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ORIGINAL ARTICLE

Identification and characterization of the myeloid differentiation factor 88 gene in yellow catfish

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Abstract

Myeloid differentiation factor 88 (MyD88) is an important adapter protein of the innate immune system, but it has never before been reported in yellow catfish (*Pelteobagrus fulvidraco*). In this study, we cloned and characterized the yellow catfish *MyD88* gene. The gene was 1230 bp in length and contained an 876-bp open reading frame which encodes a polypeptide of 291 amino acid residues. The theoretical molecular mass and isoelectric point of this polypeptide were 33.4341 kDa and 5.17, respectively. Furthermore, bioinformatic and phylogenetic analyses grouped yellow catfish *MyD88* with *MyD88* of other fish. We found that the deduced amino acid sequence showed that the conserved N-terminal death domain and the C-terminal typical Toll/interleukin-1 receptor domain were very similar to those of other fish. Moreover, reverse transcription PCR showed that yellow catfish *MyD88* is ubiquitously expressed in all tissues examined, with highest expression levels observed in the spleen and lowest levels in the intestine. Importantly, *MyD88* was shown to be significantly up-regulated in the intestines after 30-day dietary supplement of *Clostridium butyricum*. Collectively, these results indicate that yellow catfish MyD88 has a conserved structure and is probably an important component of innate immunity in yellow catfish. This study is the first to identify and characterize *MyD88* in yellow catfish, thereby providing a reference for further research into the yellow catfish innate immune system.

Keywords Yellow catfish · MyD88 · TLR · *Clostridium butyricum* · Intestine · Innate immunity

Introduction

Myeloid differentiation factor 88 (MyD88), a crucial component of innate immunity, is a major downstream adaptor protein of Toll-like receptors (TLRs). MyD88 protein contains an N-terminal death domain (DD), an intermediate domain, and a C-terminal Toll/interleukin receptor (TIR) domain (Neill 2003). Both DD and TIR domains are the decisive functional domains of MyD88 (Li et al. 2005; Loiarro et al. 2009).

TLRs are a family of transmembrane receptors that can recognize various pathogen-associated molecular patterns such as peptidoglycans, double-stranded viral RNAs, and lipoproteins (Akira 2003; Brownlie and Allan 2011; Hawlisch and Köhl 2006). Responding to invading pathogen signals, MyD88 is first recruited to activate TLRs then binds to their TIR domain. MyD88 and interleukin-1 receptor-associated kinases (IRAKs) bind together using their DDs (Honda and Taniguchi 2006; Neumann et al. 2007). This initiates a signaling cascade which activates nuclear factor (NF)-κB and mitogen-activated protein and triggers the transcription of multiple immune effectors (Wang et al. 2014; Wesche et al. 1997). In the absence of MyD88, TLRs is not able to regulate downstream factors such as interferon regulatory factor-3 and interferon (IFN)-β (Fischer et al. 2010; Hoshino et al. 2002; Siednienko et al. 2011).

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Yellow catfish (*Pelteobagrus fulvidraco*), a fish species native to the Asia-Pacific region that is widely farmed in China, is well known for its high nutritive and commercial values. In 2012, the production of yellow catfish in China reached 256–650 tons with a net value exceeding 300 million USD (Zhu et al. 2014, 2015). However, pathogens and disease pose a big threat to yellow catfish health during the artificial feeding and breeding process. Consequently, molecular immunity studies are very important to help identify ways to overcome these problems, but genomic data are lacking so target gene sequences are often unavailable from the current databases.

The *MyD88* gene and its information have been known in few aquatic species, such as zebrafish, common carp, and Japanese flounder (Am et al. 2006; Kongchum et al. 2011; Takano et al. 2006), but basic information of yellow catfish *MyD88* has not yet been reported. In this study, we cloned and analyzed the full-length cDNA of yellow catfish *MyD88* for the first time. Furthermore, we evaluated its tissue expres*sion* patterns by reverse transcription (RT)-PCR, and determined that dietary supplementation of *Clostridium butyricum i*nduces *MyD88* expression in the intestine of yellow catfish.

Materials and methods

Materials

Yellow catfish were gained from Nanchang Academy of Agricultural Science aquatic farm. A total of 180 15-day-old healthy yellow catfish were picked out and randomly divided into two groups which received the basal diet either with or without 2×10^8 colony-forming units/g of Clostridium butyricum. Each group allowed for three replicates of the treatment, with 30 fishes per replicate. On day 45, two fish per cage were killed and samples of tissue were collected, including heart, liver, spleen, brain, gills, intestine, kidney, and muscle. These were snap-frozen in liquid nitrogen, then stored at − 80 °C until required for RNA isolation. All protocols used in this study were approved by the animal ethics committee of S20153354 (MS-222 was used for fish anesthetization).

RNA extraction, reverse transcription, and real-time PCR

For the extraction of total RNA, approximately 100 mg of tissue was homogenized in 1 ml of TRIzol (Takara, Japan) and processed for extraction according to the manufacturer's instructions. Total RNA was reverse transcribed using the PrimeScript® RT reagent kit with gDNA eraser (Takara Bio Inc., Dalian, China). Real-time PCR analysis was performed to examine the expression of different genes, using primers designed with Primer 5 software and synthesized by TaKaRa (Table 1). Real-time fluorescent measurements were conducted on the iQ5 real-time PCR detection system (Bio-Rad, USA). A tenfold dilution series of cDNA was included in each run to construct a relative standard curve to determine the PCR efficiency. All experiments also contained a negative control and samples were analyzed in independent runs. Real-time PCR data were analyzed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) to calculate the relative level of mRNA in each sample, which was expressed as a ratio relative to the 18S gene.

Cloning the cDNA of yellow catfish MyD88

Based on conserved sequences, two degenerate primers were chosen to clone the middle fragment of yellow catfish *MyD88* cDNA (Table 1). The missing 5′ and 3′ ends were obtained by rapid amplification of cDNA ends (RACE)

Table 1 The chemical composition of MyD88 cloned fragment

Amino acid	Number count	% by frequency	% by weight	Amino acid	Number count	% by frequency	% by weight
Ala (A)	15	5.2	3.46	Lys (K)	17	5.8	6.43
Arg (R)	17	5.8	7.66	Met (M)	7	2.4	2.70
Asn (N)	7	2.4	2.39	Phe (F)	11	3.8	4.70
Asp (D)	24	8.2	8.26	Pro (P)	17	5.8	5.06
Cys (C)	10	3.4	3.13	Ser (S)	28	9.6	7.61
Gln (Q)	13	4.5	4.91	Thr (T)	16	5.5	4.93
Glu (E)	16	5.5	6.09	Trp (W)	5	1.7	2.64
Gly (G)	5	1.7	0.97	Tyr (Y)	12	4.1	5.62
His (H)	2	0.7	0.80	Val (V)	22	7.6	6.67
Ile (I)	19	6.5	6.45	Pyl (O)	0	0.0	0
Leu (L)	28	9.6	9.50	Sec (U)	0	0.0	0

using gene-specific primers (Table 1). RACE was performed using the cDNA amplification kit (Clontech, USA) according to the manufacturer's instructions. All PCR products were separated by electrophoresis on a 3% agarose gel and purified using the E.Z.N.A.® Gel Extraction Kit (Omega Bio-Tek, Norcross, GA, USA). The purified fragments were cloned into the pMD-19T vector (Takara Bio, Inc.), and six were selected for sequencing with an ABI PRISM 3100 DNA sequencer (Applied Biosystems, Foster City, CA).

Multiple sequence alignment and phylogenetic analysis

The yellow catfish MyD88 amino acid sequence was used as a template to identify homologous vertebrate sequences with the BLAST bioinformatics alignment tool. Homologous sequences from different species were used for multiple sequence alignment with CLC Genomics Workbench ver. 8 software. The multiple sequence alignment output was color-coded according to identity. A phylogenetic tree was constructed by MEGA 6 software using the neighbor-joining algorithm (Kelley et al. 2016), and the reliability of the branching was tested using bootstrap resampling with 1000 pseudo-replicates.

Bioinformatic analysis of protein sequence

The ProtParam tool (http://web.expasy.org/protparam/) was used to analyze the molecular weight, theoretical isoelectric point value (pI), amino acid composition, atomic composition, instability index, aliphatic index, and grand average of hydropathicity (GRAVY) of the protein sequence. The NetPhos 2.0 server (http://www.cbs.dtu.dk/services/NetPhos/) produced neural network predictions for serine, threonine, and tyrosine phosphorylation sites.

Protein secondary structure and three-dimensional (3D) structure prediction

The secondary structure of the MyD88 protein was analyzed by CLC Genomics Workbench ver. 8 software. The protein sequence was used to predict a-helices and b-strands, and protein kinase C phosphorylation sites and N-glycosylation sites were marked. DNA-binding sites were predicted by BindN (http://bioinfo.ggc.org/BindN/). The 3D structure of the protein was predicted by the I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) based on homology structure modeling. The structural similarity of two protein models was measured by the TM-score and RMSD from the I-TASSER server. Similarities between the predicted structure and template sequence were superimposed using the Pymol program (http://pymol.sourceforge.net). Global and per-residue model quality was assessed using C-scoring, which provides a confidence score for estimating the quality of predicted models by I-TASSER by determining the significance of threading template alignments and convergence parameters of structural assembly simulations. C-scores are typically in the range of (Hoshino et al. 2002; Livak and Schmittgen 2001), with higher C-scores signifying a model with a high level of confidence.

Results

Cloning and sequence analysis of yellow catfish MyD88

The complete yellow catfish *MyD88* cDNA of 1230 bp was obtained by RACE technology. It consisted of an 876-bp open reading frame (ORF), preceded by a 143-bp 5′ untranslated region (UTR) and followed by a 211-bp 3′UTR (Fig. 1). Based on the deduced polypeptide sequence, the ORF encoded a putative protein of 291 amino acids. Two conserved domains were identified using the Pfam program (Finn et al. 2000): an N-terminal DD domain at positions 36–112 and a C-terminal TIR domain at positions 164–256.

Multiple sequence alignment with representative MyD88 amino acid sequences revealed that MyD88 proteins from different species were highly conserved (Fig. 2). Disulfide bonds formed between the following cysteine residues (Cys 61, Cys 63, Cys 187, Cys 198, Cys 211, Cys 239, and Cys 269) were found to maintain high stability in all compared proteins.

Phylogenetic analysis of MyD88

To investigate the phylogenetic relationships of *MyD88* among different vertebrate species, a neighbor-joining tree was constructed using MEGA 6 software with the *p*-distance method. As shown in Fig. 3, yellow catfish MyD88 was clustered within the fish subgroup and is closely related to MyD88 of *Ictalurus punctatus*. This suggests that our cloning of yellow catfish *MyD88* was successful, and that it is conserved among vertebrates.

Bioinformatic analysis of yellow catfish MyD88

Chemical analysis and determination of the amino acid composition of the MyD88 protein were performed using ProtParam. MyD88 was shown to contain 291 amino acids, to have a molecular weight of 33.4341 kDa, and a theoretical PI of 5.17 (Table 1). The calculated GRAVY of − 0.212 indicated that yellow catfish MyD88 is a hydrophilic protein. The total number of negatively charged residues (Asp + Glu) was 40 and that of positively charged residues (Arg + Lys) was 34.

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1     AATAACGTAACGTTTGCACGCCGTTTAGTCAGTTTAGACTGAAATGACTTAAAAACAGGGAGCGGTTTATAGTTTTATTATCGCCGTAAA
91    CGACAATTTAATACAACAAGAAGAACAAAGTTTTTACACTTTGTTTTTTTATC

144   ATGGCGTCAAGTCCGTATTCTTCATCACCATCACCATCATCATCATCATCATCACCATCTGTGGATTATGACTTGATTCCAGTCATCGCC
1      M  A  S  S  P  Y  S  S  S  P  S  P  S  S  S  S  S  S  P  S  V  D  Y  D  L  I  P  V  I  A
234   CTGAACTACAGCGTGAGGAAAAAACTGGCTTTGTATTTAAACCCCATTAACACGGTGGCAGCAGACTGGACGGATATCGCTGAGAAAATG
31     L  N  Y  S  V  R  K  K  L  A  L  Y  L  N  P  I  N  T  V  A  A  D  W  T  D  I  A  E  K  M
324   GACTTCAGCTACCTGGAGATTAAAAACTATGAGAAGTTGGAGAATCCCACCAAGAAGCTGCTGGAGACCTGGCAGACCAGAGCAGGGGCC
61     D  F  S  Y  L  E  I  K  N  Y  E  K  L  E  N  P  T  K  K  L  L  E  T  W  Q  T  R  A  G  A
414   ACGGTGGACAAACTGGTGTCCATCCTGGAGCAGGCAGAGAGGAAAGACATCATCTCAGACCTGCAGCCACTCATAGATGAAGACTGCAGG
91     T  V  D  K  L  V  S  I  L  E  Q  A  E  R  K  D  I  I  S  D  L  Q  P  L  I  D  E  D  C  R
504   ATTTATGTGGAAAGACAAAAGGAGCTTCCTGTTCAGGTGCCCGAGGTGGACAGCAGAGACCAGTGTCGAGGAATCACTGTAAATGACGGT
121    I  Y  V  E  R  Q  K  E  L  P  V  Q  V  P  E  V  D  S  R  D  Q  C  R  G  I  T  V  N  D  G
594   CACATACCGGAGATGTTCGACGCTTTCATTTGCTACTGCCAGAGCGACTTCCAGTTCGTCCATGAGATGATCAGACAGCTGGAGCAGACA
151    H  I  P  E  M  F  D  A  F  I  C  Y  C  Q  S  D  F  Q  F  V  H  E  M  I  R  Q  L  E  Q  T
684   GATTACAACCTGAAGCTGTGTGTGTTTGACCGCGACGTCCTGCCCGGGACGTGTGTTTGGACCATCACCAGCGAGCTCATTGAGATGAGG
181    D  Y  N  L  K  L  C  V  F  D  R  D  V  L  P  G  T  C  V  W  T  I  T  S  E  L  I  E  M  R
774   TGTAAGAGGATGGTGGTTGTCATTTCTGATGATTACTTGGACAGCGATGCCTGTGACTTCCAGACTAAATTCGCCCTCAGTCTTTGCCCA
211    C  K  R  M  V  V  V  I  S  D  D  Y  L  D  S  D  A  C  D  F  Q  T  K  F  A  L  S  L  C  P
864   GGTGCTCGGACCAAGCGGCTGATCCCTGTGGTCTACAAGCAAATGAAGAGGCCTTTTCCCAGCATTCTGCGCTTCCTGACAGTGTGTGAC
241    G  A  R  T  K  R  L  I  P  V  V  Y  K  Q  M  K  R  P  F  P  S  I  L  R  F  L  T  V  C  D
954   TACACCAGACCCTCCACACAGTCCTGGTTCTGGGTTCGACTGGCCAAAGCCTTGTCCCTGTCCTGA
271    Y  T  R  P  S  T  Q  S  W  F  W  V  R  L  A  K  A  L  S  L  S  *

1020  GCGTCACACATCCAGCTCGCTGACTGACTGCCCCAGCTCTTATTATTGTTCACACACACACACTGCTACACAATCTGAACATTTCTGGTA
1110  ACAAATGAATTTTTCTTTTCTAGCTGCAGAACCCAAATATTTTGTTGCAAATGTCCCAGCTGTTGAATTGATGGCTTATGTAAGAGTTCT
1200  AAAAAGAAATTGAAGTAAAAAAAAAAAAAAA
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Fig. 1 Complete *MyD88* nucleotide sequence encoding the deduced amino acid sequence of Myd88. The asterisk represents the termination codon

BLASTP homology searches were used to compare the predicted yellow catfish MyD88 amino acid sequence with other MyD88 sequences. As shown in Table 2, yellow catfish MyD88 shared a high level of similarity with MyD88 of different species: 88.45% with *Ictalurus punctatus*, 79.7% with *Astyanax mexicanus*, and 75.18% with *Cyprinus carpio*. The predicted MyD88 protein pI and molecular weight were also shown to be very similar to those of different fish species.

MyD88 secondary structure analysis was carried out using the CLC Genomics Workbench ver.8 program (Abolnik 2015). Yellow catfish MyD88 was predicted to consist of 291 amino acids and to be composed of 12 α-helices and eight β-strands. The predicted three protein kinase C phosphorylation sites, one *N*-glycosylation site, and nine DNA-binding sites are shown in Fig. 4. Potential phosphorylation sites were analyzed using the NetPhos tool, which identified 15 serine, five threonine, and six tyrosine sites (Table 3). Because DNA-binding protein structures often recognize their target sequences and execute their function via helices, the tertiary and quaternary structures of the protein domains may hold the helices in a fixed structural arrangement in the unbound state (Horne et al. 2007; Huffman et al. 2002). Figure 5 shows that the predicted 3D structure of yellow catfish MyD88 is in accordance with the 2D results.

Tissue expression analysis of yellow catfish MyD88

To evaluate the tissue expression pattern of *MyD88*, we examined its basal expression levels in eight tissues of adult yellow catfish. As shown in Fig. 6, *MyD88* was ubiquitously expressed in all tissues examined. However, expression levels were variable, with the highest expression detected in the spleen and kidney and the lowest in the liver and intestine.

Effect of Clostridium butyricum treatment on the expression of intestinal MyD88

Clostridium butyricum is a bacterium that naturally occurs in the intestinal tract of humans and animals, and is beneficial to its host as a probiotic. We compared *MyD88* expression levels in the intestine of control group and *Clostridium butyricum* treatment group yellow catfish. As shown in Fig. 7, *Clostridium butyricum* treatment significantly upregulated the expression of intestinal *MyD88*.

Discussion

MyD88 acts as a crucial downstream mediator of TLRs, and is involved in the recognition of danger signals and induction of the innate immune response (Ishibashi et al. 2012; Warner

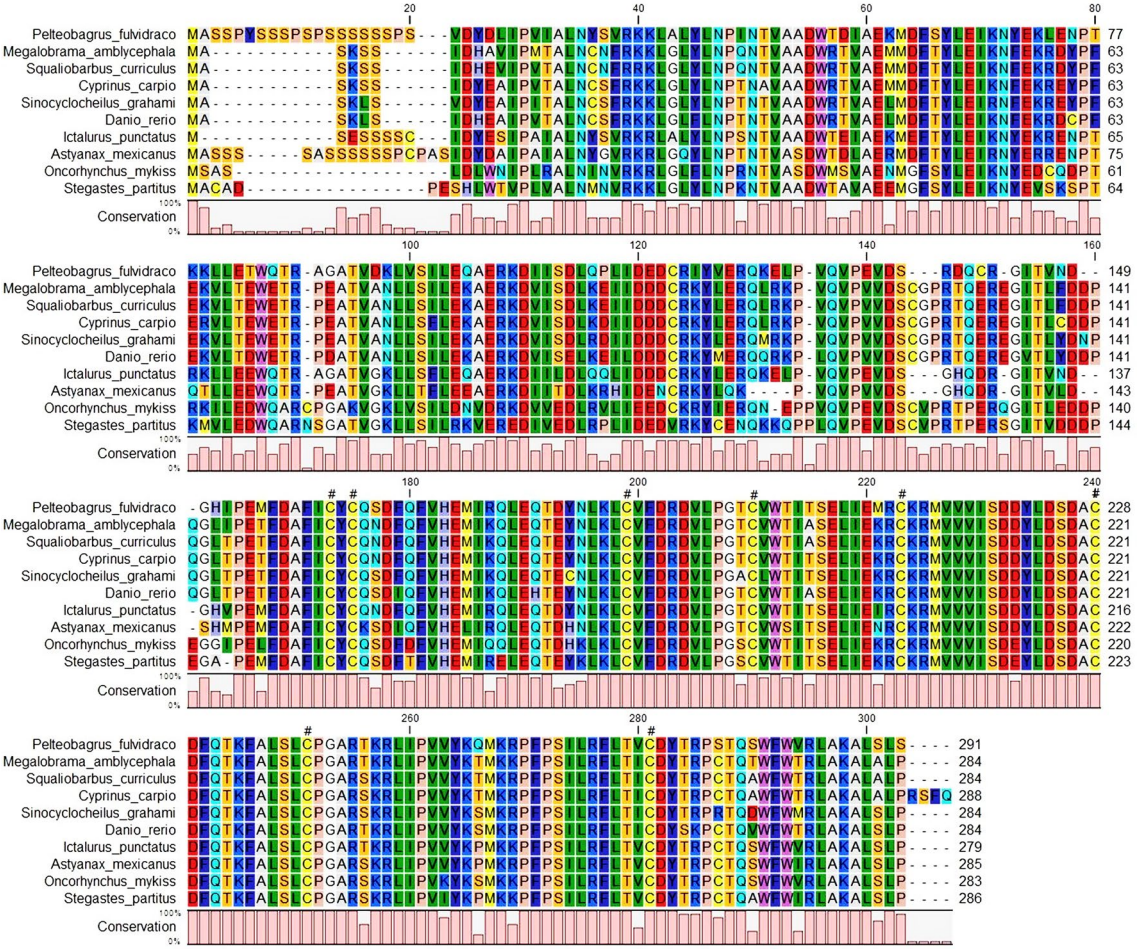

Fig. 2 The MyD88 amino acid sequence from *Pelteobagrus fulvidraco* was compared with respective proteins from nine different species (*Ictalurus punctatus, Astyanax mexicanus, Cyprinus carpio, Megalobrama amblycephala, Sinocyclocheilus grahami, Squaliobarbus curriculus, Danio rerio, Oncorhynchus mykiss*, and *Stegastes partitus*). The alignment was generated using the multiple sequence alignment function of CLC Genomics Workbench ver.8. The height of the pink bar beneath the residues represents the level of conservation of that residue

and Nunez 2013). The TLR signaling pathway is the main component of the mammalian innate immune system (Yao et al. 2009), and has also been identified in bony fish such as zebrafish, yellow croaker, and carp (Kongchum et al. 2011; Meijer et al. 2004; Qian et al. 2013).

In the present study, yellow catfish *MyD88* was cloned and analyzed for the first time. Multiple sequence alignment showed that it has a similar sequence to *MyD88* of other fish, and that an accordant molecular structure that contains the conserved N-terminal DD and C-terminal TIR domain seen in mammals and other fish (Goto and Imler 2012; Kongchum et al. 2011; Tran et al. 2015; Whang et al. 2011). This suggests that like MyD88 of other species, the TIR domain of yellow catfish MyD88 binds with TLRs and transmits danger signals to downstream factors such as IRAK4 and fas-associated protein with death domain using its C-terminal DD (Deguine et al. 2014; Janssens and Beyaert 2002).

A truncated splice variant of MyD88 was previously unable to elicit inflammatory responses because of its failure to recruit IRAK-4. This indicated that MyD88 variants may be able to mediate the innate immune response (Mendoza-Barberá et al. 2009). MyD88 was also shown to protect against the excessive production of IFN-β by restricting TLR3 signaling through a hitherto unknown mechanism to date (Gasse et al. 2007; Siednienko et al. 2011a, b).

Phylogenetic analysis revealed that yellow catfish MyD88 has a closer relationship with MyD88 of other fish rather than of mammals and birds. It also shared similar molecular characteristics with other fish MyD88, including chemical composition, isoelectric point value, and molecular weight. Interestingly, secondary structural analysis revealed that it is rich in phosphorylation sites, N-glycosylation sites, and DNA-binding sites, which reflect high molecular activity and interaction with other molecules. Therefore, we believe

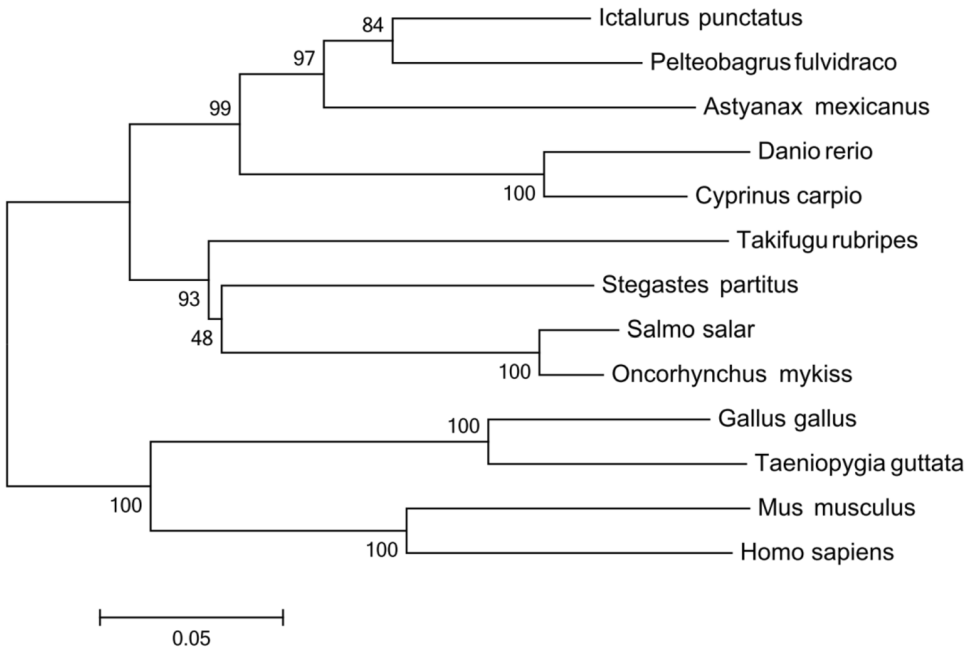

Fig. 3 Phylogenetic tree of *Pelteobagrus fulvidraco* MyD88 and potentially related fish species. The protein sequence of *Pelteobagrus fulvidraco* MyD88 was compared with MyD88 sequences from representative fish species. A phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap replicates. The GenBank accession numbers are as follows: *Ictalurus punctatus*: NP_001187207.1; *Astyanax mexicanus*: XP_007253619.1; *Cyprinus carpio*: ADE20131.1; *Megalobrama amblycephala*: AKC45380.1; *Sinocyclocheilus grahami*: AHK05779.1; *Squaliobarbus curriculus*: AKM20823.1; *Danio rerio*: NP_997979.2; *Oncorhynchus mykiss*: CDG03206.1; *Stegastes partitus*: XP_008295948.1; *Homo sapiens*: AAC50954.1; *Mus musculus*: NP_034981.1

Table 2 Comparison of yellow catfish (*Pelteobagrus fulvidraco*) MyD88 with other fish species

Species	NCBI reference sequence	No. of residues	Identity (%)	*E*-value	pI	Molecular weight (kDa)
Pelteobagrus fulvidraco		291	100	0	5.17	33.43
Ictalurus punctatus	NP_001187207.1	279	88.45	9.00E – 180	5.31	32.42
Astyanax mexicanus	XP_007253619.1	285	79.7	8.00E – 157	6.01	32.78
Cyprinus carpio	ADE20131.1	288	75.18	2.00E – 147	5.74	33.51
Megalobrama amblycephala	AKC45380.1	284	74.82	1.00E – 146	5.89	33.03
Sinocyclocheilus grahami	AHK05779.1	284	75.53	1.00E – 146	5.74	33.12
Squaliobarbus curriculus	AKM20823.1	284	74.47	2.00E – 146	5.66	33.05
Danio rerio	NP_997979.2	284	73.05	2.00E – 143	5.79	32.86
Oncorhynchus mykiss	CDG03206.1	283	73.67	3.00E – 143	5.33	32.69
Stegastes partitus	XP_008295948.1	286	75.46	2.00E – 142	5.49	32.83

that yellow catfish MyD88 may be one of the most active factors in the yellow catfish immune system.

The yellow catfish *MyD88* expression profile was determined by RT-PCR and shown to be widely expressed in all tissues examined, but with the highest expression in the spleen and the lowest in the intestine (Fig. 6). This result is in accordance with the yellow croaker and rock bream, indicating a role for MyD88 in innate immune defense (Whang et al. 2011; Yao et al. 2009).

Clostridium butyricum has been widely used to prevent disturbances of microflora in the intestinal tract, to treat pathogen-induced intestinal disease, enhance the humoral immune response, and improve animal production (Goyal et al. 2016; Lin et al. 2016; Zhou et al. 2010). As such, it is an effective microbial feed additive (Sun et al. 2011; Yan et al. 2013). *Bifidobacterium breve* was also reported to have a positive effect on the immune system (Ezendam

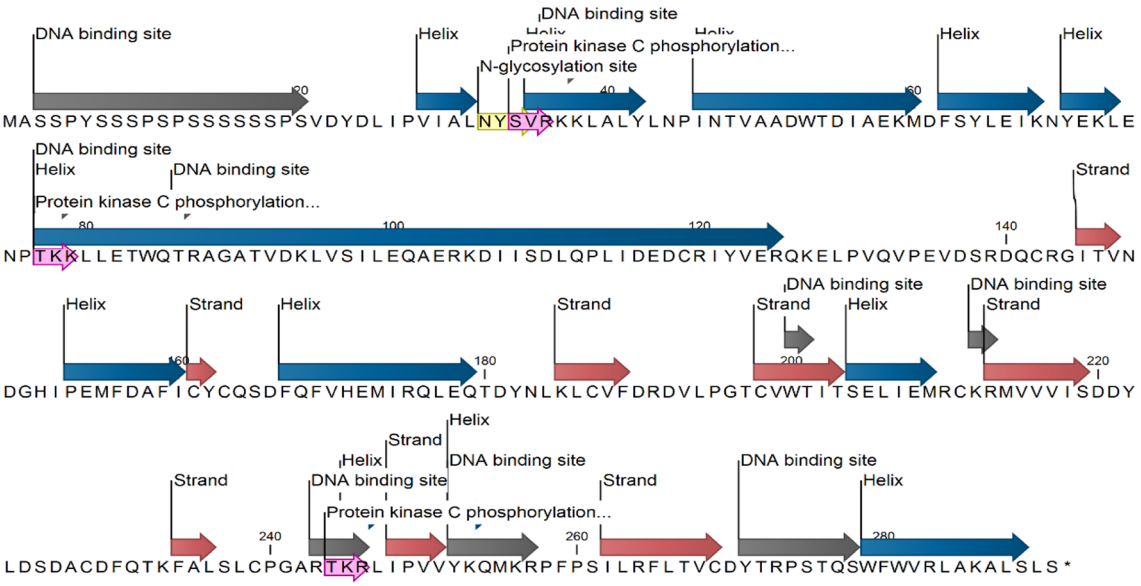

Fig. 4 The predicted secondary structure and annotated functional sites of the yellow catfish MyD88 sequence based on the CLC Genomics Workbench ver. 8 program

Table 3 The prediction results of phosphorylation and *N*-glycosylation sites by the NetPhos 2.0 Server

Position	Context	Score	Pred	Position	Context	Score	Pred
7	SSPYSSSPS	0.974	*S*	237	KFALSLCPG	0.906	*S*
8	SPYSSSPSP	0.673	*S*	275	YTRPSTQSW	0.959	*S*
9	PYSSSPSPS	0.819	*S*	86	ETWQTRAGA	0.604	*T*
11	SSSPSPSSS	0.994	*S*	91	RAGATVDKL	0.652	*T*
13	SPSPSSSSS	0.972	*S*	244	PGARTKRLI	0.713	*T*
14	PSPSSSSSS	0.987	*S*	267	LRFLTVCDY	0.903	*T*
15	SPSSSSSSP	0.938	*S*	276	TRPSTQSWF	0.638	*T*
16	PSSSSSSPS	0.968	*S*	6	ASSPYSSSP	0.866	*Y*
17	SSSSSSPSV	0.797	*S*	23	PSVDYDLIP	0.778	*Y*
18	SSSSSPSVD	0.994	*S*	64	MDFSYLEIK	0.681	*Y*
20	SSSPSVDYD	0.902	*S*	122	DCRIYVERQ	0.596	*Y*
63	KMDFSYLEI	0.914	*S*	182	EQTDYNLKL	0.581	*Y*
97	DKLVSILEQ	0.714	*S*	222	ISDDYLDSD	0.975	*Y*

and van Loveren 2007), while the probiotic bacterium *Lactobacillus casei* was found to induce activation of the gut mucosal immune system through innate immunity (Galdeano and Perdigón 2006). Importantly, depending on MyD88 expression, *Clostridium butyricum* activates the TLR2 signaling pathway and induces the secretion and expression of NF-kB, IL-6, IL-8, and tumor necrosis factor-α (Gao et al. 2012). In this study, *MyD88* expression was shown to be up-regulated in the intestine in yellow catfish receiving a dietary supplement of *Clostridium butyricum*. This observation indicates that *Clostridium butyricum* is able to regulate the immune system of yellow

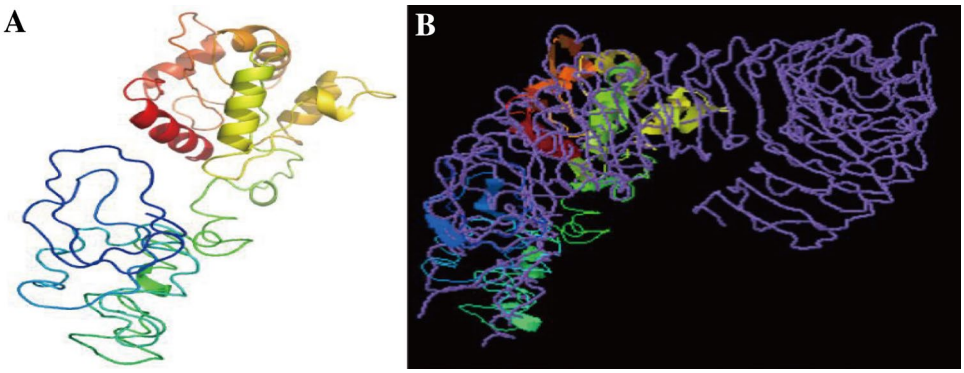

Fig. 5 The predicted 3D structural model and the main chain interface structure. **a** Predicted 3D structural model of *Pelteobagrus fulvidraco* MyD88. **b** Superimposed prediction model and native cartoon structures of *Pelteobagrus fulvidraco* MyD88 and *Homo sapiens* TLR5. The rainbow structure represents *Pelteobagrus fulvidraco* MyD88. The purple line is the alpha carbon backbone of *Homo sapiens* TLR5

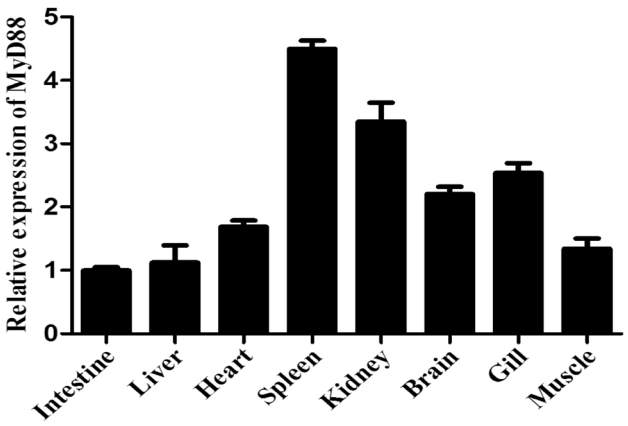

Fig. 6 Yellow catfish *MyD88* mRNA expression analysis was performed in the heart, liver, spleen, brain, gills, intestine, kidney, and muscle. Highest *MyD88* mRNA expression was detected in the spleen, and lowest expression was detected in the liver

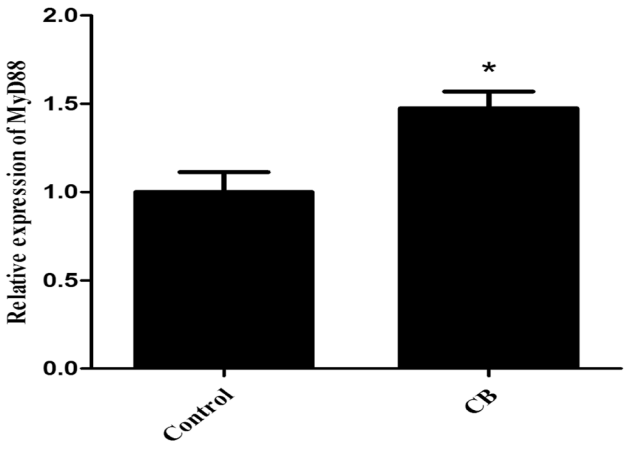

Fig. 7 *Clostridium butyricum* (CB) induced *MyD88* expression in the intestine of yellow catfish. Asterisk represents a significant difference between groups

catfish through *MyD88* gene and the *MyD88* gene is likely an important component of the immunity of yellow catfish.

In conclusion, we cloned yellow catfish MyD88 for the first time and performed bioinformatic as well as expression analyses. Our finding suggests that the MyD88 gene may have similar functions in the yellow catfish and it is probably to play an important role in the immune system. This work provides basic knowledge about yellow catfish MyD88 that will be useful for further study in this field.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

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