Fish and Shellfish Immunology 93 (2019) 251–257

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Molecular cloning, characterization, and expression of a C-type lectin from *Scylla paramamosain,* which might be involved in the innate immune response

Weijia Zhang[a,b], Zhouyi Zhang[a,b], Changkao Mu[a], Ronghua Li[a], Yangfang Ye[a], Hao Zhang[a,**], Weiwei Song[a], Ce Shi[a], Lei Liu[a], Chunlin Wang[a,*]

[a] Key Laboratory of Applied Marine Biotechnology, Ministry of Education, Ningbo University, Ningbo, 315211, China
[b] Collaborative Innovation Center for Zhejiang Marine High-efficiency and Healthy Aquaculture, Ningbo University, Ningbo, 315211, China

ARTICLE INFO

Keywords:
C-type lectin
Scylla paramamosain
Agglutinating activity
Innate immunity

ABSTRACT

C-type lectins (CTLs) have characteristic carbohydrate recognition domains (CRDs) and play important roles in the immune system. In the present study, a new CTL, *Sp*CTL5, was identified from the hepatopancreas of the mud crab *Scylla paramamosain*. The open reading frame of *Sp*CTL5 comprised 762 bp, encoding a polypeptide of 253 amino acids with a putative signaling peptide of 20 amino acids. The predicted *Sp*CTL5 protein contained a single CRD. *Sp*CTL5 transcripts were distributed in all examined tissues, with the highest level being detected in the hepatopancreas. Upon challenging with *Vibrio alginolyticus*, the mRNA levels of *Sp*CTL5 in the hepatopancreas were up-regulated. The recombinant protein of *Sp*CTL5 could agglutinate three Gram-positive bacteria and three Gram-negative bacteria in the presence of Ca^{2+}. Furthermore, hemagglutination analysis showed that the recombinant protein of *Sp*CTL5 can agglutinate rabbit erythrocytes. This study indicated that *Sp*CTL5 acts as a pattern recognition receptor for the innate immune response which protects *S. paramamosain* from bacterial infection. Moreover, these findings also provide information to further our understanding of the innate immunology of invertebrates.

1. Introduction

The innate immune system is the first line of inducible host defense against bacterial, fungal, and viral pathogens [1,2]. Lectins are a type of pattern recognition receptor (PRR) that participate in pathogen recognition and other defense responses [3–5], and as such, play significant roles in non-self recognition and the clearance of invading pathogens by the innate immune system [6,7]. Among the lectin superfamily, C-type lectins (CTLs) are particularly important, having a diverse range of functions that include important roles in innate immune response [8]. CTLs have at least one characteristic carbohydrate recognition domain (CRD). These domains consist of 115–130 amino acids with a well-defined structure stabilized by two or three bonded pairs of sulfide groups, and bind to the carbohydrate residues of foreign pathogens [9,10]. CTLs have been demonstrated to be Ca^{2+}-dependent pattern recognition receptors that are involved in the development of invertebrates and the regulation of their innate immune responses, such as nodule formation, phagocytosis, microbial agglutination, antiviral responses, and encapsulation [11].

The mud crab *Scylla paramamosain* is one of the most valuable marine species in China. It is widely cultured in brackish and seawater ponds along the coast of southeastern China [12]. Furthermore, with the advancement of artificial breeding techniques, the *S. paramamosain* aquaculture industry has rapidly expanded. However, several bacterial and viral pathogens that infect marine crabs, such as *Vibrio alginolyticus,* have been reported [13].

A number of CTLs have been isolated from decapod crustaceans, including five CTLs from *S. paramamosain*. These are *Sp*Lec1, *Sp*Lec2, *Sp*-lectin3, *Sp*-lectin4, and *Sp*CTL-B. Quantitative real-time analyses have revealed that *Sp*Lec1, *Sp*Lec2 [14], and *Sp*-lectin4 [15] were expressed highest in the hepatopancreas. Furthermore, *Sp*Lec1, *Sp*Lec2, *Sp*-lectin3 [15], *Sp*-lectin4, and *Sp*CTL-B [4] were significantly up-regulated in the hepatopancreas after being exposured to *Vibrio parahemolyticus*. In the same study, antimicrobial assays showed that recombinant *Sp*CTL-B inhibits the growth of all the Gram-positive and Gram-negative bacteria examined [4].

* Corresponding author. School of Marine Sciences, Ningbo University, 818 Fenghua Rd., Ningbo, 315211, China.
** Corresponding author. School of Marine Sciences, Ningbo University, 818 Fenghua Rd., Ningbo, 315211, China.
E-mail addresses: zhanghao@nbu.edu.cn (H. Zhang), wangchunlin@nbu.edu.cn (C. Wang).

https://doi.org/10.1016/j.fsi.2019.07.035
Received 17 April 2019; Received in revised form 12 July 2019; Accepted 12 July 2019
Available online 15 July 2019

In this study, a new CTL, *Sp*CTL5, was isolated from the hepatopancreas of *S. paramamosain*, and its structural features as well as its hemagglutination and bacterial agglutination activities were analyzed. The tissue distribution and expression profile of *Sp*CTL5 in the hepatopancreas of *V. alginolyticus*-challenged *S. paramamosain* were also investigated.

2. Materials and methods

2.1. Immune system challenging and sample collection

One hundred healthy *S. paramamosain* (50 ± 10 g) were purchased in the crab fishing area of Ningbo in Zhejiang Province, China. These crabs were temporarily cultured at 22–23 °C for a week in 25‰ salinity seawater before injecting with *V. alginolyticus*. All crabs were fed with clams, and the seawater was changed every day. The *V. alginolyticus* used to inject the crabs was provided by our laboratory.

The crabs were divided into two groups. Each challenge group crab received an injection of 0.5 mL living *V. alginolytcus* suspended in sterilized saline solution (pH 7.0, 10^6 CFU mL^{-1}) into the arthrodial membrane of its back walking leg. The control group crabs received an injection of 0.5 mL sterilized saline solution. Then, the processed crabs were returned into their former environment and five individuals were randomly sampled at 0, 3, 6, 12, 24, and 48 h.

Tissues including the stomach, heart, gonad, eyestalk, hemocytes, muscle, and hepatopancreas of five non-challenged crabs were collected. The hemolymphs were collected in tubes containing ice-cold ACD anticoagulant buffer (1.32% sodium citrate, 0.4% citric acid, 1.47% glucose) and centrifuged at 3,000 rpm and 4 °C for 10 min to collect the hemocytes for RNA extraction.

2.2. Total RNA extraction and first-strand cDNA synthesis

Total RNA was extracted via rapid extraction with the TRIzol reagent (TaKaRa, Japan). The quality of RNA was assessed using a microplate reader at A260/280. A total of 1 μg RNA from the hepatopancreas was reversely transcribed with M-MLV reverse transcriptase (Promega, USA) for *Sp*CTL5 cloning. For the other samples, the first-strand cDNA was synthesized for qRT-PCR.

2.3. Cloning the cDNA of SpCTL5

Partial cDNA sequence of *Sp*CTL5 was obtained from transcriptome sequencing after challenging with *V. alginolyticus* for 6 h. The specific primer F1 and R1 (Table 1) was designed based on the ORF of *Sp*CTL5. The following reaction conditions for PCR were utilized: 30 cycles of denaturation at 95 °C for 10 s; annealing at 60 °C for 5 s; and extending at 72 °C for 30 s. The PCR product was cloned into pMD 19-T vector (TaKaRa, Japan) and then transformed into *Escherichia coli* DH5α (Novagen). Positive recombinant clones were identified by PCR screening with F1 and R1 primers, and subsequently sequenced by a commercial company (Sangon, Shanghai, China).

2.4. Bioinformatics analysis

The fragment was sequenced and confirmed to be a CTL sequence using the online BLAST program (http://www.ncbi.nlm.nih.gov/blast/). The deduced amino acid sequence was obtained with the ORF finder (http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi). Multiple protein sequence alignment was performed using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). ExPASy (http://web.expasy.org/compute-pi/) was used to predict the molecular weight and isoelectric point (pI) of *Sp*CTL5. MEGA 7.0 was used to produce the phylogenetic tree, and the neighbor-joining (NJ) method was used for phylogenetic analysis. Signal peptides and protein domains were analyzed using the SMART program (http://www.smart.emblheidelberg.de/).

2.5. Expression and purification of recombinant SpCTL5 (rSpCTL5)

The pair of primers F2 and R2 (Table 1) that included *Nde* I and *Xho* I restriction enzyme sites were designed to amplify the cDNA fragment encoding the ORF of *Sp*CTL5. The PCR product was subcloned into pET 21a (+) previously cut with the same restriction enzymes *Nde* I and *Xho* I (NEB), and then the constructed expression plasmid, pET-21a (+)-*Sp*CTL5, was transformed into *E. coli* Origami (DE3) (Novagen). Screened positive clones (DE3) were cultured (37 °C, 220 rpm) in LB liquid medium containing 50 mg mL^{-1} ampicillin and 1% glucose until an OD_{600} of 0.4–0.6 was reached. Then, cells were induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM for protein expression.

The culture was further incubated at 37 °C for 4 h. Cells were harvested by centrifugation at 6,000 rpm for 5 min at 4 °C, re-suspended in 1 × PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) containing 1% Triton X-100, and sonicated at 4 °C for 10 min using a program of 3 s sonication and 3 s interval under 40% power (BILON-250Y). Cell lysates were centrifuged at 10,000 rpm for 15 min at 4 °C to collect the supernatant. The recombinant *Sp*CTL5 proteins were verified by 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then purified using Ni-Agarose resin (CWBIO, China) according to the manufacturer's instructions.

The purified proteins were processed using 6, 4, 2, 1, 0 M urea gradient dialysis. The dialysis buffer contained 50 mM Tris-HCl, 50 mM NaCl, 10% glycerol, 1% glycine, 1 mM EDTA, 0.2 mM oxidized glutathione, and 2 mM reduced glutathione [16]. Protein concentrations were determined with the BCA protein assay kit (Novagen, Hilden, Germany) according to the manufacturer's instructions.

2.6. Bacterial agglutination and hemagglutination assays

The bacteria agglutination assay was performed according to a method previously described by Liu et al. [17]. The Gram-positive bacterial strains *Bacillus subtilis*, *Micrococcus lysodeik*, and *Staphylococcus aureus* and the Gram-negative bacterial strains *Necrotizing fasciitis*, *V. alginolyticus*, and *Chryseobacterium indologenes* were chosen to verify the bacterial agglutination activity of *Sp*CTL5. After staining with fluorescein isothiocyanate (FITC), the 5 μl bacteria were incubated with

Table 1
Oligonucleotide primers used in the experiment.

Primer	Sequence (5′-3′)	PCR objective
F1	GCGTTGCAAGTTGTTCCA	ORF amplification
R1	GTGCCCCAGAGCATCATA	ORF amplification
F2	CATATGGATGTGTCGGCGAGGGTTAACGA	Expression
R2	CTCGAGTTAGTGGTGGTGGTGGTGGTGATACAATATTTGACAAACGA	Expression
F3	GCCATCCAACCTCACTCACAA	qRT-PCR
R3	CCACAGTCTTCTCGGCAATCA	qRT-PCR
F4	CTACAAGTGTGGTGGCATCG	qPCR of internal control
R4	CGTCGATGATGGTCACGTAG	qPCR of internal control

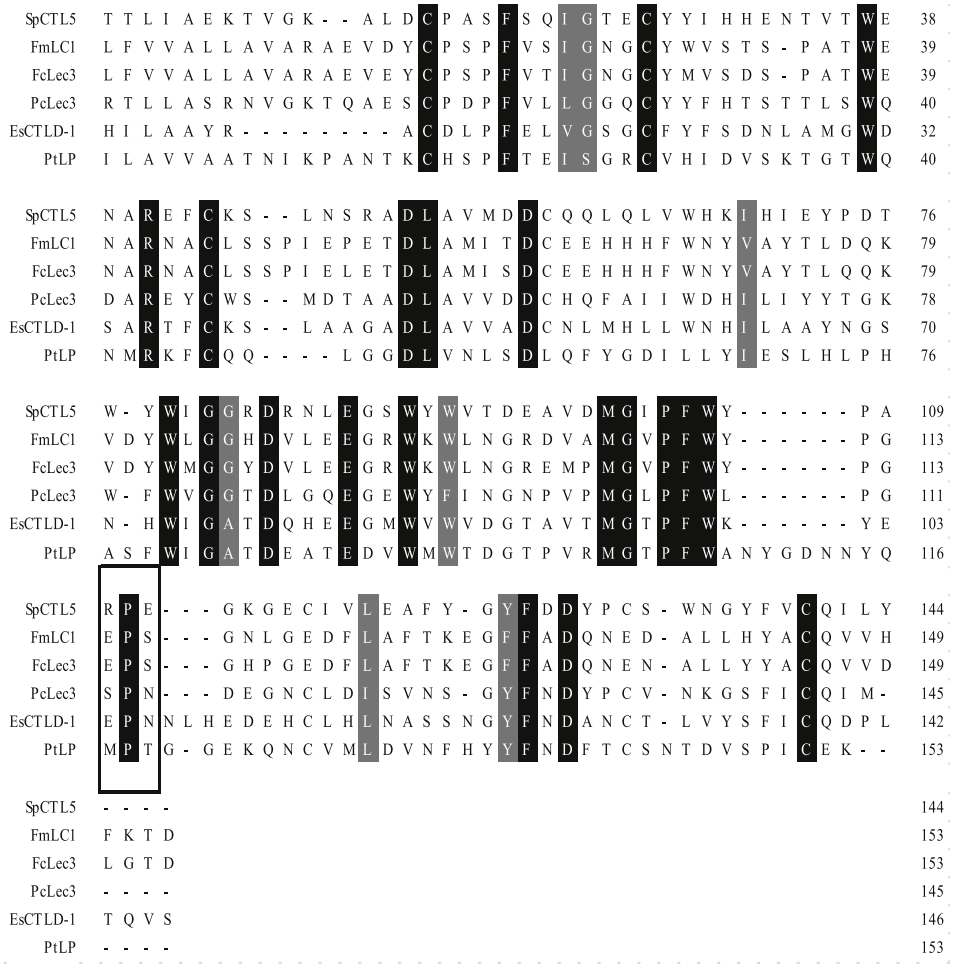

Fig. 1. Multiple sequence alignment of CRD in *Sp*CTL5 and other CTLs. The sequences are as follows: *Sp*CTL5, *Fm*LC1 (*Fenneropenaeus merguiensis*, KC894157.1), *Fc*Lec3 (*Fenneropenaeus chinensis*, EU834292.1), *Pc*Lec3 (*Procambarus clarkia*, JX844151.1), *Es*CTLD-1 (*Eriocheir sinensis*, ADK66338.1), *Pt*LP (*Portunus trituberculatus*, ACC86854.1). The relationships between the residues are indicated as follows: non-similar residues, black letters on a white background; block of similarity, white letters on a grey background; identical residues, white letters on a black background. The suger-binding motif of all lectins were enclosed.

20 μl r*Sp*CTL5 or 20 μl bovine serum albumin (BSA) protein at room temperature for about 45 min. The results were then observed with a positive fluorescent microscope (Nikon, Japan).

Hemagglutination assays were performed according to the method described by Lourenço et al. [18]. Rabbit hemolymph was collected with medical blood vessels containing sodium citrate and then the erythrocytes were obtained via centrifugation (3,000 rpm, 3 min). These erythrocytes were washed thrice with TBS-Ca^{2+} buffer (50 mM Tris-HCl, 10 mM $CaCl_2$, 100 mM NaCl, pH 7.5), trypsinized, and then suspended in 2% TBS-Ca^{2+} buffer. Then, 25 μL *Sp*CTL5 protein and 25 μL cell suspension were mixed and cultured for 1 h in a 96 well microtiter plate. A blank control containing 25 μL cell suspension cultured without *Sp*CTL5 was employed. Furthermore, 25 μL *Sp*CTL5 protein were mixed and cultured with 25 μL cell suspension containing EDTA (10 mmol L^{-1}) to detect whether the protein function is calcium dependent. Then, hemagglutination results were obtained using an optical microscope (Nikon, Japan).

2.7. qRT-PCR analysis

Time-course expression pattern analysis of *Sp*CTL5 after *V. alginolytius* challenging and tissue distribution characterization were performed using the qRT-PCR method. A pair of fluorescent quantitative primers F3 and R3 (Table 1) were designed for relative quantification. The gene *elongation factor 1 α* with primers F4 and R4 (Table 1) were used as the internal control gene [19]. qRT-PCR was carried out using Go Taq® qPCR Master Mix (Promega, Beijing, China) in a Light-Cycler 480 (Roche, USA) according to the manufacturer's instructions. The total reaction volume was 20 μL containing 10 μL of GoTaq® qPCR Master Mix, 1.5 μL of the ten-fold diluted cDNA, 0.5 μL (10 mM) each of the forward and reverse primer, and 7.5 μL of Nuclease-Free Water. The amplification procedure included a denaturation step of 95 °C for 30 s, and 40 cycles of 95 °C for 5 s, 60 °C for 20 s, followed by a melting curve analysis from 65 °C to 95 °C. Each sample was run in triplicate. The relative expression levels of *Sp*CTL5 were calculated via the $2^{-\Delta\Delta Ct}$ method [20]. All data were expressed as means ± SE. IBM SPSS statistics 22 was used for statistical analysis. The data were analyzed via one-way ANOVA analysis using OriginPro 8.5 followed by T test. Values were considered statistically significant if $P < 0.05$.

3. Results

3.1. cDNA cloning and sequence analysis of SpCTL5

A nucleotide sequence representing the complete cDNA sequence of *Sp*CTL5 was obtained from the amplified fragments. The ORF of 762 bp encodes 253 amino acids, and the predicted theoretical pI and the molecular weight of *Sp*CTL5 are 4.81 and 28.915 kDa, respectively. It is predicted that amino acid residues 1–20 would represent the signal peptide region of *Sp*CTL5. SMART program analysis show that *Sp*CTL5 contains a CRD domain ranging from Cys^{124} to Gln^{250} with six conserved cysteine residues. However, the CRD does not contain a typical

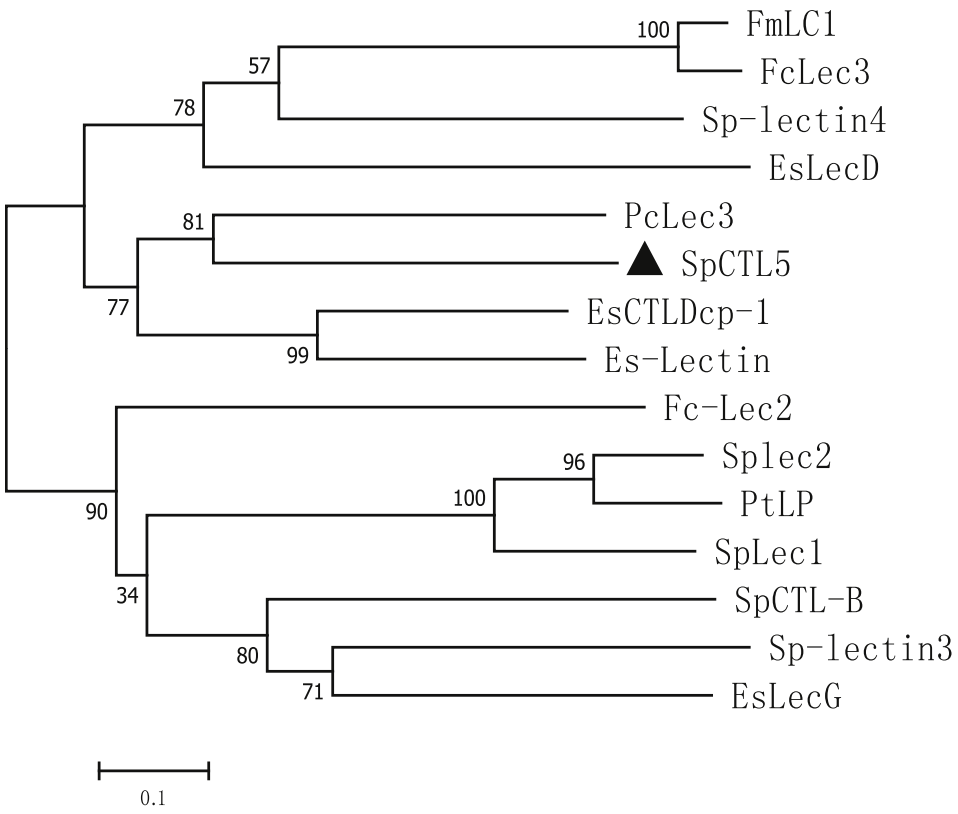

Fig. 2. Phylogenetic tree analysis of *Sp*CTL5 and other CTLs from different species screened from BLAST. Bootstrap trials are replicated 1000 times to derive the confidence value. The protein sequences are as follow: *Sp*CTL5, *Sp*Lec1 (*S. paramamosain*, HQ325747.1), *Sp*Lec2 (*S. paramamosain*, HQ325748.1), *Sp*-lectin3 (*S. paramamosain*, JX094505.1), *Sp*-lectin4 (*S. paramamosain*, KJ631743.1), *Pc*Lec3 (*P. clarkia*, JX844151.1), *Es*CTLDcp-1 (*E. sinensis*, ADK66338.1), *Es*-Lectin (*E. sinensis*, ADB10837.1), *Es*LecD (*E. sinensis*, AGG23537.1), *Es*LecG (*E. sinensis*, JF799934.1), *Fm*LC1 (*F. merguiensis*, KC894157.1), *Fc*Lec3 (*F. chinensis*, EU834292.1), *Fc*-Lec2 (*F. chinensis*, EU834289.1), *Pt*LP (*P. trituberculatus*, ACC86854.1).

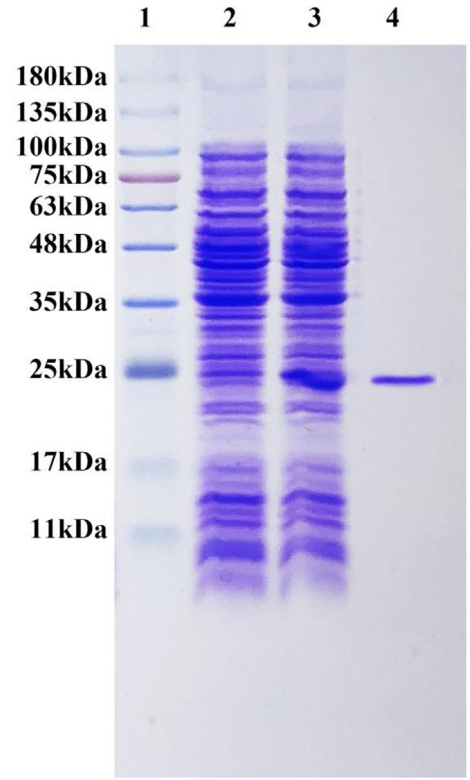

Fig. 3. SDS-PAGE analysis for r*Sp*CTL5. Lane 1: protein molecular standard; lane 2: negative control without IPTG induction; lane 3: expression of r*Sp*CTL5 after IPTG induction; lane 4: purified r*Sp*CTL5.

EPN or WND motif for carbohydrate binding. Fig. 1 shows that some amino acid residues, such as the cysteine residues, are highly conserved in different species.

3.2. Homology and phylogenetic analysis

Multiple sequence alignment analysis of *Sp*CTL5 against the CTLs from various species show that they differ in amino acid composition (Fig. 1). BLAST analysis show that the deduced amino acid sequence is similar to that of CTLs from other species, revealing a 49% sequence identity with *Pc*Lec3 (*Procambarus clarkia*, JX844151.1), a 40% sequence identity with *Es*CTLDcp-1 (*Eriocheir sinensis*, ADK66338.1), and a 35% sequence identity with *Fm*LC1 (*Fenneropenaeus merguiensis*, KC894157.1).

The phylogenetic tree prepared with 1,000 bootstrap tests is shown in Fig. 2. *Sp*CTL5 is clustered with *Pc*Lec3 (*P. clarkia*), which is some distance from the positions of the other CTLs from *S. paramamosain*, such as *Sp*Lec1, *Sp*Lec2, *Sp*-lectin3, *Sp*-lectin4, and *Sp*CTL-B in the phylogenetic tree.

3.3. Expression and purification of recombinant SpCTL5

In the prokaryotic expression system, the recombinant protein of *Sp*CTL5 (r*Sp*CTL5) was expressed as a fusion protein with an additional His-tag. The mature *Sp*CTL5 protein is estimated to have a molecular weight of 26.66 kDa with a theoretical pI of 4.81. It was successfully induced by adding IPTG and was detected using 15% SDS-PAGE (Fig. 3). The concentration of the purified r*Sp*CTL5 was ca. 126.28 μg mL^{-1}.

3.4. Bacterial agglutination and hemagglutination activity of rSpCTL5

The experimental results for the incubation of r*Sp*CTL5 with bacteria stained with FITC showed that all tested bacteria were agglutinated, including three Gram-negative bacterial strains and three Gram-positive bacterial strains. No apparent agglutination was observed in the control group (BSA) (Fig. 4).

After rabbit erythrocytes being incubated with 126.28 μg mL^{-1} r*Sp*CTL5, agglutination was observed. However, no agglutination is

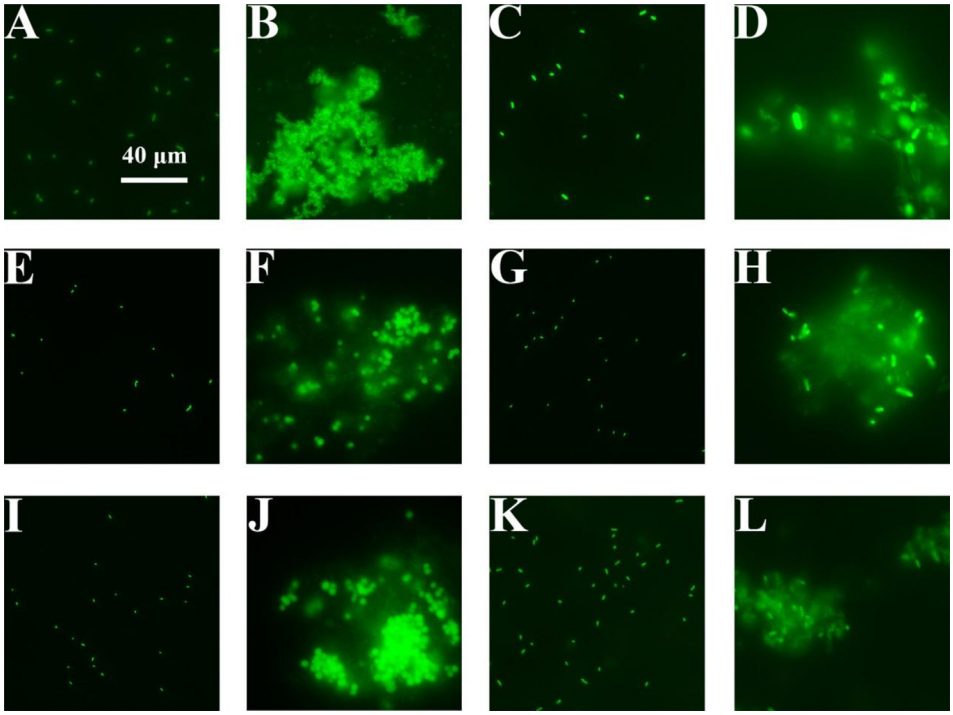

Fig. 4. Bacterial aggregation activity of r*Sp*CTL5. The magnification is 200 ×. A, B: *A. hydrophila*. C, D: *C. indologenes*. E, F: *B. subtilis*. G, H: *V. alginolyticus*. I, J: *S. aureus*. K, L: *M. lysodeikticus*; A, C, E, G, I, and K contain BSA; B, D, F, H, J, and L: 126.28 μg mL^{-1} r*Sp*CTL5 is contained.

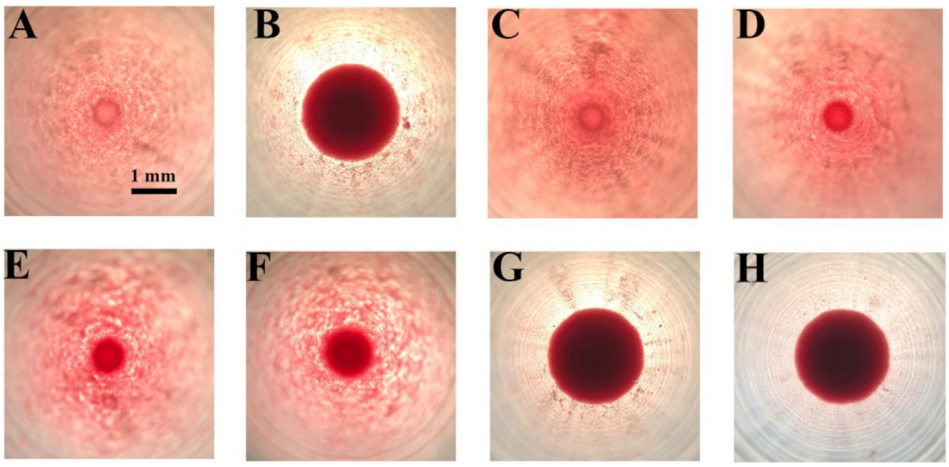

Fig. 5. Agglutination activity of r*Sp*CTL5 against rabbit erythrocytes. The magnification is 40 ×. A: 126.28 μg mL^{-1} protein is contained. B: 126.28 μg mL^{-1} protein and 10 mM EDTA are contained. C: 105.23 μg mL^{-1} protein is contained. D: 90.20 μg mL^{-1} protein is contained. E: 78.92 μg mL^{-1} protein is contained. F: 70.15 μg mL^{-1} protein is contained. G: 63.14 μg mL^{-1} protein is contained. H: No protein is contained.

observed when EDTA is added or the r*Sp*CTL5 solution is diluted more than two-fold (Fig. 5).

3.5. Tissue distribution of SpCTL5 expression

To examine the tissue distribution profile of *Sp*CTL5 , the total RNA was isolated from stomach, heart, gonad, eyestalk, hemocytes, muscle, and hepatopancreas tissues. The tissue distribution of *Sp*CTL5 transcription was then detected by qRT-PCR. *Sp*CTL5 expression was observed in all the examined tissues, with high levels expressed in the hepatopancreas and low expression levels being detected for the eyestalk, heart, muscles, gonad, stomach, and hemocytes (Fig. 6).

3.6. Temporal changes of SpCTL5 transcription after V. alginolyticus injection

To determine whether *Sp*CTL5 would be involved in the immune response to pathogen infection, temporal changes in *Sp*CTL5 transcription after *V. alginolyticus* injection were detected with qRT-PCR. As shown in Fig. 7, the expression of *Sp*CTL5 was significantly increased at 3 and 6 h after injection of *V. alginolyticus*, and the expression level recovered to a normal level by 12 h.

4. Discussion

In this study, a novel *Sp*CTL5 cDNA from *S. paramamosain* was cloned based on our transcriptomic data. The ORF of *Sp*CTL5 contains 762 bp encoding a polypeptide of 253 amino acids. *Sp*CTL5 has a CRD domain containing six conserved cysteine residues. These features of *Sp*CTL5 are similar to those of other CTLs of *S. paramamosain,* such as *Sp*Lec1 and *Sp*Lec2 [14].

It has been reported that four different types of Ca^{2+} binding sites exist in the CRD and that only the second Ca^{2+} binding site is involved in the specific binding of sugars [21]. In addition, in the CRD also contains two very distinct amino acid motifs, the Glu-Pro-Asn (EPN) motif and the Gln-Pro-Asp (QPD) motif, which have been reported to

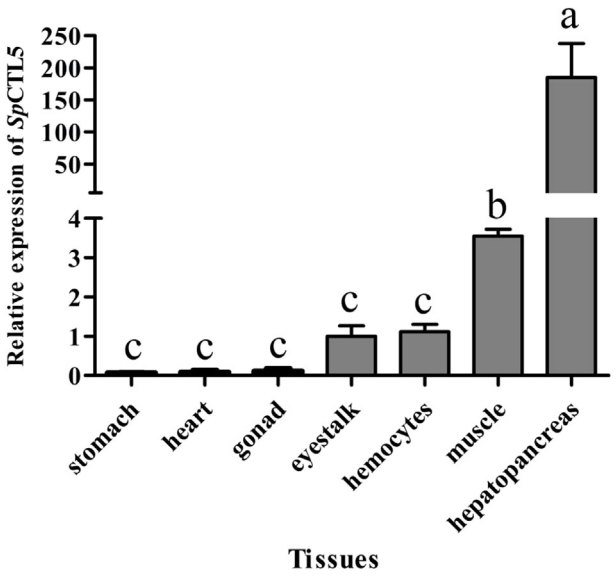

Fig. 6. Tissue distribution of the *Sp*CTL5 expression. *Sp*CTL5 transcript levels in stomach, heart, gonad, eyestalk, hemocytes, muscles, and hepatopancreas were normalized to that in eyestalk. Data are shown as mean ± S.E. ($n \geq 3$). Different letters represent significant differences ($P < 0.05$).

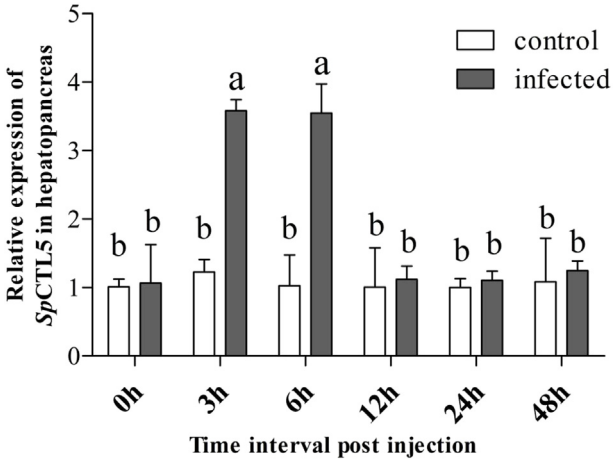

Fig. 7. Relative expression of *Sp*CTL5 in the hepatopancreas after challenging with *V. alginolyticus*. *Sp*CTL5 transcription levels in the hepatopancreas were determined at 0 h, 3 h, 6 h, 12 h, 24 h, and 48 h post injection of *V. alginolyticus* (black bars) or sterilized saline (white bars), respectively. Bars represent means ± S.E. ($n \geq 3$). Different letters represent significant differences ($P < 0.05$).

bind galactose and mannose [9]. The EPN and QPD motifs are important for combination with galactose and mannose, respectively. Accordingly, *Sp*-lectin4 [15] has a QPD motif and *Fm*LC1 [22] has an EPS motif. Furthermore, the EPN motif seems to be more diverse in invertebrate CTLs, and can be EPD, EPK, EPS, EPQ, QPG, QPS, QPN, QPT, and YPT [23]. The CRD with an EPN motif was found to have mutated into an EPS motif upon analyzing the amino acid sequence of *Sp*CTL-B. Although *Sp*CTL-B contains a hydrophobic domain at the N-terminal region, the CRD with an EPN motif preferentially binds mannose, and the QPD tripeptide motif binds galactose [4]. However, not all CTLs include the EPN motif [9]. For example, the CRDs in *Es*CTLDcp-1 from *E. sinensis* [24] and *Pt*LP from *P. trituberculatus* [25] do not contain a typical EPN or QPD motif for carbohydrate binding. Therefore, *Sp*CTL5 should be a member of the CTLs.

Based on the homologous alignment and phylogenetic analysis of the *Sp*CTL5 gene, it is considered to be highly homologous to CTLs. Furthermore, it exhibits a 49% sequence identity with *Pc*Lec3 of *P. clarkia* [26]. *Sp*CTL5 shows sequence identities below 30% to other CTLs that have been reported from *S. paramamosain*, such as *Sp*Lec1, *Sp*Lec2 [14], *Sp*-lectin3, *Sp*-lectin4 [15], and *Sp*CTL-B [4]. These results indicate that SpCTL5 is a new CTL in *S. paramamosain*.

The most important function of CTLs is to recognize and non-covalently bind specific carbohydrate ligands on cell surfaces and agglutinate cells by binding cell surface glycoproteins and glycoconjugates. *Sp*CTL5 has a wide spectrum of binding activities towards various microorganisms, and can agglutinated all the microorganisms examined.

*Sp*CTL5 is in accordance with *Sp*CTL-B of *S. paramamosain* [4], *Pt*CTL4 of *P. trituberculatus* [26], and *Fc*-hsL of *F. chinensis* [27]. However, it is different from *Pt*LP, which does not agglutinate *V. alginolyticus* or *S. aureus* [25]. This difference between *Sp*CTL5 and *Pt*LP indicates that different CTLs might have different agglutination characteristics. *Sp*CTL5 has hemagglutination activity against rabbit erythrocytes in the presence of Ca^{2+}, indicating that *Sp*CTL5 is a member of the Ca^{2+}-dependent CTL family. Thus, the present study shows that *Sp*CTL5 has both bacteria and hemocyte binding abilities, promoting phagocytosis of invasive bacteria in *S. paramamosain*.

The expression levels of CTLs in various tissues are different. In *S. paramamosain*, the highest expression is observed for the hepatopancreas [4], with some in the haemocytes, muscles, and ovaries. In *P. trituberculatus,* the expression of *Pt*CTL4 and *Pt*LP is detected in all examined tissues and is particularly highly expressed in the hepatopancreas [25,28]. Furthermore, CTL expression is widely observed in the tissues of *E. sinensis*, and consistently in the hepatopancreas, gills, accessory glands, ovaries, muscle, and thoracic ganglia [21,29]. Indeed, abundant CTL transcription is often observed for the hepatopancreas [24,30,31]. For example, in the four penaeid species *F. chinensis*, *F. merguiensis*, *L. vannamei*, and *P. monodon*, transcription of CTL is detected mainly in the hepatopancreas, although traces are found in other tissues including the hemocytes, heart, stomach, intestine, and lymphoid organs [22,27,32–34]. However, Northern blotting experiments have revealed a significant hybridization band for *Fc*lectin only in hemocytes, while no positive bands were detected in the hepatopancreas, muscle, intestine, gill, and heart tissues [35]. In the present study, the expression profiles for *Sp*CTL5 revealed that *Sp*CTL5 transcripts are mostly distributed in the hepatopancreas (Fig. 7). The hemocytes and hepatopancreas are considered to be important immune organs of crustaceans [11].

Many studies have demonstrated that CTLs play an important role in immune defense against pathogen invasion [22,26,36]. Accordingly, in the present study, *S. paramamosain* were challenged with *V. alginolyticus* in order to study the role of *Sp*CTL5 in its innate immunity. The results revealed that the expression of *Sp*CTL5 in the hepatopancreas had increased significantly at 3 and 6 h. The expression returned to normal levels at 12 h after being challenged.

In summary, this study demonstrates that r*Sp*CTL5 binds a wide range of foreign pathogens and eliminates them by inducing agglutination. Thus, *Sp*CTL5 plays an important role in the innate immunity of *S. paramamosain*.

Acknowledgement

This work was supported by National Key R&D Program of China (2018YFD0901304), the Major Sci & Tech Special Project of Zhejiang Province (no: 2016C02055–8), Ministry of Agriculture of China & China Agriculture Research System (no: CARS-48) and K. C. Wong Magna Fund in Ningbo University.

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