Molecular cloning and immune responsive expression of a novel C-type lectin gene from bay scallop *Argopecten irradians*

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Abstract  C-type lectins are Ca^{2+}-dependent carbohydrate-recognition proteins that play crucial roles in innate immunity. The cDNA of C-type lectin (AiCTL1) in the bay scallop *Argopecten irradians* was cloned by expressed sequence tag (EST) and RACE techniques. The full-length cDNA of AiCTL1 was 660 bp, consisting of a 5′-terminal untranslated region (UTR) of 30 bp and a 3′ UTR of 132 bp with a polyadenylation signal sequence AATAAA and a poly(A) tail. The AiCTL1 cDNA encoded a polypeptide of 166 amino acids with a putative signal peptide of 20 amino acid residues and a mature protein of 146 amino acids. The deduced amino acid sequence of AiCTL1 was highly similar to those of the C-type lectins from other animals and contained a typical carbohydrate-recognition domain (CRD) of 121 residues, which has four conserved disulfide-bonded cysteine residues that define the CRD and two additional cysteine residues at the amino terminus. AiCTL1 mRNA was dominantly expressed in the hemocytes of the bay scallop. The temporal expression of AiCTL1 mRNA in hemocytes was increased by 5.7- and 4.9-fold at 6 h after injury and 8 h after injection of bacteria, respectively. The structural features, high similarity and expression pattern of AiCTL1 indicate that the gene may be involved in injury healing and the immune response in *A. irradians*.

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Introduction

The bay scallop *Argopecten irradians* is a native species in the east coast of the United States and was introduced to China as an alternative species for aquaculture in 1982 [1]. It quickly became the most popular farming shellfish
in China because it can reach marketable size within a year. By the early 1990s, the annual production of the bay scallop reached about 200,000 tons in China [2]. However, since the summer of 1997, massive mortality of the bay scallop has caused catastrophic losses to scallop aquaculture, leading to a drastic decline in its production [2]. Although the actual causes for the massive scallop mortality remain unclear, deteriorated water quality, disease outbreaks, and stock degeneration due to extensive inbreeding have been considered as major attributions [3,4].

Like other invertebrates, molluscs lack the adaptive immune system that is a characteristic of vertebrates, and rely exclusively on the innate immune system in dealing with a diverse array of pathogens and stress responses [5]. Invertebrate innate immune reactions are triggered when so-called pathogen-associated molecular patterns (PAMPs) of microorganisms are detected and recognized as "non-self" by the pattern recognition receptors (PRRs) of the host [6,7]. So far, seven groups of PRRs have been identified in invertebrates, including peptidoglycan-recognition proteins (PGRP), thiorester-containing proteins (TEP), Gram-negative binding proteins (GNBP), multidomain scavenger receptors (SCR), C-type lectins (CTL), galactoside-binding lectin (galectin) and fibrinogen-like domain immunolectin [8]. As one of the PRRs, C-type lectin (CTL) can recognize and bind to terminal sugars on glycoproteins and glycolipids on the cell surface of potential pathogens, and is considered to play key roles in non-self recognition and clearance of invaders in invertebrate immunity [9,10].

For many years, C-type lectins have been well studied in vertebrates and many C-type lectin genes have been identified and cloned [11]. C-type lectins have a characteristic carbohydrate-recognition domain (CRD) with a well-defined structure stabilized by two or three pairs of disulfide bonds. The presence of these two additional cysteines at the beginning of the C-type lectin-like domain (CTLD) sequence is used to distinguish between long- and short-form CTLDs in sequence analysis [11]. However, C-type lectins have not been characterized in invertebrates until the last decade. Up to now, approximately 32 and 183 genes encoded in CTLDs have been identified in the fruit fly Drosophila melanogaster and Caenorhabditis elegans, respectively [12]. In addition, several ESTs of lectins in the hepatopancreas have also been found in Litopenaeus vannamei and L. setiferus [13,14]. Simultaneously, it has become apparent that C-type lectins are involved in a variety of innate immune reactions in invertebrates. For example, immulectin-1, an inducible C-type lectin from Manduca sexta, can stimulate prophenoloxidase activation in plasma [9,15]. Immulectin-2, as a pattern recognition receptor, enhances hemocyte encapsulation and melanization in M. sexta [16], and protects the larvae from bacterial infection [15]. An LPS-binding lectin from Bombyx mori is involved in hemocyte nodule formation [17] and a cockroach Periplaneta americana lectin plays a role as an opsonin [18]. Recently, a C-type lectin (CF-Lec-1) was also identified from Chlamys farreeri, and it displayed aggregation activity toward Escherichia coli JM109 and inhibitory effects on the growth of Micrococcus luteus and E. coli JM109 [19]. Despite the possible importance in immunity, the molecular characterization, gene cloning, and responsive expression of C-type lectins to immune challenge in the bay scallop A. irradians have not been well studied.

In this study, the putative C-type lectin gene (AiCTL1) was cloned and characterized from the bay scallop A. irradians. Furthermore, its tissue distribution and temporal expression after being injured or challenged by bacterial pathogens were also investigated.

Materials and methods

Animals, immune challenge and hemolymph collection

Bay scallops A. irradians, averaging 55 mm in shell length, were collected from a scallop farm in Sanggou Bay, Shandong Province, China and kept in aerated seawater at 15 °C for 24 h before processing. To take account of individual variability, 15 individual scallops, which were divided into three replicates, were used for each time point (2, 4, 6, 8, 16 and 32 h) in the injury and challenge experiments.

The injury experiment was performed by scalpel and a wound about 5 mm in length and 3 mm in depth was cut in the scallop adductor muscles. The bacterial challenge experiment was performed by injecting with 50 μl of Vibrio anguillarum resuspended in PBS to an OD of 0.4 (1 OD = 5 × 10⁸ bacteria ml⁻¹) into the adductor muscles of each scallop. The untreated scallops were used as the blank, while the scallops that were injected with 50 μl PBS were used as the control in the bacterial challenge experiment. The injured or injected scallops were returned to seawater tanks and 15 individuals were randomly sampled at 2, 4, 6, 8, 16, and 32 h into the experiment. The hemolymph from the blank, the control and the treatment groups was collected from the adductor muscles using a syringe. The hemolymph from all the individuals in each group was pooled and immediately centrifuged at 1000 × g, 4 °C for 10 min to harvest the hemocytes. The hemocyte pellets were immediately used for RNA extraction.

cDNA library construction and EST analysis

A cDNA library was constructed from the body of A. irradians using the ZAP-cDNA synthesis kit and ZAP-cDNA GigapackIII Gold cloning kit (Stratagene). Random sequencing of the library using T3 primer yielded 5828 successful sequencing reactions [20]. BLAST analysis of all the EST sequences revealed that one EST (EST No. rscae 5962) was homologous to the C-type lectin of Crassostrea gigas (BAF75353) and Oryzias latipes (BAB83835). This EST sequence was selected for further cloning of the C-type lectin gene from the bay scallop.

Cloning the full length cDNA of C-type lectin gene (AiCTL1)

Based on the identified EST sequence, two gene-specific primers AiCTL1-F1 and AiCTL1-R1 were designed to amplify the full-length cDNA of AiCTL1 by 5'-RACE and 3'-RACE (Table 1). PCR reaction to obtain the 5'-end of AiCTL1 cDNA was performed in a PTC-100 Programmable Thermal Controller Cycler (MJ Research) by using sense primer T3 and reverse primer AiCTL1-R1 in 25 μl of reaction volume, containing 2.5 μl of 10× PCR buffer, 1.5 μl of MgCl₂,
Cloning and immune expression of *Argopecten irradians* gene

(25 mmol l⁻¹), 2.0 μl of dNTP (2.5 mmol l⁻¹), 1 μl of each primer (10 μmol l⁻¹), 15.8 μl of PCR-grade water, 0.2 μl (1 U) of Taq polymerase (Promega) and 1 μl of cDNA mix. The PCR temperature profile was 94 °C for 5 min followed by 32 cycles of 94 °C for 30 s, 58.5 °C for 30 s, 72 °C for 1 min, and the final extension step at 72 °C for 10 min. PCR amplification of the 3′-end of *AiCTL1* was carried out using sense primer *AiCTL1*-F1 and reverse primer *T7*, and the PCR program was 94 °C for 5 min, followed by 32 cycles of 94 °C for 30 s, 59 °C for 30 s, 72 °C for 45 s, and then an additional extension at 72 °C for 10 min. The PCR products were gel-purified and cloned into pMD18-T simple vector (Takara). After being transformed into the competent cells of *Escherichia coli* JM109, the recombinants were identified through blue–white colour selection in ampicillin-containing LB plates. The positive clones were sequenced in both directions, and the resulting sequences were verified and subjected to cluster analysis.

**Table 1** Oligonucleotide primers used in the experiments

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>AiCTL1</em>-F1</td>
<td>5′-TGGACTAGTCCGGAGCCAG-3′</td>
<td>19</td>
</tr>
<tr>
<td><em>AiCTL1</em>-R1</td>
<td>5′-GACCATGCGCTGCTGGTTGGA-3′</td>
<td>21</td>
</tr>
<tr>
<td><em>AiCTL1</em>-F2</td>
<td>5′-TACTAGGTCTCCATGCTGTTGATC-3′</td>
<td>22</td>
</tr>
<tr>
<td><em>AiCTL1</em>-R2</td>
<td>5′-CTCCACACACAGTTGGTTGATC-3′</td>
<td>21</td>
</tr>
<tr>
<td><em>AF</em></td>
<td>5′-TATGCCCTCCCTCACTAGT-3′</td>
<td>20</td>
</tr>
<tr>
<td><em>AR</em></td>
<td>5′-GGGACCGTCTGGATTCTCCT-3′</td>
<td>20</td>
</tr>
<tr>
<td>T3(Vector)</td>
<td>5′-AATTACCATCTACTAAAAGG-3′</td>
<td>20</td>
</tr>
<tr>
<td>T7(Vector)</td>
<td>5′-GTAATACGACTCACTATAGGGC-3′</td>
<td>22</td>
</tr>
</tbody>
</table>

Semi-quantitative RT-PCR was carried out in a PTC-100 Programmable Thermal Controller Cycler (MJ Research) in 20 μl of reaction volume containing 2 μl of 10× PCR buffer, 1.2 μl of MgCl₂ (25 mmol l⁻¹), 1.6 μl of dNTP (2.5 mmol l⁻¹), 1 μl of each primer (10 μmol l⁻¹), 12 μl of PCR-grade water, 0.2 μl (1 U) of Taq polymerase (Promega), and 1 μl of cDNA reaction mix. The PCR program was carried out at 94 °C for 5 min, followed by 31 cycles (for *AiCTL1*) or 22 cycles (for beta-actin) of 94 °C for 30 s, 57.5 °C for 30 s, 72 °C for 45 s, and then an additional extension at 72 °C for 10 min. The PCR products were separated in 1.5% agarose gel and stained with ethidium bromide.

**Sequence analysis and phylogenetic tree construction**

Similarity searches were performed with the BLAST program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast). The protein motifs features were predicted by Simple Modular Architecture Research Tool (http://smart.embl-heidelberg.de/) [21]. Multiple alignment of the C-type lectin sequences was performed with the ClustalW Multiple Alignment program (http://www.ebi.ac.uk/clustalw/) and Multiple Alignment show program (http://www.bio-software.net/sms/index.html). Sixteen amino acid sequences of C-type lectin deposited in GenBank were used to construct the phylogenetic tree by UPGMA in Mega 3.1 (Molecular Evolutionary Genetics Analysis Version 3.1) [22] and the accession numbers of the sequences in GenBank are given in the legend to Fig. 2.

**Semi-quantitative RT-PCR analysis of *AiCTL1* gene expression**

Total RNA was extracted from above-mentioned hemocyte pellets and different tissues (adductor muscle, mantle margin, heart, gonad, digestive gland, gill and intestine) by following the manufacturer’s instructions (Invitrogen, USA). Single-strand cDNA was synthesized from 1 μg of total RNA in a final volume of 20 μl containing 50 pmol of random hexamers, 50 mmol l⁻¹ Tris–HCl, pH 8.3, 75 mmol l⁻¹ KCl, 3 mmol l⁻¹ MgCl₂, 50 mmol l⁻¹ DTT, 0.75 U of RNasin, 0.2 mmol l⁻¹ each of dATP, dCTP, dGTP, dTTP, and 200 U of MMLV reverse transcriptase (Promega). Reactions were incubated at 37 °C for 1 h, terminated by heating the mixture at 95 °C for 5 min, and subsequently stored at −80 °C. Two gene-specific primers *AiCTL1*-F2 were used to amplify a product of 357 bp. A constitutive expression gene, the beta-actin gene, was used as an internal control to verify the RT-PCR reaction and adjust the cDNA templates. The beta-actin gene was identified as an EST sequence (EST no: cl14ct22cn22) from the cDNA library of *A. irradians*. This EST sequence was 968 bp in length and showed significant similarity (score of 384; E value, −105; identities, 97%) to *Acanthopagrus schlegelii* beta-actin gene (AAR84618). Two primers, beta-actin primers AF and AR (Table 1), were designed based on the sequence of cl14ct22cn22 to amplify a 592-bp fragment of bay scallop beta-actin gene.

**Figure 1** Nucleotide and deduced amino acid sequences of *AiCTL1* gene (EU277646). The nucleotide and amino acid sequence is numbered on the left. The putative signal peptide is underlined and the cleavage site is indicated by a vertical arrow. The carbohydrate-recognition domains (CRD) are wave underlined and four conserved disulfide-bonded cysteine residues that define the CRD are indicated by black shadow, whereas two additional cysteine residues at the amino terminus are marked by gray shadow. The asterisk (*) indicates the stop codon. The classical polyadenylation signal is enclosed in a box.
Figure 2  Multiple alignment of the deduced amino acid sequence of *A. irradians* AiCT1L (EU277646) gene’s CRD with its other C-type lectin protein sequences’ CRD. The black shadowing shows identical amino acids and the gray shadowing indicates similar amino acids.
stained with ethidium bromide. To confirm the specificity of RT-PCR amplification, the RT-PCR products were purified from the gel and sequenced. Electrophoretic images and the optical densities of amplified bands were analyzed using the software of Scion Image (version B4.02). Statistical analysis was performed with commercially available statistical software (GraphPad Prism v4.0, GraphPad Software). The data were analyzed with a one-way ANOVA followed by the Student–Newman–Keul’s test. Differences were considered significant at P < 0.05.

Results

cDNA cloning and the characterization of AiCTL1 gene

Random sequencing of the bay scallop A. irradians cDNA library with T3 primer yielded 5828 EST sequences, which were clustered into 637 contigs (including 3690 singlets) and 2138 singlets [20]. One of the ESTs (no. rscae 5962) was homologous to the C-type lectin of C. gigas (BAF75353), O. latipes (BAB83835), Danio rerio (XP_708072) and Homo sapiens-1 (1DV8_A). A 660 bp nucleotide sequence representing the complete cDNA sequence of A. irradians C-type lectin (AiCTL1) gene was cloned and deposited in GenBank under accession no. EU277646. The deduced amino acid sequence is shown in Fig. 1. The complete cDNA of AiCTL1 includes a 5'-terminal untranslated region (UTR) of 30 bp, a 3'-terminal UTR of 129 bp with a canonical polyadenylation signal sequence AAT AAA and an EPN or QPD motif, which has been predicted to be important for Ca2+/carbohydrate binding [24].

The phylogenetic tree was constructed based on the 18 long-form CRD amino acid sequences of 16 C-type lectins from different species were divided into two clusters: C-type lectins from C. gigas (BAF75353), Chlamys farreri(DQ209290), Oncorhynchus mykiss (AAM21196), Penaeus monodon-1 (AAZ29608) and Penaeus monodon-2 (AAZ255889). The protein domain features of AiCTL1 using the SMART program indicated the presence of a long-form CRD at the N-terminus and the QPD motif (Gln117-Pro118, Asp119) for determining Ca2+/carbohydrate binding specificity.

The search for similarity in GenBank also revealed that AiCTL1 was highly similar to the C-type lectins from C. gigas (BAF75353) (40% identity; E = 9e-22), D. rerio (XP_708072) (28% identity; E = 7e-14), O. latipes (BAB83835) (28% identity; E = 1e-13), Gallus gallus (P02707) (28% identity; E = 6e-13) and H. sapiens (1DV8_A) (27% identity; E = 6e-13) and (1K9L_A) (26% identity; E = 1e-12). All these proteins contain at least one long-form carbohydrate-recognition domain (CRD), which has four conserved disulfide-bonded cysteine residues involved in the formation of the CRD internal disulfide bridges, two additional disulfide-bonded cysteine residues at the N-terminus, and an EPN or QPD motif, which has been predicted to be important for Ca2+/carbohydrate binding [24]. The sequence similarity and the common structure features strongly suggested that AiCTL1 should be a new member of the C-type lectin superfamily.

A phylogenetic tree was constructed based on the 18 long-form CRD amino acid sequences of 16 C-type lectins using the neighbour-joining method (Fig. 3) and showed that C-type lectins from different species were divided into two clusters: C-type lectins from C. gigas (BAF75353), Chlamys farreri(DQ209290), Oncorhynchus mykiss (AAM21196), Penaeus monodon-1 (AAZ29608) and Penaeus monodon-2 (AAZ255889). The sequences were extracted from A. irradians (this study) and from GenBank (see Fig. 2). The tree was obtained by bootstrap analysis with the neighbour-joining method and the numbers on the branches represent bootstrap values for 1000 replications.
D. rerio (XP_708072), Salmo salar (AAAT77222), O. latipes (BAB83835), G. gallus (P02707), Canis lupus familiaris (XP_542118), H. sapiens (1DV8_A and 1K9I_A) and the bay scallop A. irradians (EU277646) formed one cluster (group 1), they all were categorized into the DC-SIGN group [25], while C-type lectins from shrimp, including Penaeus monodon (AAQ75589, AB197373 and AAZ29608), P. semisulcatus (AB197372) and Litopenaeus vannamei (AB197374) formed another cluster (group 2) (Fig. 3).

Tissue distribution and expression of AiCTL1

Tissue distribution
The transcript of the AiCTL1 gene was dominantly expressed in the hemocytes and weakly detected in the gill, but was not detected in the intestine, heart, digestive gland, gonad, mantle margin and adductor muscle of normal scallop A. irradians (Fig. 4). The weak signal detected in the gill was suggested to be due to the existence of infiltrating hemocytes [4,26].

AiCTL1 gene expression after injury
The temporal expression of the AiCTL1 gene after injury was measured by semi-quantitative RT-PCR and a clear time-dependent expression pattern was observed (Fig. 5). At 2 h after injury, there was no significant difference between blank and injury samples. At 4 h after injury, the expression of the AiCTL1 gene was up-regulated gradually, and reached the peak at 6 h. The expression of the AiCTL1 gene increased 5.7-fold compared to the blank at 6 h. After 8 h, the AiCTL1 expression dropped progressively and recovered to the original level by 16 h. Analysis of variance indicated that the AiCTL1 gene expression at 4, 6 and 8 h post-injury were significantly higher ($P < 0.001$) than at other time points.

Figure 5 mRNA expression of AiCTL1 in hemocytes at different time points after adductor muscle injury by RT-PCR analysis. ‘mRNA expression of AiCTL1/β-actin (%)’ indicates the ratio obtained from the density of the band of AiCTL1 mRNA expression standardized with that of the β-actin mRNA expression level. B, blank group.

AiCTL1 expression after bacterial challenge
The temporal expression of the AiCTL1 gene after bacterial challenge is shown in Fig. 6. At 2 and 4 h post-injection, there was no significant difference in AiCTL1 gene expression among the control, blank, and challenged samples. At 6 h after injection, the expression of the AiCTL1 gene was up-regulated in the challenged group. At 8 h, the expression reached the highest level and was increased 4.9-fold compared to the blank. After 16 h, the expression dropped and recovered to the original level by 32 h. Analysis of variance indicated that the AiCTL1 gene expression at 6, 8 and 16 h post-challenge was significantly higher ($P < 0.01$) than at other time points.

Figure 6 mRNA expression of AiCTL1 in hemocytes at different time points after bacterial challenge by RT-PCR analysis. ‘mRNA expression of AiCTL1/β-actin (%)’ indicates the ratio obtained from the density of the band of AiCTL1 mRNA expression standardized with that of the β-actin mRNA expression level. B, blank group; C, control group.

Discussion
In this study, a cDNA encoding a C-type lectin AiCTL1 was identified from the bay scallop A. irradians by EST and RACE techniques. The CRD of AiCTL1 share high structural similarity with those of the transmembrane C-type lectin receptors, but it was predicted to be a secreted protein.
Similar results have been reported in the Japanese flounder Paralichthys olivaceus C-type lectin JFCTL [27] and the Atlantic salmon Salmo salar serum lectin SSL [28]. However, the AiCTL1 gene was expressed in hemocytes, while the JFCTL and SSL genes were transcribed in liver [27] and kidney [28], respectively, and then were secreted into the blood stream as a plasma protein and played immune functions [27]. The hemocytes and hepatopancreas are considered as important tissues for immune defense in invertebrates [29]. The circulating hemocytes play defense roles not only by direct sequestration and killing of infectious agents, but also by synthesis and exocytosis of a battery of bioactive molecules in immune defense [26].

The hepatopancreas also contains highly specialized cells and phagocytes that also function in cellular immune responses [13]. Some C-type lectins of invertebrates have been reported that were exclusively expressed in hemocytes, such as the Fclectin from Fenneropenaeus chinensis [26], the PmA and Pmlec from P. monodon [30,31]. However, in other reports about C-type lectins, the hepatopancreas is the sole tissue of these genes’ expression in normal bodies, such as LvLT from Litopenaeus vannamei [32] and Fc-hsL from Fenneropenaeus chinensis [29]. It may have some functional implication in the different expressions of these lectins.

While C-type lectin proteins have been classified into several groups, the bay scallop C-type lectin AiCTL1 was categorized into the DC-SIGN group (Fig. 3), indicating functional similarity of these proteins in the immune system [33]. DC-SIGN, which was first found in human dendritic cell (DC)-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN; CD209), is a type II transmembrane C-type lectin with a single C-terminal CRD [33]. It can bind to “self” glycan ligands found on human cells [33], as well as to “foreign” glycans derived from bacterial or parasitic pathogens [34], and can play an important role in the innate immune system by cell–cell contact and recognition of pathogens [33–35]. The AiCTL1 contains a long-form CRD at the N-terminus and the galactose-type carbohydrate-binding motif, Gln-Pro-Asp (QPD) [24]. The groups in the C-type superfamily are defined according to sequence and evolutionary relationship, but they also show distinct binding preferences either to mannose-type or to galactose-type sugars [36]. C-type lectins can bind galactose or similar sugars by the QPD motif in place of the EPN (Glu-Pro-Asn) motif [24,36]. The presence of an EPN motif makes the AiCTL1 an exception among the group DC-SIGN, which frequently contain the EPN motif and bind to mannose-type ligands [34]. In an evolutionary sequence framework, the conversion of binding motifs in different groups of C-type lectins has been reported. For example, the chicken hepatic lectin is highly homologous to mammalian receptor lectins, which generally contain a QPD motif and bind galactose or similar sugars [37]. However, the chicken hepatic lectin sequence reveals an EPN motif and was found to bind preferentially to mannosetype GlucNac [37,38]. Conversely, the carp collectin, evolved in a typically mannoside-binding collectin progenitor gene, contains a QPD motif and was suggested to bind to galactose-type sugars [39]. These examples reveal the remarkable plasticity and the divergent evolution in carbohydrate binding [37].

AiCTL1 was expressed constitutively in hemocytes and the transcription of the AiCTL1 gene after injury or V. anguillarum challenge was clearly time-dependent. At 2 h after injury and 2–6 h after V. anguillarum challenge, there were no significant differences in the AiCTL1 expression among the blank, control, and treated samples. The mRNA expression of the AiCTL1 gene was up-regulated after injury and injection, increased by 5.7- and 4.9-fold at 6 h after injury and 8 h after injection of V. anguillarum, respectively, and then dropped progressively. The C-type lectin, CFlec-1, from the Zhikong scallop Chlamys farreri, was moderately expressed in the gill and gonad, and the expression of CFlec-1 mRNA in hemocytes of bacterial challenge also decreased from 2 to 8 h post-injection, and then recovered to the original level from 16–32 h post-injection [19]. Therefore, AiCTL1 and CFlec-1 are expressed in different tissues and exhibit different expression profiles. An antiviral C-type lectin, PmA, from P. monodon, also contains a QPD motif [30,31]. PmA was highly expressed in the hepatopancreas, and its expression was slightly decreased in the hepatopancreas from 6 h to 1 day post-infection. The expression of PmA was up-regulated as white spot syndrome virus (WSSV) load was increased. PmA expression started to increase at 2 days, and reached a peak at 4 days post-infection. After 5 days, PmA expression began to decline. AiCTL1 and PmA also cluster in different groups (Fig. 3). These results suggest that the expression of C-type lectins in different tissues or with different patterns may determine their in vivo functions. AiCTL1 is induced by injury and bacterial challenge, suggesting that AiCTL1 may be involved in injury healing and the immune response in molluscs.

In summary, a putative galactose-binding C-type lectin AiCTL1 was cloned from the bay scallop A. irradians. The mRNA encoding AiCTL1 was mainly found in the hemocytes. The up-regulated mRNA expression of AiCTL1 after injury or bacterial challenge indicates that the AiCTL1 gene is inducible and may be involved in wound healing and antibacterial defenses. However, biochemical characters and roles of AiCTL1 in the bay scallop defence mechanisms require further study.

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