, reads number per gene ≥ 5 and fold change ≥ 2).

CYP gene	ESC liver		ESC intestine			Columnaris gill		
	3 days	14 days	3 h	24 h	3 days	4 h	24 h	48 h
*CYP*1C1	**16.84**	1.81	–	–	–	–	–	–
*CYP*2K17	–	–	**2.26**	1.07	1.27	–	–	–
*CYP*2M1	**−2.96**	1.20	–	–	–	–	–	–
*CYP*2R1	**−6.80**	1.10	1.20	**−2.50**	−1.93	–	–	–
*CYP*2X1	−1.07	**−2.31**	**−5.65**	**−3.76**	**−4.66**	–	–	–
*CYP*2X20	**−14.78**	−1.85	–	–	–	−1.43	**−2.73**	**−2.57**
*CYP*2X21	**−31.69**	**−2.01**	–	–	–	–	–	–
*CYP*2X22	**−52.52**	1.08	–	–	–	–	–	–
*CYP*2Y8	**−2.56**	−1.00	–	–	–	–	–	–
*CYP*2AA14	–	–	−1.26	−1.90	**−2.23**	–	–	–
*CYP*2AD6	–	–	–	–	–	1.83	1.07	**2.88**
*CYP*2AD8	**2.32**	−1.02	–	–	–	–	–	–
*CYP*3A128	**3.26**	**2.03**	–	–	–	–	–	–
*CYP*3C5	**5.93**	1.58	–	–	–	–	–	–
*CYP*4F60	**5.29**	**7.98**	–	–	–	–	–	–
*CYP*4F66	**2.17**	1.71	–	–	–	–	–	–
*CYP*4T16	**2.41**	−1.44	**2.75**	**2.76**	**2.58**	1.65	1.98	**3.25**
*CYP*4V7	**−32.48**	−1.06	–	–	–	–	–	–
*CYP*7A1	**−11.72**	−1.78	–	–	–	–	–	–
*CYP*7D1	**10.19**	**2.09**	–	–	–	–	–	–
*CYP*8A1a	**27.60**	**2.29**	–	–	–	–	–	–
*CYP*8A1b	**30.23**	1.67	–	–	–	–	–	–
*CYP*8B5	**2.05**	1.41	–	–	–	–	–	–
*CYP*8B6	**2.14**	1.39	–	–	–	–	–	–
*CYP*8B7	**−4.68**	**−6.30**	–	–	–	–	–	–
*CYP*11A1	**2.72**	1.19	–	–	–	–	–	–
*CYP*17A1	–	–	–	–	–	−1.25	**−3.74**	**−3.39**
*CYP*19A1	–	–	–	–	–	1.88	**2.06**	1.86
*CYP*21A1	**3.35**	−1.04	–	–	–	–	–	–
*CYP*24A1	**−6.78**	**−2.15**	–	–	–	–	–	–
*CYP*26A1	**−2.87**	−1.09	1.16	**−2.67**	−1.24	−1.29	**−5.92**	**−2.90**
*CYP*27A2	**2.17**	**2.01**	–	–	–	–	–	–
*CYP*27A3	**−6.63**	1.06	–	–	–	–	–	–
*CYP*27A14	**−2.59**	−1.00	–	–	–	–	–	–
*CYP*27A17	–	–	–	–	–	**−3.93**	−1.08	−1.31
*CYP*51A1	**7.28**	**2.96**	−1.21	**−2.33**	**−2.27**	1.22	**−2.18**	**−2.13**

PCR with average correlation coefficient around 0.9 ($p < 0.001$). In the present work, these two data sets from RNA-Seq of the intestine and gill after *E. ictaluri* and *Columnare* infection, along with data set from RNA-Seq of the liver after ESC challenge, were utilized to analyze expression profiles of *CYP* genes in channel catfish after infection.

The expression patterns of *CYP* genes following *E. ictaluri* infection in the liver exhibited drastic differences as compared to the control. Up-regulation was observed 3 days after infection for a large number of *CYP*s including *CYP1C1*, *CYP2AD8, CYP3A128*, *CYP3C5*, *CYP4F60*, *CYP4F66*, *CYP4T16*, *CYP7D1*, *CYP8A1a*, *CYP8A1b*, *CYP8B5*, *CYP8B6*, *CYP11A1*, *CYP21A1*, *CYP27A2* and *CYP51A1*. This is the first report involving induction of this large number of *CYP* genes after infection. In a study conducted by Sewer et al. [14], mRNAs of all three of the *CYP*4A subfamily members (*CYP*4A1, *CYP*4A2 and *CYP*4A3) were found to be induced 2- to 6-fold in the F344 rat livers after LPS administration. Later [15], the same group observed that this induction was not unique to LPS, *CYP*4A mRNA expression levels were also induced by irritants such as SiO_2 and $BaSO_4$. Though the mechanism behind this is not clear, it is possible that these *CYP*s are involved in the synthesis of mediators of inflammation cascade. Although the detailed mechanism for the involvement of such a large number of *CYP* genes in defense responses after infection is unknown at present, the disease induced expression suggested that they are important mediators in defense. Additional research is warranted to explore how the *CYP* genes are involved.

Down-regulation of *CYP* genes was observed 3 days after infection in liver with *CYP*2M1, *CYP*2R1, *CYP*2X1, *CYP*2X20, *CYP*2X21, *CYP*2X22, *CYP*2Y8, *CYP*4V7, *CYP*7A1, *CYP*8B7, *CYP*24A1, *CYP*26A1, *CYP*27A3 and *CYP*27A14. Similar observation has been reported by Chaluvadi et al., who demonstrated that infection of mice with enteropathogenic bacterium *Citrobacter rodentium* could cause selective down-regulation of hepatic cytochrome P450 mRNA and protein levels [118]. Cui et al. also found that *CYP*4F4 and *CYP*4F5 were suppressed at early stage but induced after 24 h of LPS treatment, and reached the highest levels at 2 weeks post-injury [18]. In addition, Renton and Nicholson reported that *CYP*s were down-regulated after LPS treatment in the brain of rats [16]. In all these cases, it is hypothesized that the pattern of down-regulation *CYP*s is a pathophysiological consequence of inflammatory process, and regulated by inflammatory mediators, such as cytokines. Similar study has been reported by Morgan [11], who found that down-regulation in enzyme activities of *CYP*1A1, *CYP*2C11, *CYP*2C12, *CYP*2E1 and *CYP*3A2 was triggered by inflammation mediators including cytokines IL-1, IL-6, or tumor necrosis factor (TNF) in liver of rats. Though the mechanisms under this down-regulation of *CYP* genes have not been clearly established, several hypotheses have been proposed by Morgan [12]: firstly, the down-regulation of *CYP* genes may be associated with oxidative damage in liver; secondly, *CYP* genes down-regulation during an inflammatory response could be related to their function in metabolism of arachidonic acid; lastly, the reason for down-regulation of CYP enzymes in the liver could be related to their ability to form nitric oxide. Further work is needed to elucidate the role of *CYP*s in the inflammation response, and particularly after the bacterial infection.

5. Conclusion

In summary, we systematically identified and characterized a repertoire of 61 *CYP* genes in channel catfish and studied their expression profile after bacterial infection. While bacterial challenge altered the expression of large numbers of *CYP* genes, the mechanisms and significance of these changes are not known.

Acknowledgements

We thank Alabama Supercomputer Center for providing the computer capacity for the bioinformatic analysis. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2009-2009-35205-05101, 2010-65205-20356 and 2012-67015-19410 from the USDA National Institute of Food and Agriculture (NIFA).

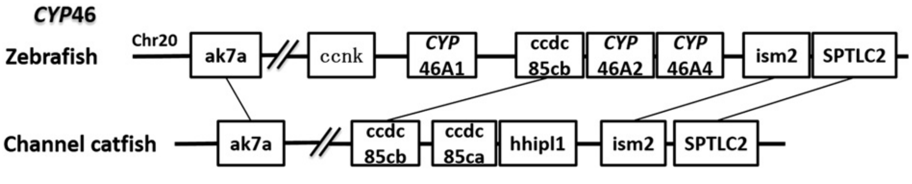

Fig. 15. Syntenic analysis of *CYP*46.

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