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Characterization of a mannose-binding lectin from channel catfish (*Ictalurus punctatus*)

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ABSTRACT

Mannose-binding lectin (MBL) is an important component of innate immunity capable of activating the lectin pathway of the complement system. A MBL gene was isolated from channel catfish (*Ictalurus punctatus*). The deduced protein contains a canonical collagen-like domain, a carbohydrate recognition domain (CRD), and a neck region similar to mammalian mannose-binding lectin. The catfish mannose-binding lectin CRD contains the EPN motif shown previously to mediate mannose specificity. The catfish mannose-binding lectin showed 30–43% identity with MBL protein sequences of rainbow trout, zebrafish, common carp, and goldfish, and 33–35% identity with sequences of mammalian species. In this study, while liver was the predominant source of mannose-binding lectin gene expression in healthy tissues, mannose-binding lectin expression in spleen rose sharply following challenge with a Gram-negative bacterium.

1. Introduction

Mannose-binding lectin is an important component of innate immunity capable of activating the lectin pathway of the complement system (Tsutsumi et al., 2005; Turner, 2003). It belongs to the calcium-dependent collagenous lectin family, or collectins, containing a carbohydrate recognition domain (CRD) and a collagen-like domain (Holmskov et al., 1994). Mannose-binding lectins have been shown to bind the 3- and 4-hydroxyl groups of appropriate sugars including *N*-acetyl-D-glucosamine (GlcNAc), mannose, *N*-acetyl-mannosamine (ManNAc), fucose and glucose (Turner, 1996, 2003). The collectin family in mammals includes mannose-binding lectin, surfactant proteins A and D (SP-A and SP-D), collectins CL-L1, CL-K1 and CL-P1, and conglutinin CL-43 and CL-46 (Ip et al., 2009). The collectins can form a structural unit of three identical polypeptide chains, bind carbohydrates on the surface of microorganisms via the C-terminal lectin/CRD, and mediate killing or phagocytosis functions (Presanis et al., 2003; Whytev, 2007). Mannose-binding lectin proteins bind and agglutinate various microorganisms including Gram-positive and Gram-negative bacteria, mycobacterium, viruses and fungi (Tsutsumi et al., 2005; Turner, 2003).

As the only member of the collectin family with the ability to activate the complement system, mannose-binding lectin activates the lectin pathway via association with mannose-binding lectin-associated serine proteases (MASPs) (Presanis et al., 2003; Russell and Lumsden, 2005; Tsutsumi et al., 2005; Turner, 2003). When binding to microorganisms, complexes of mannose-binding lectin-MASP are activated and able to cleave C2 and C4 of the complement system to form the C3 convertase, C4bC2a, which cleaves C3 into C3a and C3b. This leads to modulation of inflammation (C3a, C4a), increased opsonization via C3 receptors (CR1, CR3 and CR4) on the surface of phagocytes, and initiation of assembly of the membrane attack complex (MAC) consisting of complement components C5–C9 (Kania et al., 2010; Presanis et al., 2003; Turner, 2003). Mannose-binding lectin may also promote apoptosis and opsono-phagocytosis by a complement-independent pathway (Jack et al., 2001; Ogden et al., 2001; Turner, 2003).

Mannose-binding lectins have been reported in several fish species to date. The salmon serum lectin was the first fish lectin characterized both for structure and immune function. It exhibited antibacterial activity and lectin-enhanced macrophage activity (Ewart et al., 1999; Ottinger et al., 1999). Subsequently, mannose-binding lectins have been purified from rohu (*Labeo rohita*), rainbow trout (*Oncorhynchus mykiss*), sea lamprey (*Petromyzon marinus*), common carp (*Cyprinus carpio*), Atlantic salmon (*Salmo salar*), fugu (*Takifugu rubripes*), and turbot (*Scophthalmus maximus*)

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(Jackson et al., 2007; Mitra and Das, 2001; Nakao et al., 2006; Ourth et al., 2008; Stratton et al., 2004; Tsutsui et al., 2006a; Zhang et al., 2010). These studies have confirmed the pathogen-binding activity and the function of fish mannose-binding lectins in the lectin complement pathway of innate immunity.

In catfish, the most important aquacultured species in the United States, mannose-binding lectins have been isolated from the serum of channel catfish (*Ictalurus punctatus*) and blue catfish (*Ictalurus furcatus*) and their binding activity characterized; it was shown that levels of mannose-binding lectin correlated with resistance of catfish against infection by *Edwardsiella ictaluri* (Ourth et al., 2007). However, no molecular examination of catfish MBL sequence or gene expression has been carried out to-date. In this study, we report the cloning and characterization of a full-length cDNA of a MBL gene from channel catfish (*I. punctatus*), providing novel analysis of gene structure, homeostatic expression and regulation following pathogen exposure.

2. Materials and methods

2.1. Identification and sequencing of catfish mannose-binding lectin cDNA

Based on the cDNA sequence of MBL from zebrafish, we searched the channel catfish expressed sequence tags (ESTs) database (Li et al., 2007; Wang et al., 2010) in NCBI using BLAST. The clone CBPN25626 (GenBank ID: FD345689.1 and FD345690.1) was determined to encode the putative complete cDNA of a catfish mannose-binding lectin. The clone was re-sequenced using T7 and SP6 primers (Table 1), using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) (Xu et al., 2006).

2.2. Tissue sampling and RNA extraction

To determine gene expression in various healthy catfish tissues, samples of 11 tissues including brain, gill, heart, head kidney, trunk kidney, intestine, liver, muscle, skin, spleen and stomach from three pools (15 fish in each pool) of healthy channel catfish were collected, and flash-frozen in liquid nitrogen. The weight and length of fish used averaged 6.1 g and 9.5 cm, respectively. Fish were confirmed to be disease-free by histological and chemical analysis in the Fish Disease Diagnostic Laboratory, Auburn University. Tissues were homogenized under liquid nitrogen using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA) to extract total RNA. First-strand cDNA was synthesized using an iScript cDNA Synthesis Kit (Bio-Rad, USA).

2.3. Southern blot analysis

To determine the genomic copy number of the MBL gene in channel catfish, Southern blot analysis was conducted as previously described (Liu et al., 2010). Briefly, 10 μg of genomic DNA isolated from three individual adult channel catfish was digested with 30 units of the restriction endonucleases *Eco*R I, *Hin*d III or *Pst* I (New England Biolabs, Beverly, MA) in a 25 μl reaction at 37 °C. The digested DNA samples were electrophoresed on a 0.8% agarose gel. After electrophoresis, the gel was submerged in 0.25 N HCl for 15 min, then in denaturation and neutralization buffer for 30 min, respectively. The DNA was transferred to an Immobilon positively-charged nylon membrane (Millipore, Bedford, MA) by capillary transfer for 18 h using 20× SSC buffer. The DNA was fixed to the membrane using a UV cross-linker (Stratagene, La Jolla, CA) with the auto-crosslink settings. The membrane was hybridized with cDNA probes amplified using primers listed in Table 1. After pre-hybridized for 2 h with salmon sperm DNA and hybridized with a ^{32}P-dCTP labeled probe at 63 °C for 16 h, the membrane was washed twice with wash buffer 1 (2× SSC, 0.1% SDS) and one time with wash buffer 2 (0.5× SSC, 0.1% SDS), and then exposed to a piece of X-ray film overnight at −80 °C.

2.4. Gene structure and mapping

Using blastn and the cDNA sequence of the catfish mannose-binding lectin as a query, we searched the genomic sequence contigs generated during our ongoing whole genome sequencing project. The Spidey program (http://www.ncbi.nlm.nih.gov/spidey) was used to determine exons and introns, and microsatellite sites were identified by Perfect Microsatellite Repeat Finder (http://sgdp.iop.kcl.ac.uk/nikammar/repeatfinder.html). Microsatellite PCR primers were designed based on the flanking region of the simple sequence repeats (Table 1). A backcross hybrid family (F1-2 × Ch-6) was used for mapping, and the map was built using Join Map 4.0 (Kucuktas et al., 2009).

2.5. Phylogenetic analysis

Amino acid sequences of the mannose-binding lectin genes from various species were downloaded from NCBI. The conserved CRD domains were searched with the SMART program (http://www.smart.embl-heidelberg.de/) and analyzed using ClustalW. A phylogenetic tree of CRD domains was constructed using the neighbour-joining method within the Molecular Evolutionary Genetics Analysis (MEGA 4) package (Tamura et al., 2007). The CRD domain of *Drosophila melanogaster* C-lectin was used as the out group. Data were analyzed using Poisson correction, and gaps were removed by complete deletion. The topological stability of the neighbour-joining trees was evaluated by 10,000 bootstrapping replications.

2.6. Bacterial challenge and samples collection

The bacterial challenge was conducted as previously described (Liu et al., 2010), using fish with an average body weight of 6.1 g

Table 1
Primers used in this study.

Primer name	Primer sequence 5′-3′	Application
T7	TAATACGACTCACTATAGGG	For sequencing
SP6	ATTTAGGTGACACTATAG	
18S-F	GAGAAACGGCTACCACATCC	For quantitative real-time RT-PCR
18S-R	GATACGCTCATTCCGATTACAG	
MBL-1F	TTGTTGGAGAAAGCCACAAG	
MBL-1R	TCATTCCTGGGTAAGGCAAG	
MBL-2F	GTCTGCAGGAGGAAATCAGG	For generation of Southern blot probes
MBL-2R	GAGCTTTTGGTTTTCACATGG	
MBL-3F	GAGTTTTCCCAGTCACGACCTTCAAGCCCTCCTCAGATG	For mapping the MBL gene using microsatellites
MBL-3R	TCCTAACCAACCCCATGAAA	

and length of 9.5 cm and *E. ictaluri* as the pathogen. The bacteria were isolated from a single colony and cultured in Brain Heart Infusion broth (BHI) at 28 °C overnight. The bacterial culture was diluted with PBS (pH 7.4), and 1×10^5 cfu of bacteria in 100 μl PBS were injected intraperitoneally into the channel catfish. The same volume of PBS was injected into the fish of the control group and samples collected at 4 and 24 h, 3 and 7 days after treatment under anesthesia using tricaine methanesulphonate (MS 222) at 100 mg/L. At each time point, head kidney, trunk kidney, intestine, liver and spleen tissues were collected from 45 fish (three pools of 15 fish per pool) of both the challenge group and the control group. Then RNA was isolated and cDNA synthesized as described in Section 2.2. The cDNA samples were subsequently used for determination of gene expression by quantitative real-time RT-PCR. During the challenge, symptomatic treatment fish and control fish were collected and confirmed to be infected with *E. ictaluri* and pathogen-free, respectively, at the Fish Disease Diagnostic Laboratory, Auburn University. Limited mortalities (<10%) were observed prior to 7 days, although challenged fish manifested clinical symptoms of ESC.

2.7. Quantitative real-time RT-PCR analysis

Quantitative real-time RT-PCR was used to examine mannose-binding lectin mRNA expression in different tissues and following bacterial challenge. The reactions were performed on a Bio-Rad CFX96 (Bio-Rad, USA), using the SsoFast™ EvaGreen® Supermix kit (Bio-Rad, USA). Total cDNA (225 ng) was used in each reaction. The 18S rRNA gene was used as an internal control for normalization of expression levels. The primers used in quantitative real-time RT-PCR are listed in Table 1. The cycle time (Ct) values were compared and converted to fold differences by the relative quantification method using the Relative Expression Software Tool 384 v.1 (REST) (Pfaffl et al., 2002). Comparisons were made in the different tissues using analysis of variance (ANOVA) (SPSS 14.0 package, SPSS Inc., New York, USA). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Identification and sequencing of a catfish mannose-binding lectin cDNA

The channel catfish mannose-binding lectin cDNA contains an open reading frame (ORF) of 681 bp encoding 227 amino acids with a 139 bp 5′-untranslated region (UTR) and a 119 bp 3′-UTR. The 3′-UTR contains a typical polyadenylation signal sequence AATAAA. The signal sequence was composed of 23 amino acids as predicted by Kyte and Doolittle hydrophobicity analysis (Kyte and Doolittle, 1982). The mature protein had a pI of 5.03 and molecular weight of 24.11 kDa (GenetyxWin software). Analysis of the deduced amino acid sequence by multiple sequence alignment indicated that the mannose-binding lectin gene is moderately conserved through evolution (Fig. 1). The catfish mannose-binding lectin has 30–43% identity with fish mannose-binding lectin protein sequences from rainbow trout, zebrafish, common carp, and goldfish, and 33–35% identity with mammalian mannose-binding lectin protein sequences such as that of rat, human and cattle (Supplemental Table 1).

Fifteen Gly-Xxx-Yyy repeats (Gly36–Asn80), without interruption, constitute the collagen-like region of the catfish mannose-binding lectin gene. The neck region (Asp81–Ala100) was predicted to form a coiled helix by the software "COILS" (http://www.ch.embnet.org/), and may participate in a coiled–coil inter polypeptide chain structure. The terminal C-type carbohydrate recognition (CRD) domain comprised 125 residues (Tyr113–Glu224). The amino acid motif in the mannose-binding lectin that determines the specificity of the CRD was Glu191-Pro-Asn193 (EPN),

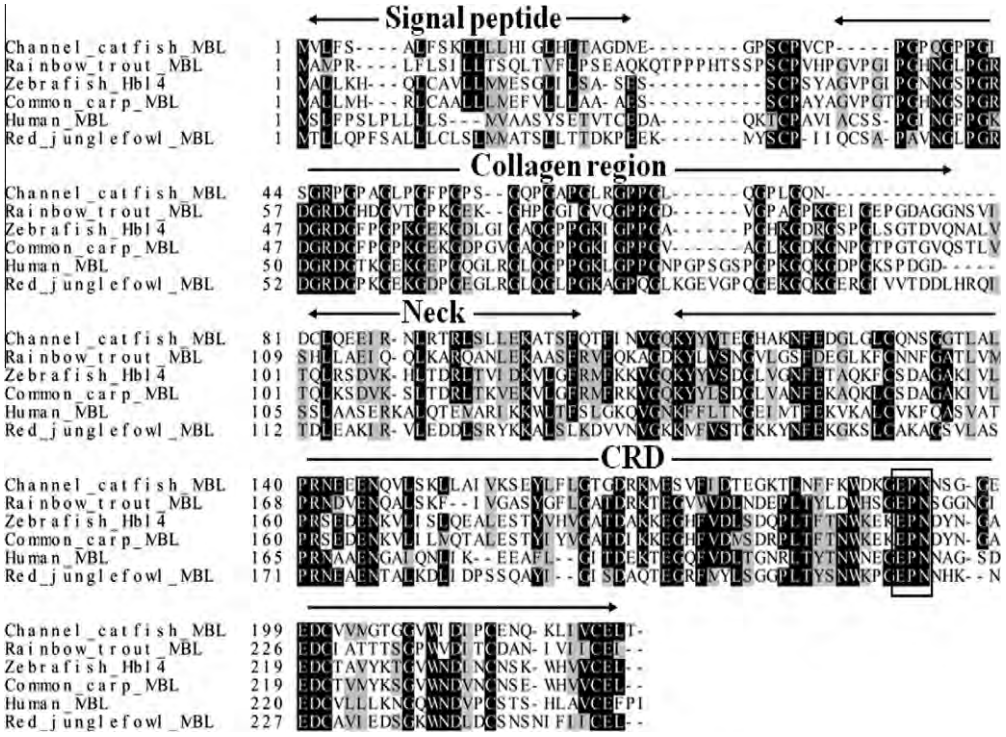

Fig. 1. Multiple sequence alignment of the deduced amino acid sequences of mannose-binding lectin (MBL) genes. The conserved and identical residues are represented by black shading, and conservative substitutions are represented by grey shading. The domain structure is indicated with arrows and lines spanning the appropriate length. EPN motif has been circled. The Genbank accession numbers of genes involved are as below, Zebrafish Hbl4, NP_001108197; Common carp MBL, NP_001108197; Rainbow trout MBL, NP_001153951; Human MBL, CAB56044; Red junglefowl MBL, AAK30298.

which dictates specificity for mannose-containing ligands (Fujita et al., 2004).

3.2. Phylogenetic analysis of mannose-binding lectin genes

A phylogenetic tree was constructed using amino acid sequences of 21 CRDs of mannose-binding lectin protein sequences of mammals, birds and fish. The CRD was predicted by the SMART program (http://www.smart.embl-heidelberg.de/). As shown in Fig. 2, channel catfish mannose-binding lectin fell into a clade that included all teleost mannose-binding lectins. All mammalian mannose-binding lectins formed their own strongly supported clade (based on bootstrap values) while the chicken mannose-binding lectin fell between the teleost and the mammalian genes. Interestingly, the mannose-binding lectin gene from the lampreys was more similar to the mammalian genes than to the teleost fish genes (Fig. 2).

3.3. Structural analysis and mapping of the mannose-binding lectin gene

By BLAST analysis of genomic sequence contigs generated from our ongoing whole genome sequencing project, we identified one contig with 100% identity with the catfish mannose-binding lectin cDNA sequence. Alignment of the genomic sequence with the cDNA sequence revealed the presence of four exons and three introns in the channel catfish mannose-binding lectin gene, similar to the situation in zebrafish and mammalian species. However, gene sizes are highly divergent, with fish having a smaller gene size of approximately 2 kb and mammalian species having genes ranging in size from 5.78 to 6.76 kb, largely due to the differences in introns sizes.

A polymorphic microsatellite in the genomic sequence contig allowed mapping of the mannose-binding lectin gene to linkage group 29 of the catfish genetic linkage map (data not shown; Kucuktas et al., 2009).

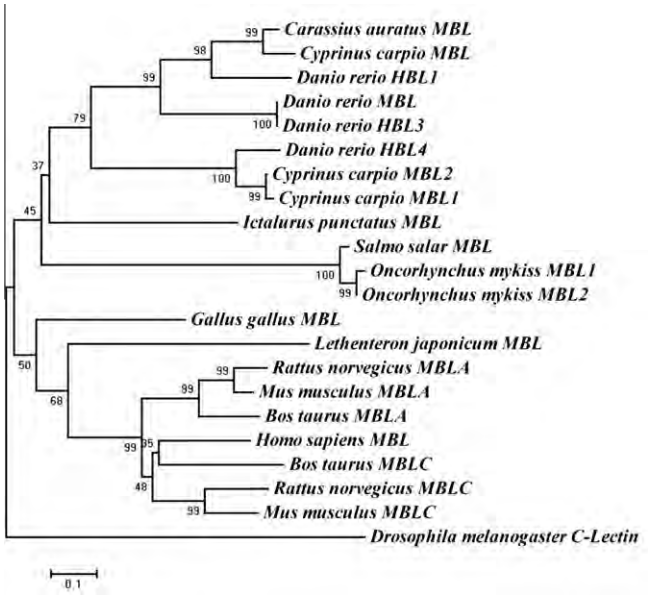

Fig. 2. Phylogenetic tree of known carbohydrate recognition domains (CRD) of mannose-binding lectin (MBL) genes with representatives of mammals, birds and fish. The CRD was predicted by the SMART program (http://www.smart.embl-heidelberg.de/). The Genbank accession numbers of genes involved are as below, *Carassius auratus* MBL, AF227739; *Cyprinus carpio* MBL, AAF63468; *C. carpio* MBL isform1, BAD02476; *C. carpio* MBL isform2, BAD02477; *Danio rerio* MBL, AAF63469; *Salmo salar* MBLC, ACN12569; *Oncorhynchus mykiss* MBL1, NP_001153951; *O. mykiss* MBL2, NP_001153952; *O. mykiss* MBL3, NP_001153953;*Gallus gallus* MBL, AAK30298; *Rattus norvegicus* MBLA, NP_036731; *Rattus norvegicus* MBLC, NP073195; *Mus musculus* MBLA, NP_034905; *Mus musculus* MBLC AAH10760; *Homo sapiens* MBL, CAB56044; *Bos taurus* MBLA, DAA14229; *Bos Taurus* MBLC, NP_776532; *Lethenteron japonicum* MBL, BAD98919; *Danio rerio* HBL1, XP_001341915; *Danio rerio* HBL3, NP_571645; *Danio rerio* HBL4, NP_001108197; *Drosophila melanogaster* C-Lectin, AAY34943. The topological stability of the neighbour-joining trees was evaluated by 10,000 bootstrapping replications, and the bootstrapping percentage values are indicated by numbers at the nodes.

3.4. Determination of genomic copy number of mannose-binding lectin

Southern blot analysis was conducted to determine the copy number of the mannose-binding lectin gene in the channel catfish genome. As shown in Fig. 3, a single band was observed with restriction enzyme digestion using *Hind* III, while two bands were detected for restriction enzymes *Eco*R I and *Pst* I. An examination of restriction sites within the genomic sequence indicated that there was one restriction site for *Eco*R I and *Pst* I and no restriction site for *Hind* III. Such restriction patterns suggest the presence of only a single copy of the mannose-binding lectin gene in the catfish genome.

3.5. Catfish mannose-binding lectin gene expression in healthy and infected tissues

Quantitative real-time RT-PCR was used to determine tissue distribution of mannose-binding lectin gene expression in healthy channel catfish. As shown in Fig. 4, catfish mannose-binding lectin is expressed most abundantly in the liver, and at relatively low levels in the brain, gill, head kidney, trunk kidney, heart, intestine, muscle, skin and spleen.

In order to determine if the catfish mannose-binding lectin is involved in responses to disease infection with the Gram-negative intracellular bacterium *E. ictaluri*, quantitative real-time RT-PCR analysis was conducted to determine the expression patterns of the mannose-binding lectin in infected liver, spleen, head kidney, intestine, and trunk kidney. As shown in Fig. 5, the expression of the catfish mannose-binding lectin was initially down-regulated in both the liver and spleen, by 3.2- and 2.3-fold, respectively, at 24 h following infection ($p < 0.05$). However, significant up-regulation of the catfish mannose-binding lectin gene expression was observed in the spleen following the initial down-regulation. The expression of the gene was induced 17.5- and 4.4-fold at 3 days and at 7 days in the spleen ($p < 0.05$), respectively. A pattern of up-regulation in the liver was also observed after initial down regulation, but was not statistically significant. The expression of channel catfish mannose-binding lectin was not significantly

Fig. 3. Southern blot analysis of catfish mannose-binding lectin (MBL) gene. Genomic DNA of three individual fish was used as marked and digested with *Eco*R I, *Hind* III, and *Pst* I. Molecular weight is shown on the left margin of the gel.

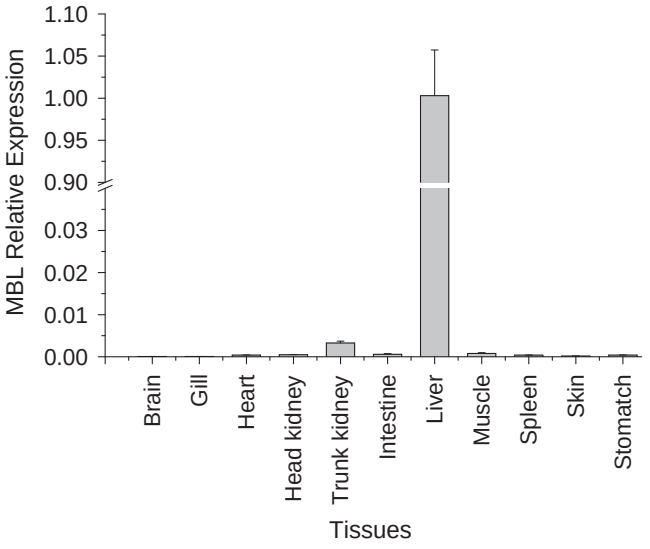

Fig. 4. Quantitative real-time RT-PCR analysis of mannose-binding lectin (MBL) gene expression in various tissues of healthy catfish. The ratio refers to the gene expression in different tissues relative to that in the liver. The 18S rRNA gene was used as an internal control.

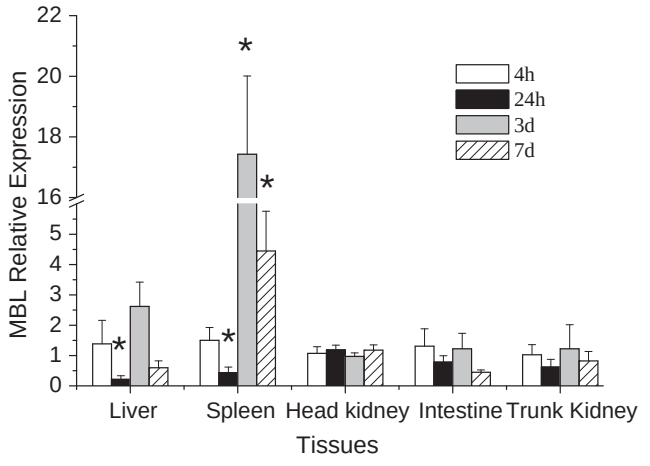

Fig. 5. Quantitative real-time RT-PCR analysis of catfish mannose-binding lectin (MBL) gene expression in liver, spleen, head kidney, intestine and trunk kidney after infection with *Edwardsiella ictaluri* at various time points (4 and 24 h, 3 and 7 days). Fold changes are expressed as the ratio of gene expression after *E. ictaluri* challenge to the control group at same time point as normalized with 18S rRNA gene. Error bars indicate standard error and asterisks indicate statistical significance ($p < 0.05$).

regulated in other tested tissues of head kidney, trunk kidney and intestine following infection.

4. Discussion

Mannose-binding lectins, along with ficolins, are critical in their ability to recognize pathogens and activate complement-mediated defense pathways. In this study, we conducted sequencing and sequence analysis of a mannose-binding lectin gene from channel catfish. We characterized the gene structure, organization, genome copy numbers, tissue expression and expression after bacterial infection.

The polymorphic nature of MBL genes in humans is well-described. Similarly, recent research demonstrated that there are multiple copies of the MBL gene in zebrafish, and that the polymorphism and copy number variation of MBL can significantly affect resistance to pathogen infection (Jackson et al., 2007). Multiple homologues of MBL also exist in rainbow trout and common carp (Kania et al., 2010; Nakao et al., 2006; Nikolakopoulou and Zarkadis, 2006). In catfish, our results from Southern blot analysis suggested the presence of only a single copy gene in the catfish genome, consistent with results of Western blot from a previous study (Ourth et al., 2007). Using antibodies against mannose-binding lectin, Ourth et al. detected a single band of 63 kDa protein in all tested catfish, presumably a trimeric protein composed of three identical polypeptides of 24 kDa, as seen in other species (Tsutsumi et al., 2005).

MBL has been described as a pattern recognition receptor and an activity factor in the complement system (Garred et al., 2000; Hoffmann et al., 1999; Nauta et al., 2003; Ogden et al., 2001; Schwaeble et al., 2002). In fish, several reports have confirmed that MBL can bind to carbohydrates on the surface of a wide range of pathogens (Jensen et al., 1997; Mitra and Das, 2001; Ottinger et al., 1999; Stratton et al., 2004), and that it can activate the complement system or act directly as an opsonin (Gonzalez et al., 2007; Nakao et al., 2006; Ourth et al., 2008). In mammals, the liver is the major source of MBL synthesis, and also a major organ in the response to immune stimulation (Kawasaki et al., 1978; Mizuno et al., 1981; Sastry et al., 1991; Takahashi et al., 2006). Our tissue expression analyses showed that MBL is expressed almost exclusively in the liver of healthy catfish. In rainbow trout (*O. mykiss*), trout MBL-1 and MBL-2 are expressed exclusively in liver and spleen, respectively (Nikolakopoulou and Zarkadis, 2006). In common carp (*C. carpio*), MBL was expressed in liver and spleen (Gonzalez et al., 2007; Vitved et al., 2000). In fugu (*T. rubripes*), the pufflectin gene is expressed in gills, oral cavity wall, esophagus, and skin. In addition, an isoform occurs exclusively in the intestine (Tsutsui et al., 2006b). In spite of predominant expression of catfish MBL in the liver, following bacterial infection the largest transcriptional responses were observed in spleen, with induction of expression reaching greater than 17-fold at 3 days following infection. Pathogen detection by circulating MBL synthesized normally in the liver may lead to downstream immune activation of MBL transcription in splenic cells (likely macrophages). In humans, post-transcriptional modification of MBL results in liver and serum products with differing abilities to activate complement. Further study is needed to examine functional differences between liver and spleen-synthesized MBL in catfish.

In conclusion, a mannose-binding lectin (MBL) gene was successfully isolated and characterized from channel catfish, *I. punctatus*. It shares important structural elements with mammalian MBL, indicating evolutionary restraints on mutation of key domains and motifs. The mRNA encoding MBL was predominantly produced in the liver. Following bacterial challenge, the expression of catfish MBL was significantly upregulated in the spleen. This study should provide a foundation for future examination of MBL binding partners, MBL-mediated complement activation, and correlation between catfish MBL production and immune fitness.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rvsc.2011.03.024.

References

Ewart, K.V., Johnson, S.C., Ross, N.W., 1999. Identification of a pathogen-binding lectin in salmon serum. Comparative Biochemistry and Physiology Part C 123, 9–15.

Fujita, T., Matsushita, M., Endo, Y., 2004. The lectin-complement pathway–its role in innate immunity and evolution. Immunological Reviews 198, 185–202.

Garred, P., Madsen, H.O., Marquart, H., Hansen, T.M., Sorensen, S.F., Petersen, J., Volck, B., Svejgaard, A., Graudal, N.A., Rudd, P.M., Dwek, R.A., Sim, R.B., Andersen, V., 2000. Two edged role of mannose binding lectin in rheumatoid arthritis: a cross sectional study. Journal of Rheumatology 27, 26–34.

Gonzalez, S.F., Buchmann, K., Nielsen, M.E., 2007. Complement expression in common carp (*Cyprinus carpio* L.) during infection with *Ichthyophthirius multifiliis*. Developmental and Comparative Immunology 31, 576–586.

Hoffmann, J.A., Kafatos, F.C., Janeway, C.A., Ezekowitz, R.A., 1999. Phylogenetic perspectives in innate immunity. Science 284, 1313–1318.

Holmskov, U., Malhotra, R., Sim, R.B., Jensenius, J.C., 1994. Collectins: collagenous C-type lectins of the innate immune defense system. Immunology Today 15, 67–74.

Ip, W.K., Takahashi, K., Ezekowitz, R., Stuart, L., 2009. Mannose-binding lectin and innate immunity. Immunological Reviews 230, 9–21.

Jack, D.L., Read, R.C., Tenner, A.J., Frosch, M., Turner, M.W., Klein, N.J., 2001. Mannose-binding lectin regulates the inflammatory response of human professional phagocytes to *Neisseria meningitidis* serogroup B. Journal of Infectious Diseases 184, 1152–1162.

Jackson, A.N., McLure, C.A., Dawkins, R.L., Keating, P.J., 2007. Mannose binding lectin (MBL) copy number polymorphism in Zebrafish (*D. Rerio*) and identification of haplotypes resistant to *L. anguillarum*. Immunogenetics 59, 861–872.

Jensen, L.E., Thiel, S., Petersen, T.E., Jensenius, J.C., 1997. A rainbow trout lectin with multimeric structure. Comparative Biochemistry and Physiology Part B 116, 385–390.

Kania, P.W., Sorensen, R.R., Koch, C., Brandt, J., Kliem, A., Vitved, L., Hansen, S., Skjodt, K., 2010. Evolutionary conservation of mannan-binding lectin (MBL) in bony fish: identification, characterization and expression analysis of three bona fide collectin homologues of MBL in the rainbow trout (*Onchorhynchus mykiss*). Fish and Shellfish Immunology 29, 910–920.

Kawasaki, T., Etoh, R., Yamashina, I., 1978. Isolation and characterization of a mannan-binding protein from rabbit liver. Biochemical and Biophysical Research Communications 81, 1018–1024.

Kucuktas, H., Wang, S., Li, P., He, C., Xu, P., Sha, Z., Liu, H., Jiang, Y., Baoprasertkul, P., Somridhivej, B., Wang, Y., Abernathy, J., Guo, X., Liu, L., Muir, W., Liu, Z., 2009. Construction of genetic linkage maps and comparative genome analysis of catfish using gene-associated markers. Genetics 181, 1649–1660.

Kyte, J., Doolittle, R.F., 1982. A simple method for displaying the hydropathic character of a protein. Journal of Molecular Biology 157, 105–132.

Li, P., Peatman, E., Wang, S., Feng, J., He, C., Baoprasertkul, P., Xu, P., Kucuktas, H., Nandi, S., Somridhivej, B., Serapion, J., Simmons, M., Turan, C., Liu, L., Muir, W., Dunham, R., Brady, Y., Grizzle, J., Liu, Z., 2007. Towards the ictalurid catfish transcriptome: generation and analysis of 31, 215 catfish ESTs. BMC Genomics 8, 177.

Liu, H., Takano, T., Abernathy, J., Wang, S., Sha, Z., Jiang, Y., Terhune, J., Kucuktas, H., Peatman, E., Liu, Z., 2010. Structure and expression of transferrin gene of channel catfish, *Ictalurus punctatus*. Fish and Shellfish Immunology 28, 159–166.

Mitra, S., Das, H.R., 2001. A novel mannose-binding lectin from plasma of *Labeo rohita*. Fish Physiology and Biochemistry 25, 121–129.

Mizuno, Y., Kozutsumi, Y., Kawasaki, T., Yamashina, I., 1981. Isolation and characterization of a mannan-binding protein from rat liver. Journal of Biological Chemistry 256, 4247–4252.

Nakao, M., Kajiya, T., Sato, Y., Somamoto, T., Kato-Unoki, Y., Matsushita, M., Nakata, M., Fujita, T., Yano, T., 2006. Lectin pathway of bony fish complement: identification of two homologs of the mannose-binding lectin associated with MASP2 in the common carp (*Cyprinus carpio*). Journal of Immunology 177, 5471–5479.

Nauta, A.J., Raaschou-Jensen, N., Roos, A., Daha, M.R., Madsen, H.O., Borrias-Essers, M.C., Ryder, L.P., Koch, C., Garred, P., 2003. Mannose-binding lectin engagement with late apoptotic and necrotic cells. European Journal of Immunology 33, 2853–2863.

Nikolakopoulou, K., Zarkadis, I.K., 2006. Molecular cloning and characterisation of two homologues of Mannose-Binding Lectin in rainbow trout. Fish and Shellfish Immunology 21, 305–314.

Ogden, C.A., de Cathehneau, A., Hoffmann, P.R., Bratton, D., Ghebrehiwet, B., Fadok, V.A., Henson, P.M., 2001. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. Journal of Experimental Medicine 194, 781–795.

Ottinger, C.A., Johnson, S.C., Ewart, K.V., Brown, L.L., Ross, N.W., 1999. Enhancement of anti-Aeromonas salmonicida activity in Atlantic salmon (*Salmo salar*) macrophages by a mannose-binding lectin. Comparative Biochemistry and Physiology Part C 123, 53–59.

Ourth, D.D., Narra, M.B., Simco, B.A., 2007. Comparative study of mannose-binding C-type lectin isolated from channel catfish (*Ictalurus punctatus*) and blue catfish (*Ictalurus furcatus*). Fish and Shellfish Immunology 23, 1152–1160.

Ourth, D.D., Rose, W.M., Siefkes, M.J., 2008. Isolation of mannose-binding C-type lectin from sea lamprey (*Petromyzon marinus*) plasma and binding to *Aeromonas salmonicida*. Veterinary Immunology and Immunopathology 126, 407–412.

Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Research 30, e36.

Presanis, J.S., Kojima, M., Sim, R.B., 2003. Biochemistry and genetics of mannan-binding lectin (MBL). Biochemical Society Transactions 31, 748–752.

Russell, S., Lumsden, J.S., 2005. Function and heterogeneity of fish lectins. Veterinary Immunology and Immunopathology 108, 111–120.

Sastry, K., Zahedi, K., Lelias, J.M., Whitehead, A.S., Ezekowitz, R.A., 1991. Molecular characterization of the mouse mannose-binding proteins. The mannose-binding protein A but not C is an acute phase reactant. Journal of Immunology 147, 692–697.

Schwaeble, W., Dahl, M.R., Thiel, S., Stover, C., Jensenius, J.C., 2002. The mannan-binding lectin-associated serine proteases (MASPs) and MAp19: four components of the lectin pathway activation complex encoded by two genes. Immunobiology 205, 455–466.

Stratton, L., Wu, S., Richards, R.C., Vanya Ewart, K., 2004. Oligomerisation and carbohydrate binding in an Atlantic salmon serum C-type lectin consistent with non-self recognition. Fish and Shellfish Immunology 17, 315–323.

Takahashi, K., Ip, W.E., Michelow, I.C., Ezekowitz, R.A., 2006. The mannose-binding lectin: a prototypic pattern recognition molecule. Current Opinion in Immunology 18, 16–23.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24, 1596–1599.

Tsutsui, S., Tasumi, S., Suetake, H., Kikuchi, K., Suzuki, Y., 2006a. Carbohydrate-binding site of a novel mannose-specific lectin from fugu (*Takifugu rubripes*) skin mucus. Comparative Biochemistry and Physiology Part B 143, 514–519.

Tsutsui, S., Okamoto, M., Tasumi, S., Suetake, H., Kikuchi, K., Suzuki, Y., 2006b. Novel mannose-specific lectins found in torafugu, *Takifugu rubripes*: a review. Comparative Biochemistry and Physiology Part D 1, 122–127.

Tsutsumi, A., Takahashi, R., Sumida, T., 2005. Mannose binding lectin: genetics and autoimmune disease. Autoimmunity Reviews 4, 364–372.

Turner, M.W., 1996. Mannose-binding lectin: The pluripotent molecule of the innate immune system. Immunology Today 17, 532–540.

Turner, M.W., 2003. The role of mannose-binding lectin in health and disease. Molecular Immunology 40, 423–429.

Vitved, L., Holmskov, U., Koch, C., Teisner, B., Hansen, S., Salomonsen, J., Skjodt, K., 2000. The homologue of mannose-binding lectin in the carp family Cyprinidae is expressed at high level in spleen, and the deduced primary structure predicts affinity for galactose. Immunogenetics 51, 955–964.

Wang, S., Peatman, E., Abernathy, J., Waldbieser, G., Lindquist, E., Richardson, P., Lucas, S., Wang, M., Li, P., Thimmapuram, J., Liu, L., Vullaganti, D., Kucuktas, H., Murdock, C., Small, B.C., Wilson, M., Liu, H., Jiang, Y., Lee, Y., Chen, F., Lu, J., Wang, W., Xu, P., Somridhivej, B., Baoprasertkul, P., Quilang, J., Sha, Z., Bao, B., Wang, Y., Wang, Q., Takano, T., Nandi, S., Liu, S., Wong, L., Kaltenboeck, L., Quiniou, S., Bengten, E., Miller, N., Trant, J., Rokhsar, D., Liu, Z., 2010. The Catfish Genome Consortium, Assembly of 500, 000 inter-specific catfish expressed sequence tags and large scale gene-associated marker development for whole genome association studies. Genome Biology 11, R8.

Whytev, S., 2007. The innate immune response of finfish - A review of current knowledge. Fish and Shellfish Immunology 23, 1127–1151.

Xu, P., Wang, S., Liu, L., Peatman, E., Somridhivej, B., Thimmapuram, J., Gong, G., Liu, Z., 2006. Channel catfish BAC-end sequences for marker development and assessment of syntenic conservation with other fish species. Animal Genetics 37, 321–326.

Zhang, M., Hua, Y., Sun, L., 2010. Identification and molecular analysis of a novel C-type lectin from *Scophthalmus maximus*. Fish and Shellfish Immunology 29, 82–88.