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A C-type lectin that inhibits bacterial infection and facilitates viral invasion in black rockfish, *Sebastes schlegelii*

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ABSTRACT

C-type lectins (CTLs) are important pattern recognition receptors (PRRs) that play vital roles in innate immunity. In teleosts, a number of CTLs have been reported, but their *in vivo* effects on host defense are still limited. In this study, a CTL homolog (SsLec1) was identified from black rockfish, *Sebastes schlegelii*, and its structure, expression and biological function was analyzed. The open reading frame of SsLec1 is 633 bp, with a 5'- untranslated region (UTR) of 36 bp and a 3′- UTR of 117 bp. The deduced amino acid sequence of SsLec1 shares the highest overall identity (73.20%) with the CTL of *Oplegnathus fasciatus*. SsLec1 possesses conserved CTL features, including a carbohydrate-recognition domain, four disulfide bond-forming cysteine residues, the mannose-type carbohydrate-binding motif, the conserved calcium binding sites and a putative signal peptide. The expression of SsLec1 was highest in liver and could be induced by experimental infection with *Listonella anguillarum*. Recombinant SsLec1 (rSsLec1) purified from *E. coli* was able to bind and agglutinate the Gram-negative fish pathogens *Vibrio ichthyoenteri* and *Vibrio vulnificus*. The agglutinating ability of rSsLec1 was abolished in the presence of mannose or ethylenediaminetetraacetic acid. Further analysis showed that rSsLec1 could enhance phagocytosis by macrophages. *In vivo* experiments indicated that rSsLec1 could inhibit bacterial infection and promote viral invasion. Taken together, these results suggest that SsLec1 is a novel CTL that possesses apparent immunoregulation property and plays a critical role in host defense against pathogens invasion.

1. Introduction

Lectins are non-enzyme protein that can bind with carbohydrates on the cell surface. They are widespread in living organisms, from microbes, plants, to animals [1]. Based on structural characteristics, lectins from animals are classified into several families [2], one of which is C-type lectins (CTLs). CTLs are a large and the most well-studied family, most of the CTLs are Ca^{2+}-dependent, and a few of CTLs are Ca^{2+}-independent [3]. The CTLs generally contain at least one carbohydrate recognition domain (CRD), which was consisted of 115–130 amino acids and folds into a double-loop spatial structure that is stabilized by the two disulfide bridges formed by four conserved cysteine residues in the CRD [3,4]. Based on carbohydrates binding specificity, CTLs are classified into two main categories, mannose-specific type and galactose-specific type, the corresponding conserved binding motif in CRD were Glu-Pro-Asn (EPN) or Gln-Pro-Asp (QPD), respectively [4].

Since CTLs can recognize and bind the specific carbohydrate structure, which is designated as pathogen-associated molecular patterns (PAMPs), on the surface of microbes, therefore, they are considered as a major class of pattern recognition receptors (PRRs) and play critical roles in triggering innate immunity against invading pathogens. By interacting with pathogens, CTLs can stimulate a variety of immune responses, including pathogen recognition [5], cell adhesion [6], phagocytosis [7], production of reactive oxygen species, cytokine release [8–10], anti-bacterial, anti-fungal or anti-viral activity [11–13], and activating the complement system [14], etc. Furthermore, CTLs exist in two forms, either as secreted soluble proteins or as transmembrane proteins [15], the former including mannose-binding protein (MBP), a member of secreted CTLs, that can activate the complement system through the lectin pathway [16], the latter are known to function in

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dendritic cell activation and antigen uptake [17,18].

In teleosts, a number of CTLs have been identified so far. Such as CTLs from ayu (*Plecoglossus altivelis*) [19], tongue sole (*Cynoglossus semilaevis*) [20], grass carp (*Ctenopharyngodon idella*) [21], orange-spotted grouper (*Epinephelus coioides*) [22], rainbow trout (*Oncorhynchus mykiss*) [23], Atlantic salmon (*Salmo salar*) [24], Japanese eels (*Anguilla japonica*) [25], common carp (*Cyprinus carpio*) [26–29], Japanese flounder (*Paralichthys olivaceus*) [30], and turbot (*Scophthalmus maximus*) [31], etc. Most of these CTLs are known to be able to interact with microorganisms or different PAMPs, and some of them could enhance phagocytosis, whereby playing important roles in innate immunity [19,20,31]. However, the *in vivo* effects of CTLs on fish defense against pathogens infection are still limited.

Black rockfish (*Sebastes schlegelii*) is cultured widely in China, Korea and Japan as an economic fish species. However, the fish has long been suffered from serious diseases at present, and the immune mechanism of black rockfish responses to pathogens infection is very limited. In this study, a new CTL homolog from black rockfish (SsLec1) was identified and characterized. The tissue distribution and expression pattern of SsLec1 post-bacterial infection was examined. The agglutination activity and opsonization ability of recombinant SsLec1 (rSsLec1) was investigated. Moreover, the role of rSsLec1 in defense against bacterial and viral infection was analyzed. These results will be helpful to further understanding the biological functions of teleosts CTLs in innate immunity.

2. Materials and methods

2.1. Fish

Black rockfish (*Sebastes schlegelii*) (average 8.2 ± 1.3 g) were purchased from a commercial fish farm in Shandong Province, China and maintained at 20 °C in aerated seawater. Fish were acclimatized in the laboratory for two weeks before experimental manipulation. Before experiment, fish were randomly sampled and verified to be absent of pathogens in tissues as reported previously [32,33]. Before tissue collection, fish were euthanized with an overdose of tricaine methanesulfonate (Sigma, St. Louis, MO, USA) as reported previously [34].

2.2. Bacterial and viral strains

Listonella anguillarum C1 was kindly provided by Doctor Cheng of Qingdao Agricultural University; fish megalocytivirus, infectious spleen and kidney necrosis virus (ISKNV), and *Vibrio vulnificus* PR1 were kindly provided by Doctor Li of Pearl River Fishery Research Institute, Chinese Academy of Fishery Sciences. *Escherichia coli* DH5α was purchased from Transgene (Beijing, China), *Vibrio ichthyoenteri* 1A00059 was purchased from Marine Culture Collection of China. *Pseudomonas putida* C1 and *Streptococcus agalactiae* G1 were preserved in the laboratory. *S. agalactiae* G1 was cultured in Brian Heart Infusion (BHI) medium, other strains were cultured in Luria-Bertani broth (LB) medium. *S. agalactiae* G1 and *E. coli* DH5α were cultured at 37 °C, all other strains were cultured at 28 °C.

2.3. Cloning of SsLec1

A cDNA library of black rockfish head kidney and spleen was constructed according to the method reported by Wang et al. [35]. Briefly, the bacterial fish pathogen *L. anguillarum* was cultured at 28 °C to the mid-logarithmic phase, then washed and resuspended in phosphate-buffered saline (PBS). Five black rockfish (~450 g) as described above were randomly selected, and 5×10^7 colony forming units (CFU) (diluted in 1 ml PBS) of *L. anguillarum* was administered via intraperitoneal injection. At 24 h post-infection, tissues were collected under sterile conditions. Total RNA was isolated with an RNAprep Tissue Kit (Tiangen, Beijing, China), and a cDNA library was constructed with the Super SMART PCR (Polymerase Chain Reaction) cDNA synthesis kit (Clontech, Mountain View, CA, USA) according to the manufacturer's instructions. The subsequent DNA sequence analysis showed that one of the clones contained the full-length cDNA of *SsLec1*, with 5'- and 3'-untranslated regions (UTRs).

2.4. Sequence analysis

The cDNA and amino acid sequence of SsLec1 were analyzed using the BLAST program at the National Center for Biotechnology Information (NCBI). Signal peptide search and domain search were performed with the simple modular architecture research tool (SMART) version 4.0. The molecular mass and theoretical isoelectric point (pI) were predicted using DNAman software package (Lynnon Biosoft, Quebec, Canada).

2.5. Quantitative real time reverse transcription-PCR (qRT-PCR) analysis of SsLec1 expression

2.5.1. SsLec1 expression in fish tissues under normal physiological conditions

Blood, liver, gills, spleen, kidney, heart, muscle, brain and intestine were taken aseptically from five fishes and used for total RNA extraction with the RNAprep Tissue Kit (Tiangen, Beijing, China). One microgram of total RNA was used for cDNA synthesis with the Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). qRT-PCR was carried out in a LightCycler 96 system (Roche Applied Science, North Carolina, USA) using the SYBR ExScript qRT-PCR Kit (Takara, Dalian, China) as described previously [31]. The primers used to amplify *SsLec1* are *SsLec1*RTF1 and *SsLec1*RTR1 (Table 1), Melting curve analysis was carried out at the end of each PCR to confirm the specificity of PCR products. The black rockfish elongation factor 1α (SsEF1A) gene was used as an internal control (GenBank accession no: KF430623), which was previously proved as an appropriate internal control for qRT-PCR normalization [36]. The expression level of *SsLec1* was analyzed using comparative threshold cycle method ($2^{-\Delta\Delta CT}$). The primers used to amplify *SsEF1A* are *SsEF1A*F1 and *SsEF1A*R1 (Table 1). All data are given in terms of relative mRNA levels to that of tissue in which *SsLec1* expression was the lowest.

2.5.2. SsLec1 expression in fish tissues in response to bacterial infection

To examine *SsLec1* expression in response to bacterial infection, *L. anguillarum* was cultured as above and resuspended in PBS to 1×10^7 colony forming units (CFU)/ml. Black rockfish were divided randomly into two groups (30 fishes/group), and injected intraperitoneally (i.p.) with 100 μl of *L. anguillarum* or PBS (control). Fish were sacrificed at 4 h, 8 h, 12 h, 24 h, and 48 h post-infection, and

Table 1
PCR primers used in this study.

Primers	[a]Sequences (5′–3′)	Target genes
EF1AF1	5′- AACCTGACCACTGAGGTGAAGTCTG-3′	*EF1A*
EF1AR1	5′- TCCTTGACGGACACGTTCTTGATGTT-3′	
*SsLec1*RTF1	5′-AGGAGACTGGAGATGGGTGGA-3′	*SsLec1*
*SsLec1*RTR1	5′-CGGACAGGTGGTTGTTTGG-3′	
*SsLec1*F2	5′- GATATCCTCATCGGCTTGTTGGC-3′	*SsLec1*
*SsLec1*R2	5′- GATATCCTGGGGCAGGATGGC-3′	

[a] Underlined nucleotides are the enzyme restriction sites.