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Expression and functional characterization of a mannose-binding lectin-associated serine protease-1 (MASP-1) from Nile tilapia (*Oreochromis niloticus*) in host defense against bacterial infection

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ABSTRACT

Mannose-binding lectin-associated serine protease-1 (MASP-1), a multifunctional serine protease, plays an important role in innate immunity which is capable of activating the lectin pathway of the complement system and also triggering coagulation cascade system. In this study, a MASP-1 homolog (OnMASP-1) was identified from Nile tilapia (*Oreochromis niloticus*) and characterized at expression and inflammation functional levels. The open reading frame (ORF) of *OnMASP-1* is 2187 bp of nucleotide sequence encoding a polypeptide of 728 amino acids. The deduced amino acid sequence has 6 characteristic structures, including two C1r/C1s-Uegf-BMP domains (CUB), one epidermal growth factor domain (EGF), two complement control protein domains (CCP) and a catalytic serine protease domain (SP). Expression analysis revealed that the *OnMASP-1* was highly expressed in the liver, and widely exhibited in other tissues containing intestine, spleen and kidney. In addition, the *OnMASP-1* expression was significantly up-regulated in spleen and head kidney following challenges with *Streptococcus agalactiae* and *Aeromonas hydrophila*. The up-regulations of OnMASP-1 mRNA and protein expression were also demonstrated in hepatocytes and monocytes/macrophages *in vitro* stimulation with *S. agalactiae* and *A. hydrophila*. Recombinant OnMASP-1 protein was likely to participate in the regulation of inflammatory and migration reaction by monocytes/macrophages. These results indicated that OnMASP-1, playing an important role in innate immunity, was likely to involve in host defense against bacterial infection in Nile tilapia.

1. Introduction

Complement system, as the first line of the host defense against microbial intruders, is not only a major effector of innate immunity, but also an important bridge between innate and adaptive immunity [1,2]. The process of complement activation and its products can mediate a series of important biological effects, such as cell lysis, opsono-phagocytosis, inflammatory response, clearance of immune complexes and cell debris [3,4]. The complement system includes more than 50 plasma and cell-surface proteins that is activated by the classical pathway (CP), alternative pathway (AP) and lectin pathway (LP) [2,5,6]. Among them, LP is a critical pathway, especially in infants and children, providing a direct defense against microbial infections [2]. The LP is initiated when mannose-binding lectin (MBL), collectin 11 (CL-K1) or ficolins (H-ficolin, L-ficolin or M-ficolin), in complex with the MBL-associated serine proteases (MASPs) and MBL-associated proteins (MAp 19 and MAp 44), binds to appropriate targets [7,8].

MASPs belong to the serine-protease superfamily and exist as zymogens in the plasma, including MASP-1, MASP-2 and MASP-3 [2,8]. In general, different types of MASPs combine with different oligomeric states of MBL to form MBL-MASP compounds. It has been shown previously that MASP is important in the host defense mechanism. MASP is activated when the MBL binds to the carbohydrates or the acetylated pathogen-associated molecular patterns (PAMPs) on microorganism surfaces, and then activate the complement system through a series of enzymatic reactions [1,2,9]. MASP-1 was discovered in 1992 as a C1s-like serine protease [10]. MASP-1 contains two C1r/C1s-Uegf-BMP domains (CUB), one epidermal growth factor domain (EGF), two complement control protein domains (CCP) and a catalytic serine protease domain (SP) [2,5]. It is chiefly synthesized in hepatocytes and is considered to be a promiscuous protease, stemming from the wide substrate binding groove, which is strongly resembled trypsin and this

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structure also determines its functional diversity [2,11].

In the past few years, the functions of MASP-1 in mammals has received increasing attention. In human, MASP-1 activates zymogen MASP-2 and cleaves C2 to produce C2a and C3 convertase, which plays crucial role in the initial stage of LP [2,5,12,13]. MASP-1 also has thrombin-like activity and is an essential component in the activation of thrombin substrates, which plays a role in the coagulation, cleaving factor XIII and fibrinogen, and mediating the formation of cross-linked fibrin [2,14,15]. Moreover, MASP-1 is concerned to be essential in cell activation and inflammation regulation [6,15]. However, the study on the MASP-1 in teleost was extremely limited. There was only one report of MASP-1 in grass carp (*Ctenopharyngodon idella*), which was mainly concentrated on the gene clone and expression pattern of *MASP-1* upon *A. hydrophila* challenge *in vitro* and *in vivo* [16]. Heretofore, the functional study of teleost MASP-1 in macrophages activation and inflammatory response is still unclear, including Nile tilapia (*O. niloticus*).

In this study, we reported the expression and functional characterization of a MASP-1 homolog (OnMASP-1) from Nile tilapia, which belonged serine protease family. The full length of *OnMASP-1* was cloned and analyzed. The mRNA expression of *OnMASP-1* was explored in different tissues and cells after bacterial infection. The protein concentration of OnMASP-1 was examined in serum and cell culture supernatant after infection by ELISA. Moreover, the function analyses of (r)OnMASP-1 on inflammatory and migration reaction in monocytes/macrophages were demonstrated. The results indicated that OnMASP-1 is likely to play roles in host immune defense against bacterial infection in *O. niloticus*.

2. Materials and methods

2.1. Animals

Nile tilapia, about 80 ± 10 g (for challenge experiment), 300 ± 20 g (for cell separation) were obtained and cultured as previously described [9,17,18]. The Balb/c female mice of 6-weeks-old were obtained from Guangdong Medical Laboratory Animal Center (Guangzhou, China).

2.2. Sample collection

In order to explore the expression of *OnMASP-1* in healthy fish, the tissues including liver, head kidney, hind kidney, muscle, thymus, spleen, skin, gills, intestine, and peripheral blood were collected, immediately frozen by liquid nitrogen and stored at −80 °C.

The challenge experiment was performed by intraperitoneal injection with 100 μL live bacteria (*S. agalactiae* (ZQ0910) or *A. hydrophila* (BYK00810) in PBS with a final concentration of (1×10^7 CFU/mL) [17–20].

2.3. Cloning and analysis of MASP-1 sequence

The full length sequence of *OnMASP-1* gene was cloned according to the predicted sequence of *O. niloticus MASP*-1 mRNA (GenBank accession XM_005462739). All primers were listed in Table 1. The sequence analysis referred to the previous description [9,17,18].

2.4. Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed as previously described [9,17,21]. Fold change of *OnMASP-1* gene expression levels were normalized against β actin using the $2^{-\Delta\Delta Ct}$ method [22].

2.5. Hepatocytes and monocytes/macrophages

The hepatocytes were isolated and cultured according to the previous descriptions [17,18,23,24]. Briefly, the livers were dissected and digested for 20 min through 0.1% collagenase (Sigma, USA). The cells were washed and re-suspended including 3 mL red blood cell lysis buffer (KangWei, China) on the ice 3 min. After it, the cells were re-suspended and adjusted to 5×10^5 cells/mL within the culture DMEM medium (Gibco, USA).

The head kidney monocytes/macrophages were isolated according to the previous methods [18,21,25–28]. Briefly, the cells were isolated through a 54%/31% discontinuous percoll (Sigma, USA) density gradient and adjusted to 1×10^6 cells/mL with the L-15 medium (Gibico, USA).

The experiment group was stimulated with formalin-inactivated bacteria (1×10^7 CFU/mL), and the control group was only stimulated with 1 × PBS.

Table 1
Primers used in this study.

Primers	Nucleotide Sequence (5′-3′)	Purpose
MASP-1-F	AGGAGCACAGAGGAGCGGAGGAGCC	Full cDNA
MASP-1-R	CAGGGGGCAGCGTGAAGGTGACTCT	Full cDNA
M13-F	TGTAAAACGACGGCCAGT	Sequencing
M13-R	CAGGAAACAGCTATGACC	Sequencing
EMASP-1-F	CGCGGATCCAGCCTGCGCTCACCAAACTTCCCG	Protein expression
EMASP-1-R	CGCAAGCTTGGGGGCAGCGTGAAGGTGACTCT	Protein expression
GSPR	GCTGAGCACCGAGTCCGAGCGAGAA	5′RACE
NGSPR	TGGGCAGTTCGTCTCAGTAGCAG	Nested PCR
GSPF	AGAGAGTCACCTTCACGCTGCCCCC	3′RACE
NGSPF	GACAGCCAGACAAATCCATCCAAGA	Nested PCR
β-actin-F	CGAGAGGGAAATCGTGCGTGACA	Control
β-actin-R	AGGAAGGAAGGCTGGAAGAGGGC	Control
qMASP-1-F	GCTACGCCTCCCGCTCCA	RT-qPCR
qMASP-1-R	CCCAAACACCGCCCACCT	RT-qPCR
qIL-6-F	ACAGAGGAGGCGGAGATG	RT-qPCR
qIL-6-R	GCAGTGCTTCGGGATAGAG	RT-qPCR
qIL-8-F	GATAAGCAACAGAATCATTGTCAGC	RT-qPCR
qIL-8-R	CCTCGCAGTGGGAGTTGG	RT-qPCR
qIL-10-F	TGGAGGGCTTCCCCGTCAG	RT-qPCR
qIL-10-R	CTGTCGGCAGAACCGTGTCC	RT-qPCR
qMIF-F	CACATCAACCCTGACCAAAT	RT-qPCR
qMIF-R	GCCTGTTGGCAGCACC	RT-qPCR

2.6. Expression and purification of (r)OnMASP-1

The expression primers were designed with restriction sites (*Bam*H I and *Hin*d III), EMASP-1-F and EMASP-1-R (Table 1). The ligation product (pET-32a-MASP-1) was obtained and transformed into *E. coli* BL21 (DE3) (TianGen, China). The (r)OnMASP-1 was expressed and purified using the previous descriptions [9,17,18]. The purified (r)OnMASP-1 was detected by a 12% SDS-PAGE gel electrophoresis.

2.7. Preparation of mouse polyclonal antibodies (pAb) against (r)OnMASP-1

The (r)OnMASP-1 was as antigen to immune mice. After the third injection, the antibody titers reached a level of 350,000 units/mL. To verify the specificity of the pAb to OnMASP-1, western blotting was performed by detecting Nile tilapia serum at 2 d after *S. agalactiae* stimulation and supernatant of hepatocytes at 48 h after *S. agalactiae* stimulation.

2.8. ELISA to determine OnMASP-1 concentration

The OnMASP-1 expression in serum, and culture supernatant of hepatocytes and macrophages/monocytes was detected by a competitive inhibition ELISA after bacteria stimulations [29,30]. Briefly, the 96-well plate (Corning, USA) was coated with (r)OnMASP-1 2 μg/mL at 4 °C overnight and blocked for 2 h at 37 °C. The serum (2-fold dilution),

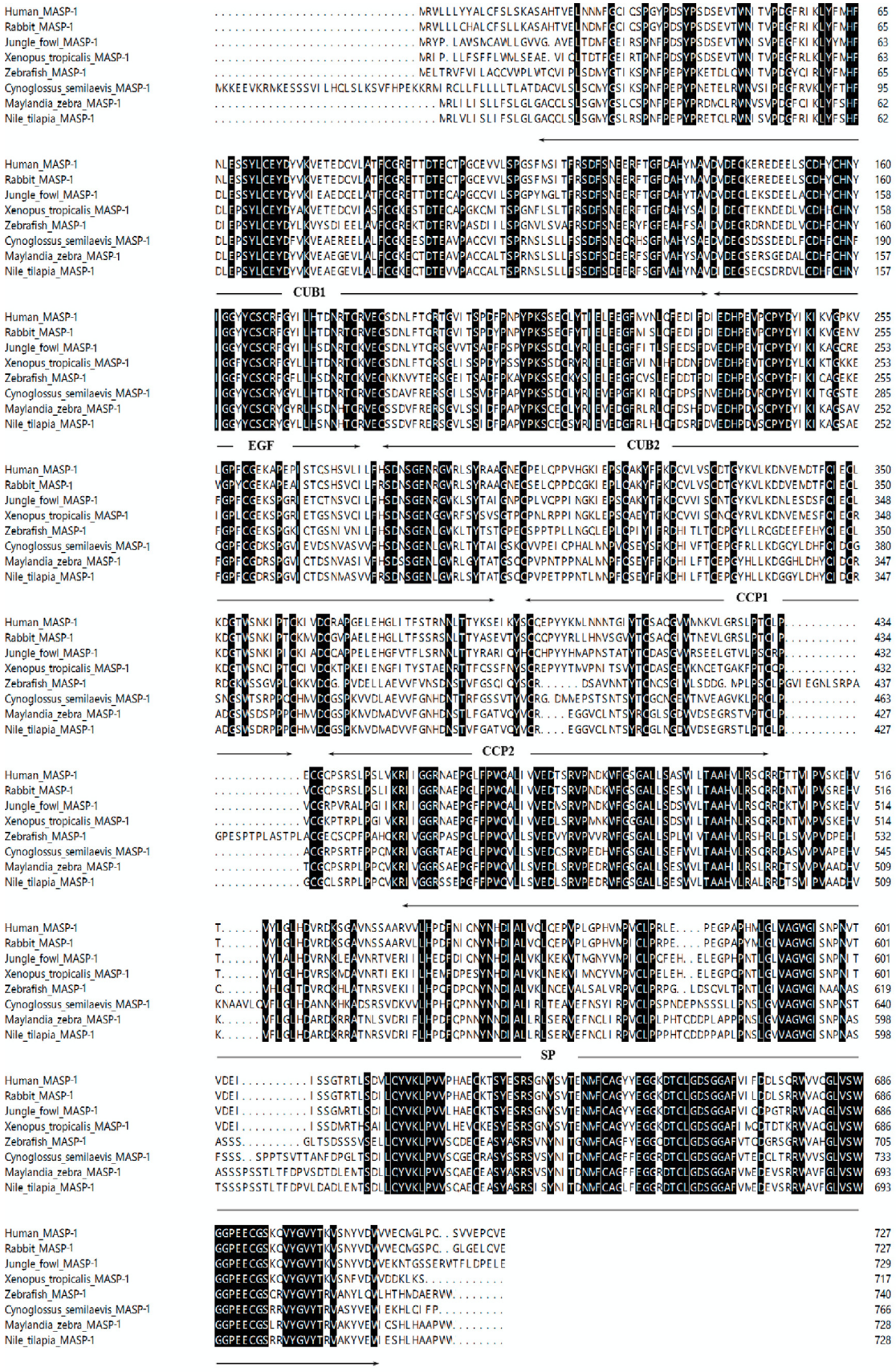

Fig. 1. Multiple sequence alignment of the deduced amino acid sequence of MASP-1 among different species. The conserved and identical residues are represented by black shading. The domain structure is indicated with arrows and lines spanning the appropriate length. The GenBank accession numbers of genes involved are as below, Human MASP-1, AAI06947; Rabbit MASP-1, XP_008264859; Jungle fowl MASP-1, NP_998751.1; *Xenopus tropicalis* MASP-1, NP_001090874; Zebrafish MASP-1, XP_001341936.1; *Cynoglossus semilaevis* MASP-1, XP_008307429.1; *Maylandia zebra* MASP-1, XP_014266317.1.

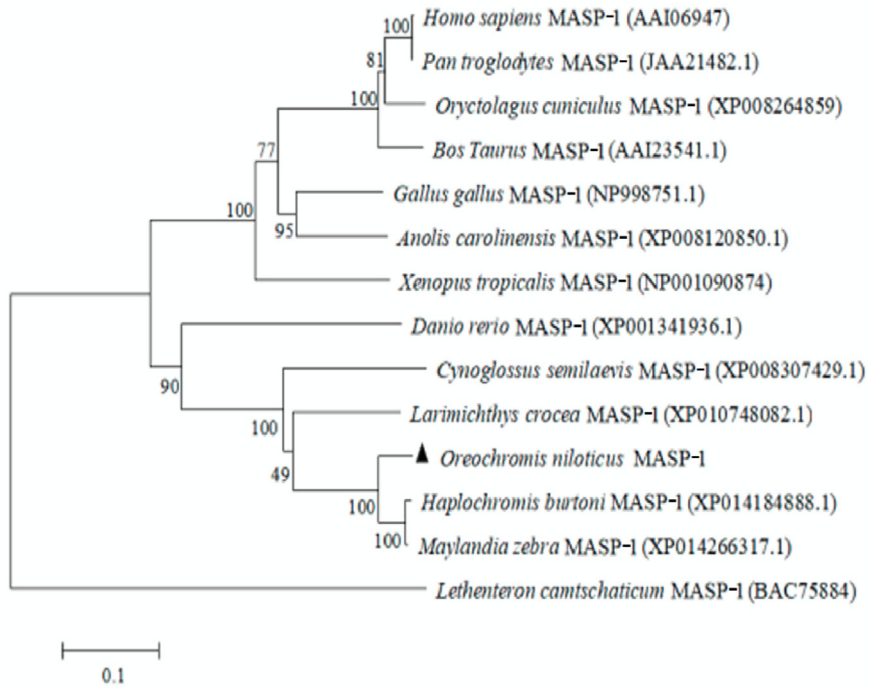

Fig. 2. Phylogenetic tree of MASP-1 family members constructed using the NJ method by MEGA 6 program based on the alignment of the MASP-1s performed with the Clustal W method. Numbers at each branch indicated the percentage bootstrap values on 1,000 replicates.

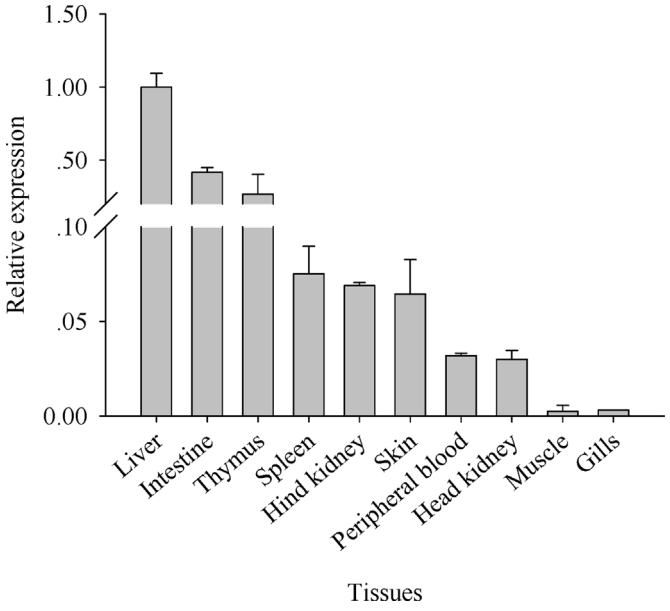

Fig. 3. Tissue distribution of *OnMASP*-1 mRNA in normal Nile tilapia. The ratio refers to the gene expression in different tissues relative to that in liver and target gene expression is normalized against β-actin. The error bars represent standard deviation of three replicate samples.

culture supernatant and mouse *anti*-OnMASP-1 pAb (1:3200, the optimal dilution determined previously) were placed in each well and incubated for 1 h. The secondary antibody was horseradish peroxidase-conjugated goat anti-mouse IgG antibody (1:2000) (Southern Biotech, USA). Then, the reaction was measured at O.D. 405 using a microplate reader (Thermo, USA) and calculated from standard curves pre-made.

2.9. Inflammation and migration reaction

The experiment was performed as described previously [31]. Briefly, the expressions of *IL-6*, *IL-10*, *IL-8* and *MIF* were detected in monocytes/macrophages after stimulation of (r)OnMASP-1 and pET-32a.

To investigate the effects of (r)OnC1INH on the expression of *IL-6*, *IL-10*, *IL-8* and *MIF* on induced by (r)OnMASP-1, the transcripts were tested according to the previous descriptions [6]. Briefly, The cells were pre-incubated with (r)OnC1INH (5 μg/mL) for 30 min and then treated with (r)OnMASP-1 (5 μg/mL) for 6 h or 24 h. The cells were collected and examined by qRT-PCR. In addition, The (r)OnC1INH and pET-32a (endotoxin removal) were obtained in our previous study [9,32].

2.10. Statistics

Statistical analyses were performed using SPSS 17.0 software and expressed as mean ± standard deviation. The $p < 0.05$ was considered statistically significant by different letters (a, b, c) or asterisks (*).

3. Results

3.1. Sequence analysis of OnMASP-1

The full length of *OnMASP-1* gene was 2579 bp, including a 5′-untranslated region (UTR) of 84 bp and a 3′-untranslated region (UTR) of 308 bp (Fig. S1). Among them, the 3′-UTR had a polyadenylation signal AATAA and a polyadenylation tail. The ORF of *OnMASP-1* was 2187 bp and encoded 728 amino acid (aa) residues, containing a 121 aa CUB1, a 44 aa EGF, a 113 aa CUB2, a 62 aa CCP1, a 62 aa CCP2 and a 277 aa SP domain (Fig. 1). Moreover, the OnMASP-1 had a putative signal peptide (residues 1–15). The mature OnMASP-1 protein (residues 15–728) had a deduced molecular mass of 79.79 kDa and a predicted isoelectric point of 4.81.

Multiple sequences alignments of the amino acid sequences showed

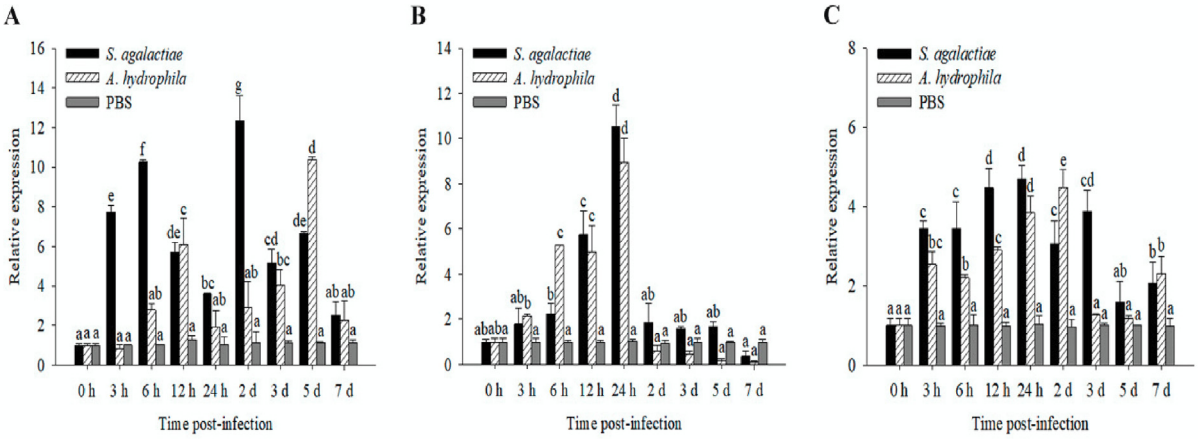

Fig. 4. Temporal mRNA expression of *OnMASP-1* transcript in liver (A), spleen (B) and head kidney (C) after *S. agalactiae* and *A. hydrophila* challenges. The mRNA level of *OnMASP-1* gene was normalized to that *β*-actin and fold units were calculated deciding the values of the vaccinated tissues by PBS. The error bars represent standard deviation (n = 3) and significant difference was indicated by different letters (a, b, c). ($p < 0.05$).

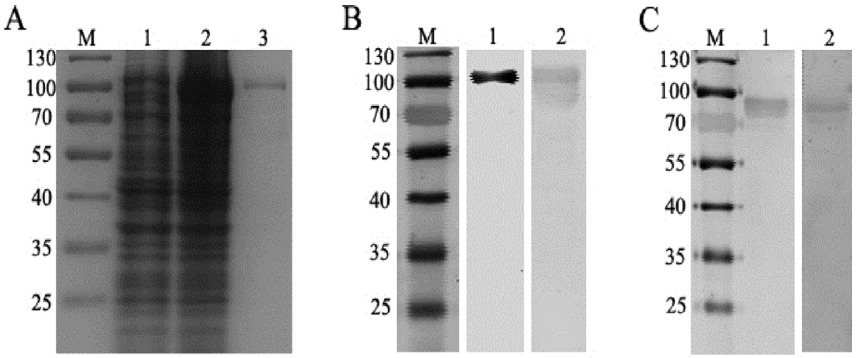

Fig. 5. Purification and Western blot analysis of (r)OnMASP-1. (A) Lane M, markers; Lane 1, the bacteria liquid before IPTG induction; Lane 2, the (r)OnMASP-1 was induced with 1 mM IPTG at 37 °C for 6 h; Lane 3, purified (r)OnMASP-1 fusion protein. (B) Western blot analysis of (r)OnMASP-1, Lane 1, anti-His tag mouse monoclonal antibody as the primary antibody; Lane 2, anti-(r)OnMASP-1 mouse polyclonal antibody as the primary antibody. (C) Western blot analysis of OnMASP-1 in serum and cell supernatant. Lane 1, the serum at 2 d after *S. agalactiae* stimulation; Lane 2, the supernatant was harvested from hepatocytes *in vitro* culture at 48 h after *S. agalactiae* stimulation.

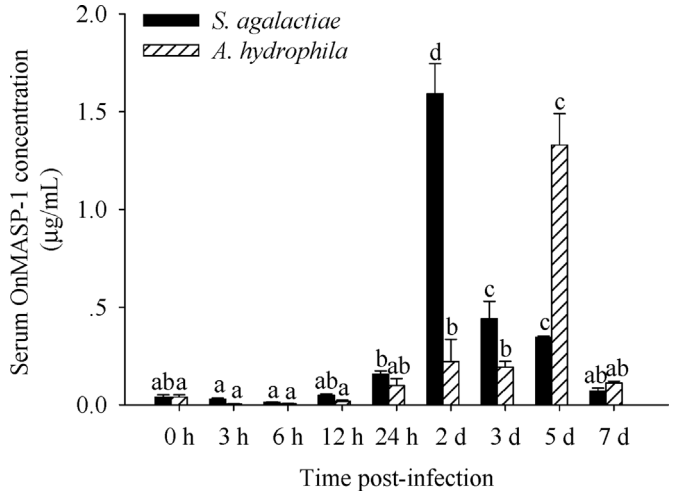

Fig. 6. The dynamic change of serum OnMASP-1 concentration after *S. agalactiae* and *A. hydrophila* stimulation. The error bars represent standard deviation (n = 3) and significant difference was indicated by different letters (a, b, c). ($p < 0.05$).

that OnMASP-1 was considerable consensus sequences with other species MASP-1s. The predicted domain of OnMASP-1 was similar to other species. Moreover, the OnMASP-1 was 54.6%, 54.5%, 54.7%, 54.3%, 57.5%, 69.2% and 93.3% identical to human, rabbit, jungle fowl, *Xenopus tropicalis*, zebra fish, *Cynoglossus semilaevis* and zebra mbuna, respectively. As shown in Fig. 2, the phylogenetic tree was distinctly classified into two clusters. The MASP-1s from teleost gathered a cluster, and the OnMASP-1 was closely related to a family of *Haplochromis burtoni* and *Maylandia zebra*. Another cluster was gathered with amphibians, reptiles, birds and mammals successively. Overall, the relationships in the phylogenic tree showed in accordance with the traditional taxonomy.

3.2. Tissue distribution of OnMASP-1

The tissue distribution of *OnMASP-1* was examined by qRT-PCR in healthy fish. The transcripts of *OnMASP-1* were existed in all tissues examined (Fig. 3). The *OnMASP-1* transcript was mainly expressed in liver and was also detected in intestine, thymus and spleen, following the hind kidney, skin, peripheral blood, head kidney, and so on.

3.3. Expression patterns of OnMASP-1

The expression of *OnMASP-1* was analyzed by qRT-PCR after pathogen infecton. The *OnMASP-1* transcript was rapidly up-regulated at 6 h post-infection (p.i.) in liver after *S. agalactiae* challenge (Fig. 4A). The tendency of the *OnMASP-1* expression after *A. hydrophila* infection in liver was alike but not as prominent as that in *S. agalactiae* (Fig. 4A). In spleen, the expression of *OnMASP-1* was significantly increased at 24 h p. i. after challenges (Fig. 4B). In head kidney, the dynamic pattern of *OnMASP-1* was consistent with spleen after pathogen challenges, while *S. agalactiae* (10.5-fold) and *A. hydrophila* (8.9-fold) remarkably raised the transcripts of *OnMASP-1* in spleen higher than those in head kidney (4.7-fold and 4.5-fold, respectively) (Fig. 4C).

To explore the expression of *OnMASP-1 in vitro*, the hepatocytes and monocytes/macrophages were stimulated (Figs. 7A–1; 7B-1). In hepatocytes, the expression of *OnMASP-1* was markedly raised at 24 h p. i.

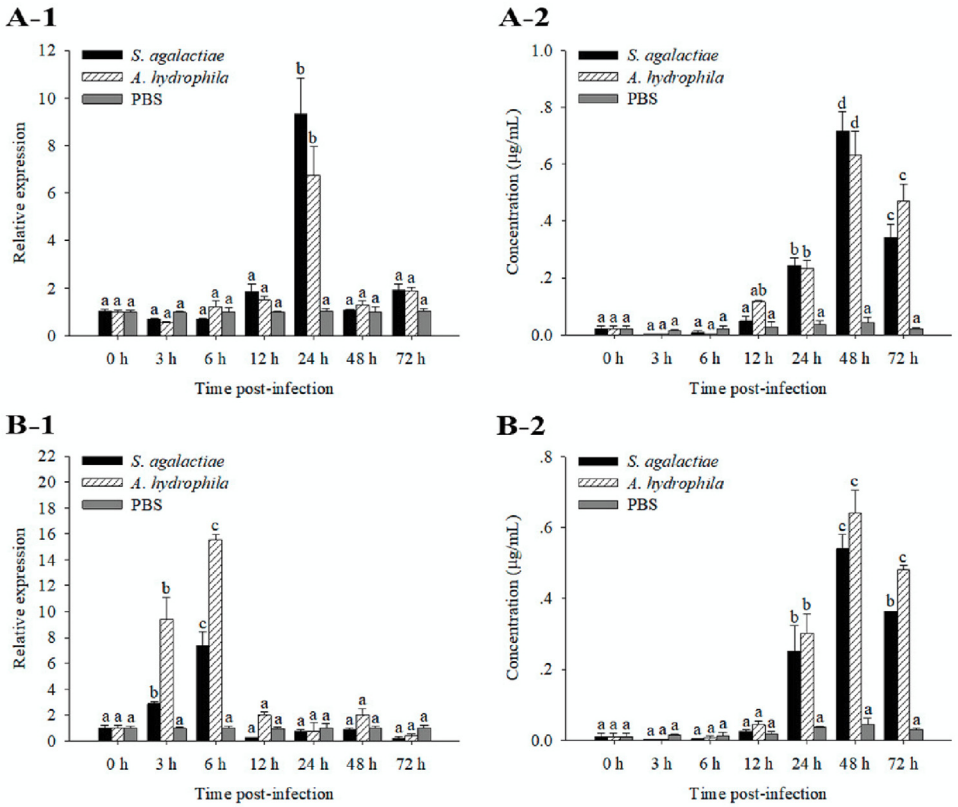

Fig. 7. The mRNA expression and protein concentration of OnMASP-1 in the hepatocytes (A) and head kidney monocytes/macrophages (B) after *S. agalactiae* and *A. hydrophila* (1×10^7 CFU/mL) challenges. (A-1, B-1) the mRNA expression of *OnMASP-1* was detected by qRT-PCR and analyzed using *β*-actin as internal reference. (A-2, B-2) OnMASP-1 concentration was assayed by a competitive-inhibition ELISA. The error bars represent standard deviation (n = 3) and significant difference was indicated by different letters (a, b, c). ($p < 0.05$).

after challenges (Figs. 7A–1). In monocytes/macrophages, the transcript of *OnMASP-1* was remarkably increased at 6 h p. i. after challenges of *S. agalactiae* (7.4-fold) and *A. hydrophila* (15.6-fold). (Figs. 7B–1).

3.4. Recombinant OnMASP-1 expression and purification

As shown in Fig. 5A, a single band (~100 kDa) corresponding to (r)OnMASP-1 could be detected. The purified (r)OnMASP-1 was used in preparation mouse pAb. To verify the pAb, the specificity of the antibody was examined by western blotting to the (r)OnMASP-1 and native OnMASP-1. A specific positive and single band about 100 kDa and 80 kDa was detected, respectively (Fig. 5B; 5C).

3.5. Dynamics of OnMASP-1 expression by stimulation in vivo and in vitro

To further investigate the expression of OnMASP-1 in tilapia serum, and culture supernatants of hepatocytes and monocytes/macrophages after pathogen infection, an ELISA test was adopted. The OnMASP-1 concentration showed significant up-regulation in serum upon challenges, and the optimal expression nearly 1.6 μg/mL (2 d) and 1.3 μg/mL (5 d), respectively (Fig. 6). In addition, the OnMASP-1 concentrations in culture media were also remarkably increased following two bacterial challenges (Figs. 7A–2; 7B-2). Moreover, the protein expression pattern of OnMASP-1 was similar to the *OnMASP-1* transcript upon challenges, but the peak expressions of OnMASP-1 after challenges appeared later than those of *OnMASP-1* in hepatocytes and monocytes/macrophages (Figs. 7A–2; 7B-2).

3.6. Effects of (r)OnMASP-1 on inflammation

The *IL-6* and *IL-10* transcripts showed significant up-regulation after (r)OnMASP-1 stimulation (Fig. 8A and 8B). Thereinto, the transcript of *IL-6* was up-regulated rapidly at 6 h p. i. (12.9-fold) (Fig. 8A), while the *IL-10* transcript was raised at 24 h p. i. (8.8-fold) (Fig. 8B). Moreover, the expression of *IL-8* and *MIF* were significant up-regulation after infection (Fig. 8C and 8D). The transcript of *IL-8* quickly increased at 3 h p. i, while the *MIF* remarkably raised at 12 h p. i. (Fig. 8C and 8D). Further, the (r)OnC1INH reduced the expressions of the cytokines at 6 h and 24 h (Fig. 9).

4. Discussion

MASP-1, as a versatile serine protease, can not only activate the complement system by a series of enzymatic catalytic reactions, but also has the functions of thrombin-like lysis activity, cell activation, and mediating inflammation and chemotaxis, which plays a crucial role in innate immunity [2,6,15]. In the current study, we explored the expression and functional characterization of *MASP-1* in *O. niloticus*, which implied that OnMASP-1 is likely to be involved in host defense against bacterial infection.

MASP-1 has similar structural domains with C1r and C1s, and is one of the serine protease superfamily. It is a single-chain polypeptide molecule, which exists in the form of proenzyme in serum and is an essential molecule in the innate immune system [5,15]. According to the amino acid sequence of OnMASP-1, the predicted structure domains mainly contains 6 domains, including CUB1, EGF, CUB2, CCP1, CCP2 and SP domain (Fig. 1). These domains are identical to human homology gene, suggesting that they have similar immune functions to pathogen infection [15,33]. Among them, the CUB regions involve binding to the MBL collagen-like region CLR [34]. The EGF-like region containing the Cd binding pattern (motif) is related to the formation of the MBL/MASP complex [5,34]. The CCP regions, a tandem repeat of complement control protein modules, involve interactions with C4 and C3 [5]. SP domain is the catalytic functional zone, in which the 3 residues (His, Asp and Ser) are highly conserved and constitute the active center of the serine protease [5,11]. Moreover, OnMASP-1 includes a signal peptide (MRLVLISLIFSLGLG), which may indicate that it

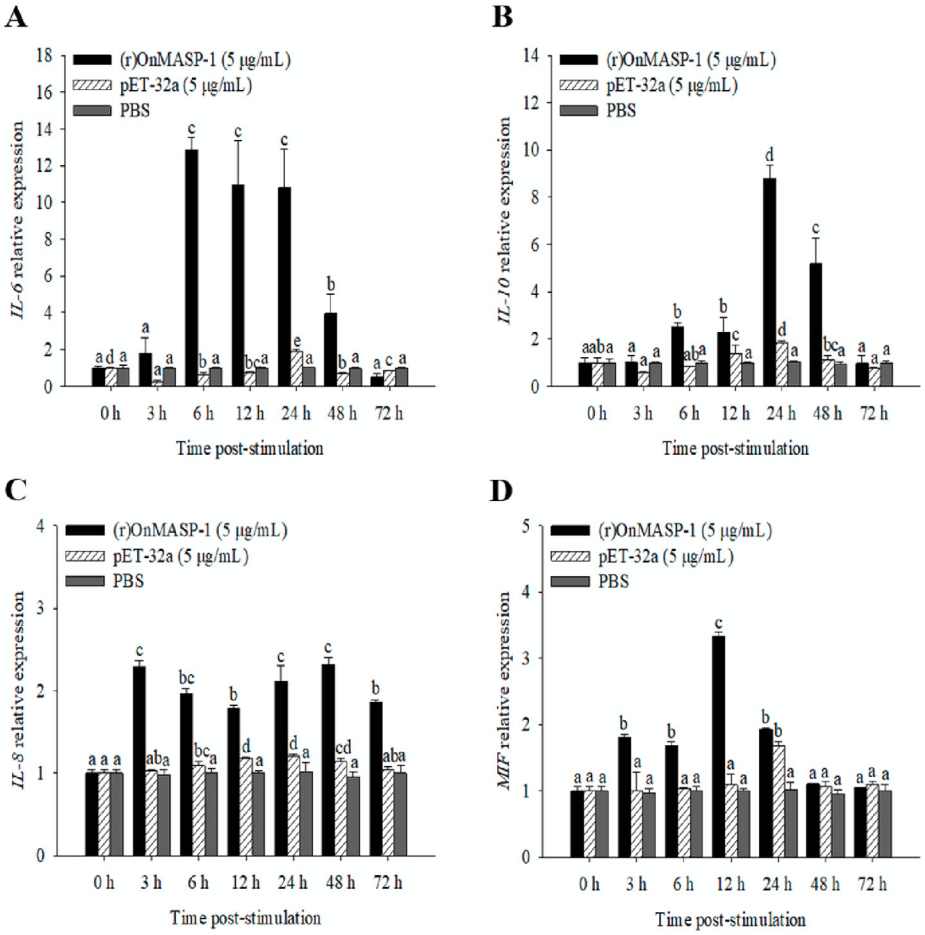

Fig. 8. The mRNA expression of *IL-6* (A), *IL-10* (B), *IL-8* (C) and *MIF* (D) from Nile tilapia in the head kidney monocytes/macrophages. Nile tilapia head kidney monocytes/macrophages were treated with (r)OnMASP-1 (5 μg/mL), pET-32a (5 μg/mL) and PBS. The mRNA level of *IL-6*, *IL-10*, *IL-8* and *MIF* gene was normalized to that of *β*-actin and fold units were calculated deciding the values of the PBS treated cells. The error bars represent standard deviation (n = 3) and significant difference was indicated by different letters (a, b, c). ($p < 0.05$).

functions outside the cell [8]. From the multiple sequence alignment and the phylogenetic tree of amino acid sequence correlated with MASP-1, the structural domain is highly conserved. We deduced that the functions of OnMASP-1 may have analogous to the other species.

Tissue distribution results showed that *OnMASP-1* widely existed in all of the tissues tested and mainly expressed in liver. We speculated that the liver was likely to be the major organ for the expression of *OnMASP-1*, which was consistent with the finding in human and grass carp [2,16]. In addition, the expression of *OnMASP-1* in the liver was rapidly increased at 6 h (10.2-fold) after stimulated with *S. agalactiae*. The results showed that the liver might be the main organ to contribute a significant *OnMASP-1* expression against pathogen infection. In teleost, the spleen and head kidney are important immune organs and play vital roles in host defense pathogen infection [9,17,35,36]. After pathogen infection, the expressions of the spleen and head kidney were significantly up-regulated, while the spleen enhanced more remarkable. It was similar to the finding in grass carp [16]. The results may imply that *OnMASP-1* could be induced by bacterial infection and act as an immune-related molecule in *O. niloticus*. This study indicate that MASP-1 probably participates in host defense against pathogen infection in *O. niloticus*.

Many serine proteases are primarily expressed and secreted in hepatocytes, including C1r and C1s [37,38]. Macrophages, as important phagocytic immune cells, are able to initiate a variety of signaling pathways in the body's non-specific cellular defense through their surface specific receptors and secretion of various cytokines when pathogenic microorganisms invade [39,40]. To explore the effects of pathogen on the expression of *OnMASP-1* at cellular level, the hepatocytes and monocytes/macrophages were stimulated *in vitro*. In hepatocytes, *OnMASP-1* showed significant up-regulation after challenges (24 h) (Figs. 7A–1). In monocytes/macrophages, the transcript of *OnMASP-1* revealed rapid and remarkable up-regulation after challenges (Figs. 7B–1). The variance of expression patterns in the hepatocytes and monocytes/macrophages *in vitro* challenges may be due to the different cell types (hepatocytes vs. monocytes/macrophages) [31]. In addition, since MASP-1 is a secretion protein, the concentration of OnMASP-1 was further analyzed in cell supernatant by ELISA. The dynamic tendency of OnMASP-1 was mostly consistent with the mRNA transcript after challenges, with a remarkable up-regulation (Fig. 7). Moreover, the OnMASP-1 concentration was also remarkably increased in serum after challenges (Fig. 6). The results were consistent with the study in humans [8,41]. The OnMASP-1 was probable an acute phase protein like MBL and CL-K1, which was able to make rapid response after bacterial infection [9,31]. When the organism is invaded by pathogen, the improvement of relative concentration of MASPs and the functional integrity may provide an effective antibacterial response [2]. For example, it activates a proteolytic cascade and ultimately forms the membrane attack complex and pathogen lysis [2]. Thus, the results indicate that OnMASP-1 probably participated in the immune defense after pathogen infection.

Monocytes/macrophages are an essential constituent of the mononuclear phagocyte system, which play roles in the non-specific cellular

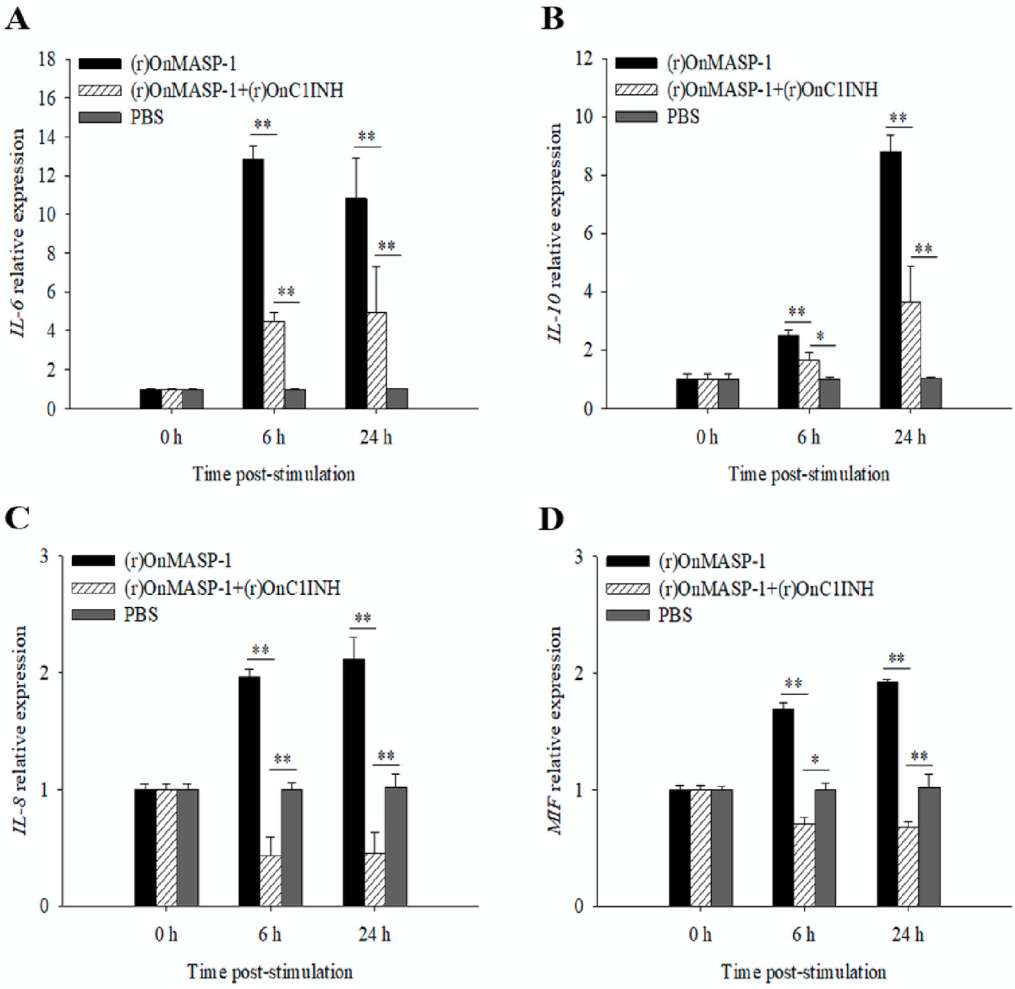

Fig. 9. The mRNA expression of *IL-6* (A), *IL-10* (B), *IL-8* (C) and *MIF* (D) from Nile tilapia in the head kidney monocytes/macrophages. The cells were pre-incubated with C1INH (5 μg/mL) for 30 min and then treated with (r)OnMASP-1 (5 μg/mL) for 6 h or 24 h. The error bars represented standard deviation (n = 3) and significant difference was indicated by asterisks (* $0.01 \leq p < 0.05$, ** $p < 0.01$).

immune defense, including inflammation, migration, phagocytosis and killing [42,43]. In inflammation, monocytes/macrophages have three major functions, including antigen presentation, phagocytosis, and immunomodulation through production of various growth factors, cytokines, and chemokines, such as interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 8 (IL-8, CXCL8), and macrophage migration inhibitory factor (MIF) [31,42,44–48]. Activated monocytes/macrophages can not only release cytokines to be associated with the regulation of immune/inflammatory responses, but also produce chemokines, which stimulate leukocyte movement and migration from the blood to tissues in the immune reaction [42,49]. In mammals, the serine proteases could take part in inflammatory response [2,6]. The transcripts of the *IL-6* and *IL-10* were significantly up-regulated after (r) OnMASP-1 stimulation. The result was accord with the finding in human MASP-1 [6]. Moreover, the transcripts of the chemokines *IL-8* and *MIF* were significantly up-regulated after challenge. The inflammatory and migration reaction can be initiated in a relatively short time within minutes or several hours, and the related cells are able to release cytokines and chemokines rapidly [42,43,49]. The production of a variety of cytokines is involved in the inflammatory response and plays vital roles in combating pathogen infection. For example, IL-6 can activate inflammatory cells and promote the synthesis of acute phase proteins including C-reactive protein and some complement components in hepatocytes, which is beneficial to the body against pathogen infection [40,46]. IL-8 can recruit neutrophil granulocytes and monocytes/macrophages to migrate to the inflammatory foci, which also enhance their phagocytic function and induce them to release inflammatory mediators, and participate in the inflammatory response [40,45]. Interestingly, the peak expressions of the *IL-10* and *MIF* appeared later compared with *IL-6* and *IL-8* after stimulation. This result indicated that the inflammatory response is likely to be a tightly ordered sequence of events. After the initial stimulus, the inflammatory factor will respond rapidly and release, causing the tissue and cells damage, degeneration, even necrosis. However, it also induces a large number of anti-inflammatory factors to promote the repair of the damaged tissue in order to reduce the damage caused by excessive inflammatory factors and achieve a new balance between the internal environment of the body [42,50,51]. The recruitment of monocytes/macrophages occurs in a relatively short period of inflammation, and the rapid release of relevant mediators in cells is the basis of this rapid response mechanism [42,52]. Moreover, we found that the (r)OnC1INH could reduce or block the expression of the cytokines after stimulation of (r)OnMASP-1. The result was similar to the finding in human MASP-1 [6]. C1INH is a serine protease inhibitor and exhibits inhibitory activities against MASP-1 and MASP-2, which plays roles in the regulation of the LP activation and inflammatory reaction [6,7]. Thus, the result implied that OnMASP-1 is likely to participate in the regulation of phagocytes migration and inflammation, in which the C1INH may

inhibit this function for a certain time.

In summary, a *MASP-1* serine protease gene (*OnMASP-1*) was resoundingly cloned and identified from *O. niloticus*, which was high conservative with other species. The *OnMASP-1* was primarily expressed in the liver. After bacterial infection, the mRNA and protein expression of *OnMASP-1* showed significant up-regulation *in vivo* and *in vitro*. This results implied that OnMASP-1 might be facilitated host defense against bacterial infection. In addition, the (r)OnMASP-1 could involve in the regulation of inflammation, while the inhibitor ((r) OnC1INH) was able to reduce these function exertion. These findings suggested that OnMASP-1 may function as a crucial serine protease, possessing apparent capacity to defense pathogen infection, and also associate with the regulation of the non-specific cellular immune defense in *O. niloticus*. Taken together, the MASP-1 is likely to play an important role in host defense of innate immunity.

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Appendix A. Supplementary data

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