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Short communication

Characterization and expression of *galectin-3* after *Streptococcus agalactiae* and *Aeromonas hydrophila* challenge in GIFT strain Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

In mammals, Galectin-3 has been revealed to be widely expressed in immune cells and played important role in immune reactions. However, Galectin-3 is frequently less reported in teleost. In the present study, a molecular characterization and expression analysis of *galectin-3* were conducted in GIFT strain Nile tilapia. The full-length cDNA is 1034 bp with 690 bp of protein coding sequences. The result of qRT-PCR showed that the mRNA of *galectin-3* was widely expressed in various tissues (heart, liver, spleen, gill, kidney, brain, intestine, skin, muscle, and ovary), and the higher expression was observed in immune-related tissues (liver and spleen). The time-course expression analysis revealed that *galectin-3* was significantly up-regulated in intestine (5 h, 50 h, and 7 d), liver (5 h, 50 h, and 7 d), spleen (5 and 50 h), head-kidney (5 and 50 h), gill (5 h and 7 d) after *Streptococcus agalactiae* challenge, and significantly up-regulated in intestine (18, 24, 36, 72, and 96 h), liver (6, 18, 24, 96 h, and 6 d), spleen (18, 24, 36, 72, and 96 h), head-kidney (6, 12, 18, 24, 36, 72, and 96 h), and gill (12, 18, 24, and 36 h) after *Aeromonas hydrophila* challenge. Taken together, these data suggest that *galectin-3* plays a role in immune responses in Nile tilapia after bacterial challenge.

1. Introduction

As a high economic valuable breeding species around the world, Nile tilapia (*Oreochromis niloticus*) widely cultivated in southern China, such as Hainan, Guangdong, Guangxi and Yunnan province et al. Due to the advantage of the fast growth, high fillet yield and easy catch, a line of tilapia known as GIFT-strain, genetically improved farmed tilapia (GIFT), was cultivated and now it is the mainly farmed species [1,2]. GIFT strain of Nile tilapia was widely farmed in China and more than 70% of the cultured area of the tilapia was GIFT strain of Nile tilapia. In recent years, with the expansion of the scale of GIFT strain Nile tilapia culture in China, the occurrence of various infectious diseases has shown an increasing trend, causing huge losses to the cultivation of Nile tilapia [3]. Especially two pathogenic bacteria, *Streptococcus agalactiae* (Gram-positive) [4–7] and *Aeromonas hydrophila* (Gram-negative) [8], have been reported mainly widespread pathogenic pathogens in Nile tilapia. *S. agalactiae*, known as group B streptococcus (GBS), was first reported in 1939 [9], and then it was recognized as the major cause of invasive neonatal infections in mammals such as *Homo spiens*, *Bos taurus*, *Felis catus*, *Canis lupus familiaris* et al. [10–12]. Besides it was also found in fish [13–16], especially in Nile tilapia with the high virulence potential [17,18]. *A. hydrophila* is a rod-shaped, typical gram-negative conditional pathogen bacterium in the aquatic environment with a high virulence to aquatics [19], and the major infectious disease is motile aeromonad septicemia in carp, tilapia, perch, catfish, and salmon [20].

The lectin family members are primordial molecules with multiple functions in vertebrates. Among these functions, the function of

Abbreviations: GIFT, Genetic Improvement of Farmed Tilapia; PBS, phosphate buffer saline; UTR, Untranslated Region; CRD, carbohydrate recognition domain; SEM, Standard Error of Mean

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Table 1
Primers and their sequences in this study.

Primer	Sequence (5′–3′)	Purpose	Product size
galectin-3-F	AGGGCGAGGCTCAGGACTTA	Partial fragment cloning	575bp
galectin-3-R	AGTGAGGGTGCGGTCTGATT	Partial fragment cloning	
galectin-3-GSP5′	AGAATCTGACGGTTCCCTTCAATCTGC	5′RACE outer primer	474bp
galectin-3-NGSP5′	CCAGCCAACCCAGCGGACCTTG	5′RACE inner primer	388bp
galectin-3 -GSP3′	AGGCAGATTGAAGGGAACCGTCAGAT	3′RACE outer primer	584bp
galectin-3-NGSP3′	CAGCTGTTTCTGACGATCAGCTTCTTGC	3′RACE inner primer	422bp
Upm-long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	RACE	
Upm-short	CTAATACGACTCACTATAGGGC	RACE	
NUP	AAGCAGTGGTAACAACGCAGAGT	RACE	
galectin-3-A	TCACCATCGCTGGAACTATCAA	qRT-PCR	159bp
galectin-3-S	CCTCTTTGCCCCACTTTTCA	qRT-PCR	
actin-F	TGGTGGGTATGGGTCAGAAAG	Internal reference	217bp
actin-R	CTGTTGGCTTTGGGGTTCA	Internal reference	

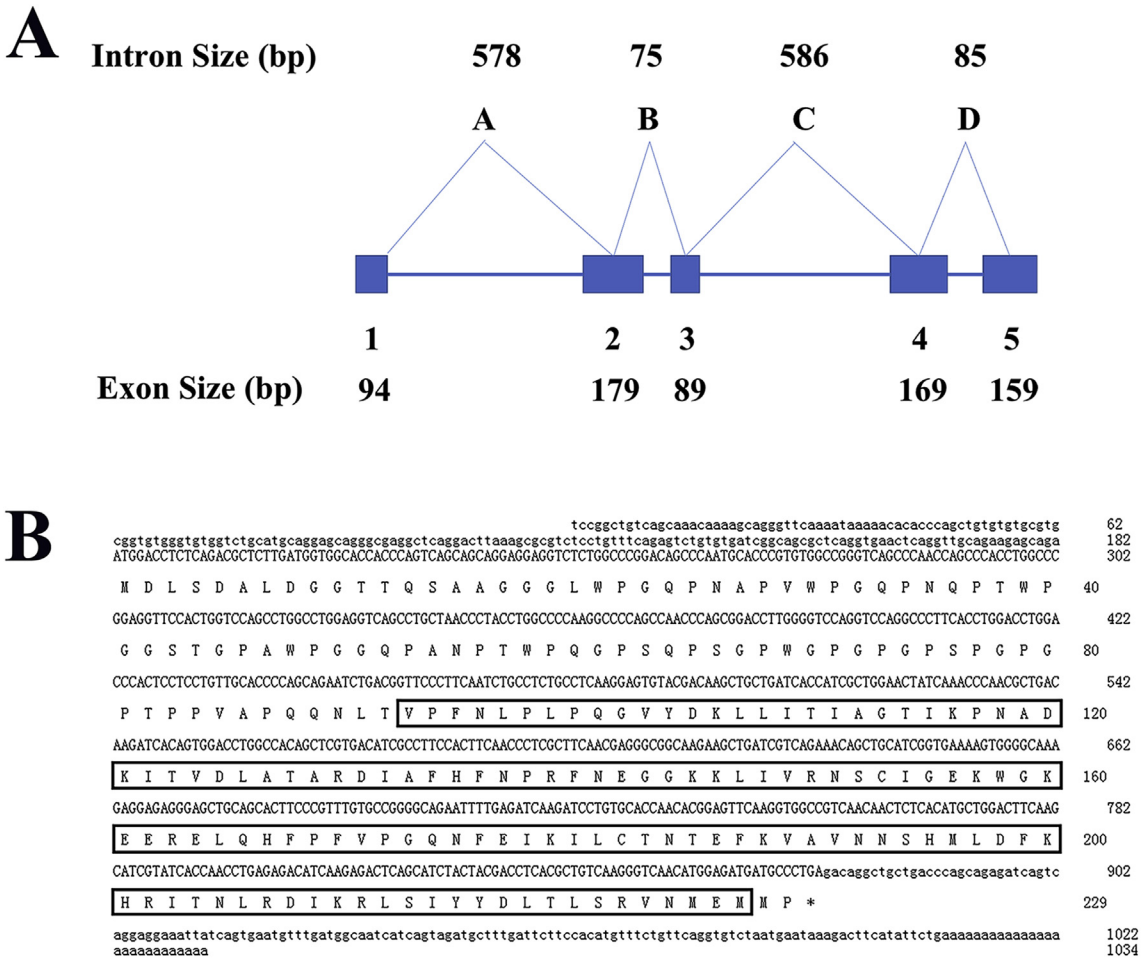

Fig. 1. Genomic structure and cDNA sequence of *galectin 3* from *O. niloticus*. (**A**) Schematic representation of the *galectin-3* genomic structure. The capital letters (A–D) above the line stand for 4 introns, and the solid blue boxes (1-5) stand for 5 exons. (**B**) cDNA and the deduced amino acid sequence of *galectin-3*. An asterisk represents the stop codon, and the lowercase for noncoding region in cDNA. Two functional domains are predicted: the C-terminal carbohydrate recognition domain (CRD) is boxed; the N-terminal end is underlined. The genome sequence of *galectin-3* is downloaded from GenBank database (Accession No. NC_031983.1). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

immunity is most outstanding one. They have been demonstrated to participate in immunity or inflammation by binding to surfaces of various infectious agents to agglutinate cell, sedimenting polysaccharide and complex carbohydrate [21–24]. So far, the five types of lectin family members have been found in fish, including Galectin, C-type lectin, Lily-type lectin, Rhamnose-lectin and Intelectin [24]. Galectins are S-type lectins, as kinds of pattern recognition receptors can molecular recognize relevant pathogens specifically and participate in a series of biological immune activity such as antibody opsonization, cytophagy, complement activation and death of microbes and so on [25,26]. In vertebrates, genes of 16 galectin family members have been found and divided into three basic types based on the structural features of carbohydrate recognition domain (CRD): the prototype galectins (*galectin-1*, *2*, *5*, *7*, *10*, *11*, *13*, *14*, *15*, *16*) with the globular CRD; The chimera *galectin-3*; The tandem repeat galectins (*galectin-4*, *6*, *8*, *9*, *12*) with two CRDs [25–27]. Galectin-3 is the only chimera *galectin* with one

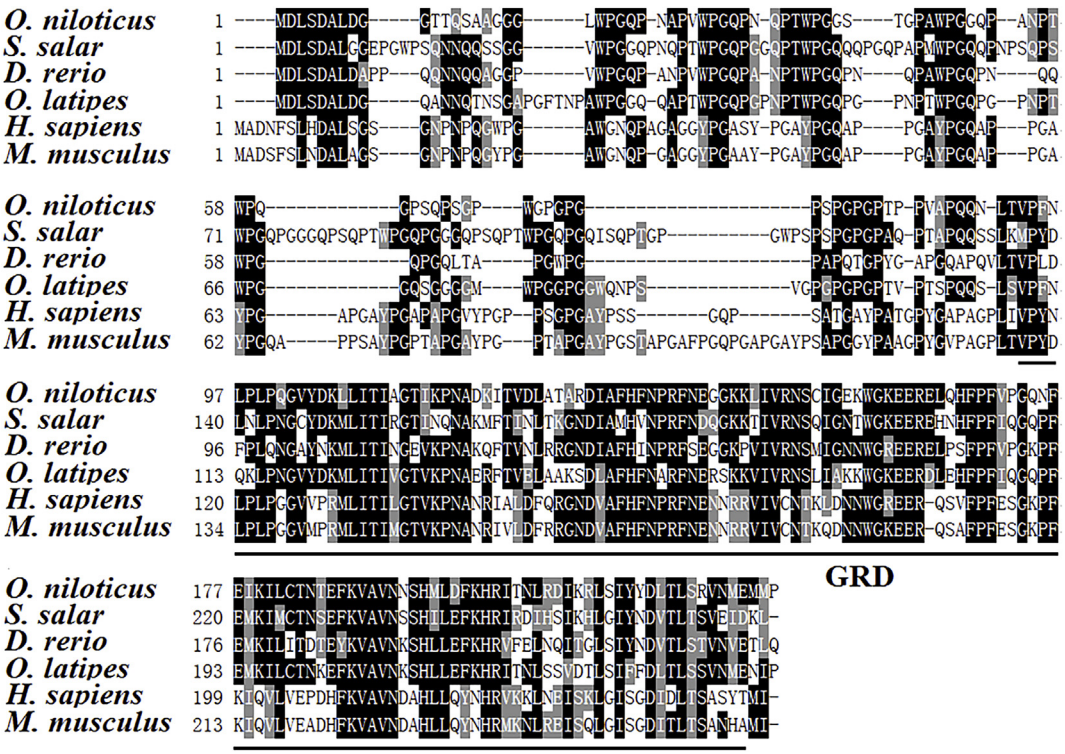

Fig. 2. Multiple alignments of the deduced amino acid sequences of *galectin3* gene. The conserved amino acids and domains are marked in black and solid lines, respectively. The corresponding sequence information is showed in Table S1. GRD, Galactoside-binding domain which is one kind of CRD (carbohydrate-recognition domain) is underlined.

C-terminal carbohydrate recognition domain connected to the N-terminal end. It was first observed on the outer membrane of macrophages and named Mac-2 antigen and later described as IgEBP (or εBP), CBP35, CPB30, HL29, RL-29, hL-31 and LBP by different research groups [28–32]. In mammals, galectin-3 has been revealed to be widely expressed in immune cells (activated T cells, B cells and inflammatory macrophages) [33,34] and played important roles in T-cell apoptosis [35–37], inflammation [38,39], and tumor [40]. In *galectin-3* knock-out mice, the number of immune cells was significantly reduced following the infection [41]. In fish, Galectins were purified from the skin mucus of the Japanese conger (*Conger myriaster*), participates in innate immunity on the intra- and the extra-body surface of the conger [42]. In addition, two homologous *galectin-3* genes were reported in channel catfish and revealed to play vital role in catfish mucosal immunity [43].

In this study, the *galectin-3* was selected based on the transcriptomic data from Nile tilapia following the *S. agalactiae* challenge [7]. This study was conducted to dissect the functionality of *galectin-3* in *O. niloticus*, we have cloned the full-length cDNA of *galectin-3* and determined its expression profile in different tissues. To further illustrate the immune function of *galectin-3*, we analyzed *galectin-3* expression in immune-related tissues after *S. agalactiae* and *A. hydrophila* challenge.

2. Materials and methods

2.1. Experimental animals

GIFT strain Nile tilapia (body weight, 50 ± 0.45 g) was obtained from Guangxi Academy of Fishery Sciences. The fish were cultured in a flow-through system for two weeks before experimental challenge. Ten tissues (heart, liver, spleen, gill, kidney, brain, intestine, skin, muscle, and ovary) were collected from three untreated individuals, and transferred to liquid nitrogen and stored at −80 °C for RNA extraction. Moreover, 70 individuals were sampled and cultured in a flow-through system at room temperature for two weeks, and challenged with *S. agalactiae* and *A. hydrophila*, respectively. The culture methods for the bacterial *S. agalactiae* and *A. hydrophila* were conducted as previously described [7]. According to the previous study, the final concentration of *S. agalactiae* and *A. hydrophila* used for intraperitoneal injection was set at 1.83×10^7 and 1.8×10^7 colony-forming units (cfu) [7], respectively, and PBS was used as negative control. According to characteristic of *S. agalactiae*'s proliferation, in the previous study, the sampling time-points of *S. agalactiae* treated group were set at 0, 5, 50 h, and 7 d after injection [7], and the sampling time-points of *A. hydrophila* treated group were set at 6, 12, 18, 24, 36, 72, 96 h, 6 d, and 7d following the previous study on *A. hydrophila* [44]. Five immune-related tissues (intestine, liver, spleen, head-kidney, and gill) were collected at each time points after bacterial challenge for total RNA extraction (three individuals for each group at each time point).

2.2. RNA extraction, cDNA synthesis, and RACE PCR

The total RNA was isolated from various tissues using TRIzol reagents (Omega, USA) according to the manufacturer's instructions. The cDNA was synthesized using the cDNA synthesis reagent kit (TaKaRa Bio Inc., Otsu, Japan). To obtain the full-length cDNA of *galectin-3*, the RACE PCR was conducted using Smart RACE cDNA amplification kit (Clontech Inc., CA, USA) according to the manufacturer's instructions. The nested PCR was conducted with the universal primer (UPM) and the universal primer (NUP), and the conditions of PCR amplifications were performed described as before [45]. All the primers used in this study were displayed in Table 1. The products of PCR amplifications were electrophoresed on a 1.0% agarose gel and purified using Gel Extraction Kit (Tiangen, Beijing, China).

2.3. Bioinformatics sequence analysis

Amino acid sequence of Galectin-3 was predicted using the DNAStar 7.0 software (DNAStar, Madison, WI, USA). The conserved domains

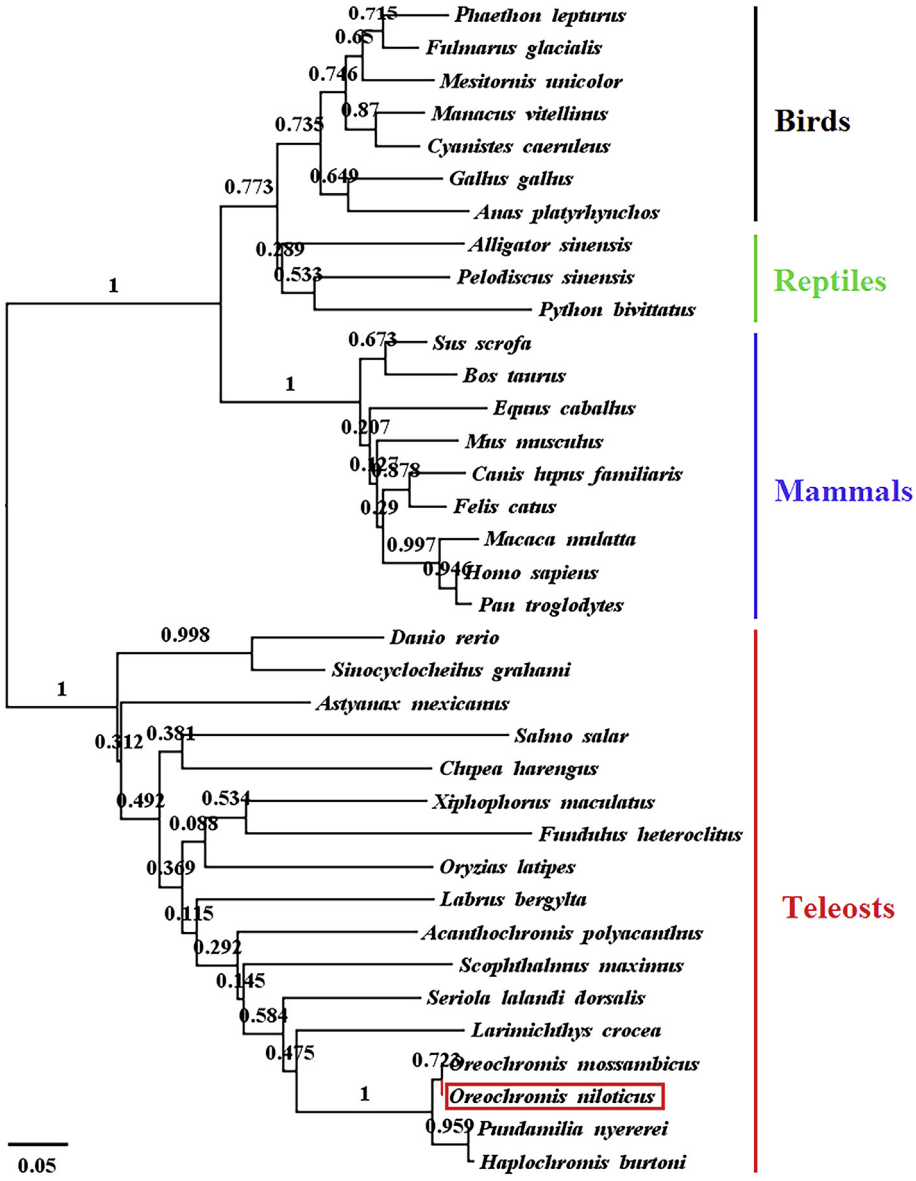

Fig. 3. Phylogenetic analysis of *galectin-3* using neighbor-joining (NJ) method. Bootstrap values are shown at the nodes. The corresponding sequences information is displayed in Table S1.

were analyzed using online software SMART (Simple Modular Architecture Research Tool) (http://smart.embl-heidelberg.de/smart/set_mode.cgi?NORMAL=1). The genomic structure of *galectin-3* was analyzed by the online software Splign (http://www.ncbi.nlm.nih.gov/sutils/splign/splign.cgi?textpage=online&level=form) with default parameters. Multiple alignments of the deduced amino acid sequences were conducted using MEGA v6.0 and the online software BoxShade (https://embnet.vital-it.ch/software/BOX_form.html).

2.4. Phylogenetic analysis

The neighbor-joining (NJ) phylogenetic tree was constructed using MEGA v6.0 software method with the default parameters [46]. The amino acid sequences of Galectin-3 used in this study were downloaded from the GenBank database (http://www.ncbi.nlm.nih.gov/), and the GenBank accession numbers of sequences were displayed in Table S1. The final phylogenetic tree was edited with FigTree v1.4 software (http://tree.bio.ed.ac.uk/software/figtree/).

2.5. Real-time quantitative PCR

Quantitative real-time PCR (qRT-PCR) was performed using the Takara SYBR® green I Premix Ex Taq™ system (TAKARA, Dalian, China) in a 20 μl standard reaction volume. The *β-actin* of Nile tilapia was used as suitable endogenous control. The primers used in the qRT-PCR were specifically designed based on the sequences of the target genes and listed in Table 1. The amplification procedure of the qRT-PCR was conducted as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s, and disassociation curve analysis for target specificity as previously described [43]. The relative expression of *galectin-3* was calculated with the $2^{-\Delta\Delta Ct}$ method [47]. All the samples were amplified in triplicate, and the differences of expression levels were analyzed using the SPSS v18.0 software (IBM, New York, USA). Values are indicated as means ± SEM (Standard Error of Mean), and statistically significant differences are represented with different letters ($P < 0.05$), * ($P < 0.05$) or ** ($P < 0.01$).

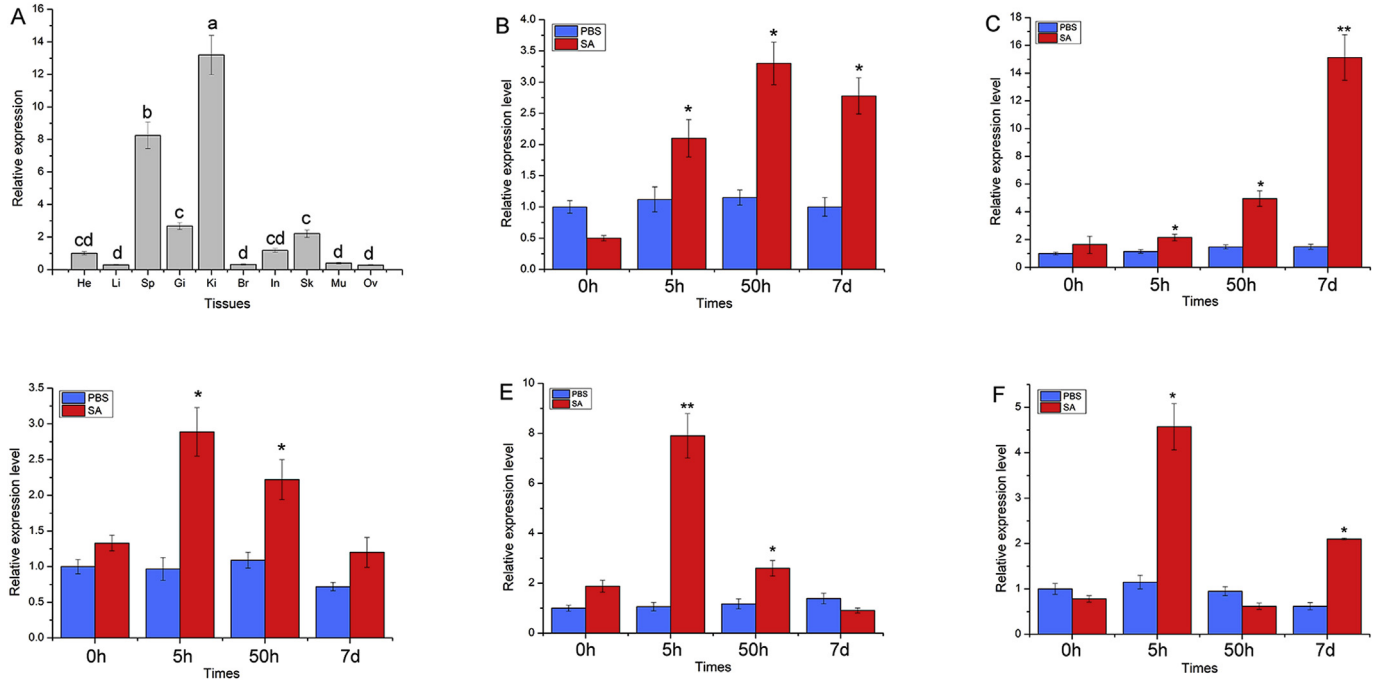

Fig. 4. Expression levels of *galectin-3* in various tissues and in immune-related tissues upon *S. agalactiae* infection. (**A**) Relative mRNA expression levels of *galectin-3* in various tissues. (**B**) Relative mRNA expression of *galectin-3* in the intestine at different time points after *S. agalactiae* challenge. (**C**) Relative mRNA expression of *galectin-3* in the liver at different time points after *S. agalactiae* challenge. (**D**) Relative mRNA expression of *galectin-3* in the spleen at different time points after *S. agalactiae* challenge. (**E**) Relative mRNA expression of *galectin-3* in the head-kidney at different time points after *S. agalactiae* challenge. (**F**) Relative mRNA expression of *galectin-3* in the gill at different time points after *S. agalactiae* challenge. He: heart; Li: liver; Sp: spleen; Gi: gill; Ki: kidney; Br: brain; In: intestine; Sk: skin; Mu: muscle; Ov: ovary. SA: *S. agalactiae* injected group; PBS: control group. Values are indicated as the means ± SEM (N = 3). The expression levels with the same letters are not significantly different ($P < 0.05$). *$P < 0.05$, **$P < 0.01$.

3. Results and discussions

3.1. Gene cloning and characterization analysis

The full-length cDNA of *galectin-3* was obtained from *O. niloticus* in this study. It is 1034 bp in length, containing a 182 bp 5′ untranslated region (UTR), a 690 bp open reading frame (ORF), and a 162 bp 3' UTR (Fig. 1B). To illustrate the genomic structure of *galectin-3*, we mapped the ORF sequence of *galectin-3* on genomic sequence (GenBank accession No. NC_031983.1). The result showed that the *galectin-3* gene was consisted of 5 exons (1-5) and 4 introns (A-D) (Fig. 1A). Moreover, the *galectin-3* encoded a 229 amino acid residues (aa) putative protein with the 24.81 kDa predicted molecular weight and the 7.98 isoelectric point (PI). Two functional domains were predicted in *galectin-3* from *O. niloticus*, one C-terminal carbohydrate recognition domain (CRD) and one N-terminal end (Fig. 1B), which were typical domains in Galectin-3 family [25–29]. Galectin-3 is the only chimera *galectin* with one CRD connected to the N-terminal end, and the CRD was conserved in vertebrates (Fig. 2). The N-terminal end and the CRD coordinate to induce signaling pathways and involve in biological processes including T-cell apoptosis [35], caspase-9 activation [36].

3.2. Phylogenetic analysis

To evaluate the evolutionary relationship of *galectin-3* in different species, a phylogenetic analysis was conducted based on amino acid sequences of Galectin-3 from different species. It is clear that *O. niloticus* shares a close ancestor with *Oreochromis mossambicus*, as they form a monophyletic clade that is supported with a high posterior probability (posterior probability = 0.72; Fig. 3). It is also supported by the result of traditional morphological classification in *O. niloticus* and *Oreochromis mossambicus*, both of which belong to Genus *Oreochromis*. Additionally, birds, reptiles, and mammals form a big clade (posterior probability = 1), and teleosts form another clade with a strong posterior probability (posterior probability = 1).

3.3. Expression profile of galectin-3

To determine the expression level of *galectin-3* in different tissues from *O. niloticus*, qRT-PCR was used to evaluate the distribution of its mRNA in a wide range of tissues (from untreated individuals) including heart, liver, spleen, gill, kidney, brain, intestine, skin, muscle, and gonad (ovary). As shown in Fig. 4A, the higher expression levels were observed in the kidney, spleen, gill, and skin, and lower transcription in other tissues. The skin and gill play a key role in filtration of suspended matter and as first barriers against the pathogen, and involved in innate and adaptive immunity of fish [48,49]. Moreover, the spleen, and kidney are also proved to be important immune organs in fish [50–53]. The high expression of *galectin*-3 mRNA in these important immune-related tissues (kidney, spleen, gill, and skin) indicates that *galectin-3* may play a role in immune response of *O. niloticus*.

To illustrate the potential roles of *galectin-3* in immune response, we determined the mRNA expression level of *galectin-3* in immune-related tissues at different time points after *S. agalactiae* challenge using qRT-PCR method. The expression levels of *galectin-3* at different sampling time points in *S. agalactiae* injected group were compared with those of PBS injection group. The results revealed that the mRNA expression levels of *galectin-3* were significantly up-regulated in five immune-related tissues including intestine (5 h, 50 h, and 7 d; Fig. 4B), liver (5 h, 50 h, and 7 d; Fig. 4C), spleen (5 and 50 h; Fig. 4D), head-kidney (5 and 50 h; Fig. 4E), gill (5 h and 7 d; Fig. 4F). In these tested tissues, the *galectin-3* displayed different expression patterns (intestine, liver, spleen, and head-kidney: up or up-down; gill: up-down-up) and different peak points (spleen, head-kidney, gill: 5 h; intestine: 50 h; liver: 7 d) during pathogen challenge. Similar to Nile tilapia *OnTLR21* and *OnTLR22* [54], the expression patterns in different tissues after

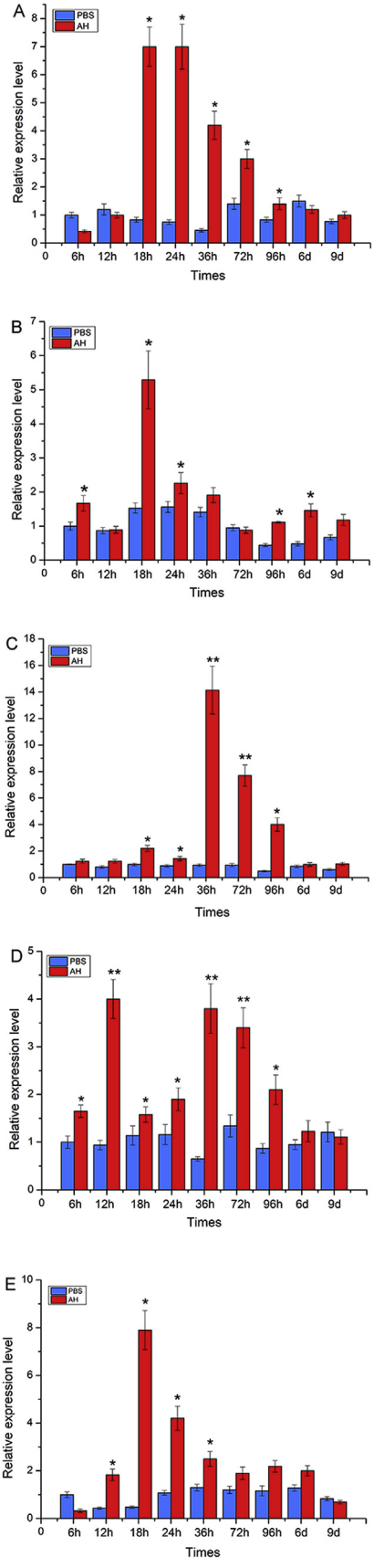

Fig. 5. Expression levels of *galectin-3* in the intestine (**A**), liver (**B**), spleen (**C**), head-kidney (**D**), and gill (**E**) at different time points after *A. hydrophila* infection. AH: *A. hydrophila* injected group; PBS: control group. Values are indicated as means ± SE (N = 3). $^{*}P < 0.05$, $^{**}P < 0.01$.

bacterial challenge were different. In Chinese tongue sole (*Cynoglossus semilaevis*), the immune-related genes *sghC1q* and *rspo2l* share the similar expression pattern that different expression profiles in different tissues after bacterial challenge [55,56]. The peak appearance of *galectin-3* expression at different time points in different tissues is similar to tongue sole *dctn5_tv1*, which is likely due to the time discrepancy of bacterial invasion or immune response in various tissues [57]. The up-regulated expression levels in these immune-related tissues after infection reveal that *galectin-3* is probably involved in immune responses against pathogenic bacterium, *S. agalactiae*. Furthermore, *O. niloticus* was also challenged with another pathogenic bacterium (*A. hydrophila*). The results showed that *galectin-3* expression was significantly up-regulated in intestine (18, 24, 36, 72, and 96 h; Fig. 5A), liver (6, 18, 24, 96 h, and 6 d; Fig. 5B), spleen (18, 24, 36, 72, and 96 h; Fig. 5C), head-kidney (6, 12, 18, 24, 36, 72, and 96 h; Fig. 5D), and gill (12, 18, 24, and 36 h; Fig. 5E). The expression patterns in three tissues (intestine, spleen, and gill) shared the common feature (up-down), and a complex expression pattern (up-down-up-down) was observed in liver and head-kidney. Although the expression patterns of *galectin-3* in these tissues between *S. agalactiae* and *A. hydrophila* treated groups showed slightly different, their overall trend displayed common feature (up or up-down). Taken together, the significant up-regulated expression of *galectin-3* after bacterial challenge indicates that *galectin-3* may involve in immune response of Nile tilapia.

4. Conclusion

In the present study, we obtained the full-length cDNA of *galectin-3* in Nile tilapia. It was 1034 bp in length with a 182 bp 5′ UTR, a 690 bp ORF, and a 162 bp 3' UTR. The *galectin*-3 mRNA was highly expressed in the kidney, spleen, gill, and skin using the qRT-PCR method. Furthermore, the *galectin-3* transcripts were up-regulated in immune-related tissues (intestine, liver, spleen, head-kidney, and gill) after *S. agalactiae* and *A. hydrophila* challenge. Overall, this study suggests that Nile tilapia Galectin-3 may play a role in immune response after bacterial challenge, but the further studies are needed to uncover its molecular mechanism in immune response.

Conflicts of interest

The authors have declared that no competing financial interests exist.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2018.12.036.

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