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RESEARCH REPORT

An i-type lysozyme (*Cf*LyzI) involved in innate immunity is essential for the survival of *Chlamys farreri* during *Vibrio* stimulation

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Abstract

Lysozymes act as key components of the innate immunity in invertebrates and play a pivotal role in early defense against invading microbe infection. In this study, an i-type lysozyme homology was identified and characterized in Zhikong scallop *Chlamys farreri* (designated as *Cf*LyzI). The full-length cDNA sequence of *Cf*LyzI contained a 702 bp open reading frame (ORF), which encoded a polypeptide of 233 amino acids and contained a typical destabilase function domain. The mRNA transcripts of *Cf*LyzI were detectable in all the investigated tissues, including hemocytes, muscle, mantle, gill, hepatopancreas and gonad with the peak level in hemocytes. Being stimulated by *Vibrio splendidus*, the mRNA transcripts of *Cf*LyzI significantly increased in hemocytes. The *Cf*LyzI-suppressed scallops turned to be more susceptible to *Vibrio*. All these results indicated that *Cf*LyzI acted as an efficient effector in the innate immunity and was also essential for hosts' survival during *Vibrio* stimulation in Zhikong scallop.

Key Words: *Chlamys farreri*; Innate immunity; Invertebrate type lysozyme

Introduction

The innate immunity, also known as non-specific immunity or in-born immunity, acts as first line for all the multicellular organisms and almost the only defense mechanism for invertebrates that protects hosts from microbial invaders (Medzhitov and Janeway Jr, 1997). Antimicrobial proteins and peptides (AMPs), also called host defense peptides (HDPs), are ancient effector molecules of innate immunity, and provide a principal first line of defense against the microbial pathogens in all living organisms (Hoffmann *et al*., 1999). Among all the identified AMPs, lysozyme (EC 3.2.1.17), also termed as *N*-acetylmuramide glycanhydrolase or muramidase is an antimicrobial enzyme forming part of the innate immunity and also regarded as an important digestive enzyme in animals, especially in ruminant artiodactyls and filter-feeding organisms (Daffre *et al*., 1994; Boman, 1995).

Lysozyme makes up a large amount of proteins and peptides and ubiquitously presents in diverse organisms ranging from human to virus (Johnson, 1998). Based on catalytic characteristics, molecular features and original sources, lysozymes could be classified into several types, including bacteria type, chalaropsis type (ch-type), chicken/conventional type (c-type), goose type (g-type), invertebrate type (i-type), phage type and plant type (Jielian *et al*., 2017). Among all the animal origin types, c-type and g-type lysozymes are present in all the vertebrates, while invertebrates mainly produce i-type lysozymes and partially produce c-type, ch-type or g-type lysozymes (Callewaert and Michiels, 2010). Moreover, i-type lysozymes exhibit multiple activities, such as chitinase, isopeptidase, muramidase and non-enzymatic antibacterial activities (Van Herreweghe and Michiels, 2012).

Zhikong scallop *Chlamys farreri* (Mollusca; Bivalvia; Pteriomorphia) is a dioecious bivalve native to the coast of China, Japan and Korea, and weightily contributes to the aquaculture industry of

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northern China (Li *et al*., 2017b). However, scallop aquaculture industry has been experiencing mass mortality during summer period and suffering from extensive economic losses in the past decades (Matozzo, 2016; Huang *et al*., 2018). The complex interactions among environment, hosts and pathogen are regarded to be the main causes for such mass mortality of cultured scallops (Wang *et al*., 2012). Lysozymes make a major contribution to the accomplishment of innate immune responses (Saurabh and Sahoo, 2008). In previous studies, a g-type lysozyme (designated as *Cf*LyzG) exhibited inhibitive effect on the growth of both Gram negative and Gram positive bacteria with more potential activities against Gram positive bacteria, and its single nucleotide polymorphisms (SNPs) were associated with resistance or susceptibility to *Vibrio* (*Listonella*) *anguillarum* (Zhao *et al*., 2007b; Li *et al*., 2009; 2013). In the present study, an i-type lysozyme gene (designated as *Cf*LyzI) have been cloned and investigated in Zhikong scallop, and the main objectives of the present study were (1) to characterize the molecular features of *Cf*LyzI (2) to validate the tissue and temporal expression patterns of its mRNA transcripts, and (3) to confirm the function of *Cf*LyzI via double strand RNA (dsRNA) mediated RNA interference (RNAi).

Materials and methods

Scallops, Vibrio *stimulation, temperature stress and samples collection*

Vibrio splendidus was cultured in liquid 2216E media (HB0132-1, HopeBiol, China) at 28 °C and 140 rpm overnight. The bacteria were collected by centrifugation at 4000 *g* for 10 min and re-suspended in filtered seawater. Approximately sixty scallops were employed for *Vibrio* stimulation assay. Scallops were purchased from a local farm in Qingdao, China, and maintained in aerated seawater at 20 °C. After acclimated for two weeks, thirty scallops were immersed with live bacteria *V. splendidus* at a final concentration of 1.0×10^8 colony forming units (CFUs) per 1 mL, defined as *Vibrio* stimulation group. The rest thirty scallops were employed as the control group. Five scallops from the two groups were randomly sampled at 0, 3, 6, 12, 24 and 48 h post stimulation, respectively. For the temperature stress assay, modified from the previous report (Ding *et al*., 2018), the scallops were randomly divided into five groups, and treated at 10 °C, 15 °C, 20 °C (as control), 25 °C and 30 °C for 7 days, respectively. Five scallops from each group were sampled. Hemocytes, muscle, mantle, gill, hepatopancreas and gonad from five untreated scallops were collected to determine the mRNA distribution of *Cf*LyzI.

RNA preparation and cDNA synthesis

Total RNA was isolated from the hemocytes and other tissues using RNAiso Plus (9108, Takara, Japan). The first-strand cDNA was synthesized with SuperScript IV Reverse Transcriptase (18090010, Thermo Fisher Scientific, USA) using the RQ1 DNase I (M6101, Promega, USA) treated total RNA as template and adaptor primer-oligo (dT) as primer (Table 1). The reactions were performed at 55 °C for 1 h, terminated by heating at 80 °C for 5 min, then a homopolymeric tail was added using dCTP (4028, Takara, Japan) and terminal deoxynucleotidyl transferase (TdT, 2230, Takara, Japan), and then stored at -80 °C till used.

Cloning the full-length cDNA of Cf*LyzI*

In our previous studies, a transcript sequence homologues to previous identified i-type lysozymes was identified in *C. farreri* via assembling and screening public available expression sequence tags and transcriptomic data (Wang *et al*., 2018b). And this transcript sequence was selected for further cloning of *Cf*LyzI. Four gene-specific primers, *Cf*LyzI-RACE-R1/2 and *Cf*LyzI-RACE-F1/2, were designed to clone the full-length cDNA of *Cf*LyzI via 5` and 3` rapid amplification of cDNA ends (RACE) technique, respectively (Table 1). All the PCR reactions was performed in a MJ Mini Personal Thermal Cycler (Bio-Rad, USA), and PCR products were purified with Monarch DNA Gel Extraction Kit (T1020S, NEB, USA), ligated into the pMD18-T simple vector (D103A, Takara, Japan), and then transformed into the competent cells *Escherichia coli* DH5α (CB101, Tiangen, China). The positive recombinants were identified using anti-ampicillin selection and confirmed by PCR screening using the universal primers M13-47 and RV-M (Table 1). Five of the positive clones were sequenced in a PRISM 3730XL automated sequencer (Thermo Fisher Scientific, USA).

Bioinformatics analysis of Cf*LyzI cDNA and deduced protein sequences*

Blast+ 2.7.1 was employed to conduct the search for sequence similarities. The deduced amino acid sequences of *Cf*LyzI were analyzed by Lasergene suite 7.1.0.44 using the EditSeq module. The presence and location of signal peptide was predicted by SignalP 4.1. SMART 7.0 was employed to analysis the function domains. Multiple sequence alignments were generated with Clustal Omega 1.2.4 and visualized by Sequence Manipulation Suite 2.0 using the multiple alignment show module.

Quantitative real-time PCR analysis of Cf*LyzI mRNA expression patterns*

The tissue and temporal expression patterns of *CfLyzI mRNA* in hemocytes were investigated by quantitative real-time PCR (qRT-PCR). All the qRT-PCR reactions were performed in a LineGene K FQD-48A Fluorescence Quantitative PCR Detection System (Bioer, China) using the SYBR premix ExTaq (RR420, Takara, Japan). All the primers for qRT-PCR were listed in Table 1. For each sample, the mRNA expression of *Cf*LyzI was normalized to that of elongation factor 1 α (EF-1α). The relative abundance of *Cf*LyzI mRNA was calculated using comparative C_T method ($2^{-\Delta\Delta Ct}$ method) as mean ± SD (Schmittgen and Livak, 2008). The data were subjected to one-way analysis of variance (ANOVA) followed by a multiple comparison using IBM SPSS Statistics 23.0.0.0, and the *p* values less than 0.05 were considered statistically significant.

Table 1 Oligonucleotide primers used in the experiments

Primer	Sequence (5`-3`)	Brief information
adaptor primer	GGCCACGCGTCGACTAGTAC	Anchor primer for 3' RACE
adaptor primer-oligo (dG)	GGCCACGCGTCGACTAGTACG_{10}HN	Anchor primer for 5' RACE
adaptor primer-oligo (dT)	GGCCACGCGTCGACTAGTACT_{17}VN	Oligo (dT) for cDNA synthesizing
*Cf*EF-1α-qRT-F	ATCCTTCCTCCATCTCGTCCT	Internal control for real-time PCR
*Cf*EF-1α-qRT-R	GGCACAGTTCCAATACCTCCA	Internal control for real-time PCR
*Cf*LyzI-CDS-F	ATGTGCATTTATTTGTATCCTAACTCT	Gene specific primer for CDS
*Cf*LyzI-CDS-R	CTAGCTGTGTGCCGAGCAACCCAT	Gene specific primer for CDS
*Cf*LyzI-dsRNA-Basic-F	GACAGATAGATAAAATACAAGCAAGAT	Gene specific primer
*Cf*LyzI-dsRNA-Basic-R	CCCACAGTCATGCCAGTAGGC	Gene specific primer
*Cf*LyzI-dsRNA-T7-F	GGATCCTAATACGACTCACTATAGGGATCCGACAGATAGATAAAATACAAGCAAGAT	Gene primer incorporated with T7 promoter
*Cf*LyzI-dsRNA-T7-R	GGATCCTAATACGACTCACTATAGGGATCCCCCACAGTCATGCCAGTAGGC	Gene primer incorporated with T7 promoter
*Cf*LyzI-qRT-F	CTTTGCCACAGGTAGCGT	Gene specific primer for real-time PCR
*Cf*LyzI-qRT-R	TTTCCCACAGTCATGCCAG	Gene specific primer for real-time PCR
*Cf*LyzI-RACE-F1	CCGGTGACCTTCATTGTTCATCCA	Gene specific primer for RACE
*Cf*LyzI-RACE-F2	CAACTGTGAGAGCTACGCACGGATCCA	Gene specific primer for RACE
*Cf*LyzI-RACE-R1	ACATCTTCAGCGTGTCCTTGTTCTCGA	Gene specific primer for RACE
*Cf*LyzI-RACE-R2	ATCGGCCTTATCTTGCTTGTATTTTAT	Gene specific primer for RACE
EGFP-dsRNA-Basic-F	CGACGTAAACGGCCACAAGT	GFP specific primer
EGFP-dsRNA-Basic-R	CTTGTACAGCTCGTCCATGC	GFP specific primer
EGFP-dsRNA-T7-F	GGATCCTAATACGACTCACTATAGGGATCCGACGTAAACGGCCACAAGT	GFP primer incorporated with T7 promoter
EGFP-dsRNA-T7-R	GGATCCTAATACGACTCACTATAGGGATCCTTGTACAGCTCGTCCATGC	GFP primer incorporated with T7 promoter
M13-47	CGCCAGGGTTTTCCCAGTCACGAC	Vector primer for sequencing
RV-M	GAGCGGATAACAATTTCACACAGG	Vector primer for sequencing

Knock-down of Cf*LyzI* in vivo *via RNAi*

T7 promoter tagged primers EGFP-dsRNA-T7-F/R and *Cf*lyzI-dsRNA-T7-F/R (Table 1) were used to amplify the cDNA fragments of enhanced green fluorescent protein (EGFP) and *Cf*LyzI, and the resultant PCR products were purified to synthesize dsRNA. The dsRNA were produced via *in vitro* transcription according to the methods previously described (Wang *et al*., 2018a; Wang *et al*., 2011), and its concentration was quantified using Nanodrop Lite (Thermo Fisher Scientific, USA) and adjusted to a final concentration of 1 mg mL^{-1}. One hundred micrograms of *Cf*LyzI dsRNA was injected into adductor of each scallop, and the control groups received an injection of 100 mg EGFP dsRNA or PBS, while the untreated scallops were employed as blank group. Post dsRNA injection, hemocytes from five scallops of each group were collected every 12 h and used for total RNA isolation and cDNA synthesis. The efficiency of gene silence was confirmed via qRT-PCR, and an optimum time point (36 h post dsRNA injection) was selected for the *Vibrio* stimulation and mortality comparison assay.

Vibrio stimulation and mortality comparison

Approximately four hundred scallops were employed for *Vibrio* stimulation and mortality comparison assay according to our previous descriptions (Wang *et al*., 2011; 2018a). These scallops were equally and randomly divided into four groups (one experimental group, two control groups and one blank group) and then each group was subdivided into three subgroups. At 36 h post dsRNA injection, the scallops were stimulated with live *V. splendidus* at the final concentration of 1×10^8 CFUs mL^{-1}. The cumulative mortalities were recorded every 12 h. The *t*-test was used to verify significant differences in mortality levels between different groups, and the *p* values less than 0.05 were considered as statistically significant.

Results

The molecular feathers of Cf*LyzI*

The complete cDNA sequence of *Cf*LyzI was obtained via 5` and 3` RACE technique and submitted to GenBank with the accession number KU361831. It comprised 880 bp, containing a 105 bp

5` untranslated regions (UTR), a 73 bp 3` UTR with a poly A tail, a polyadenylation signal site (AATAAA) and an 702 bp open reading frame (ORF). This ORF encoded a polypeptide of 233 amino acid residues with a predicted molecular mass at approximately 25.19 kDa and a theoretical isoelectric point (pI) of 7.35. A signal peptide (from M^{1} to A^{31}) and a typical destabilase domain (from S^{119} to M^{227}) were revealed in the deduced amino acid sequence. Additionally, two specific motifs (from C^{122} to C^{127} and from W^{130} to K^{153}) were also revealed (Figure 1A). Multiple sequence alignment of *Cf*LyzI with *Cf*LyzG showed that the homology between these two lysozymes was rather low (Figure 1B). While pairwise sequence alignment showed that *Cf*LyzI exhibited higher homology to its invertebrate counterparts, for examples, *Cf*LyzI exhibited similarities of 60 % to i-type lysozyme of *Chlamys islandica* (CAB63451), 53 % to that of *Crassostrea rivularis* (ADY38955) and 45 % to that of *Mytilus galloprovincialis* (AJQ21515).

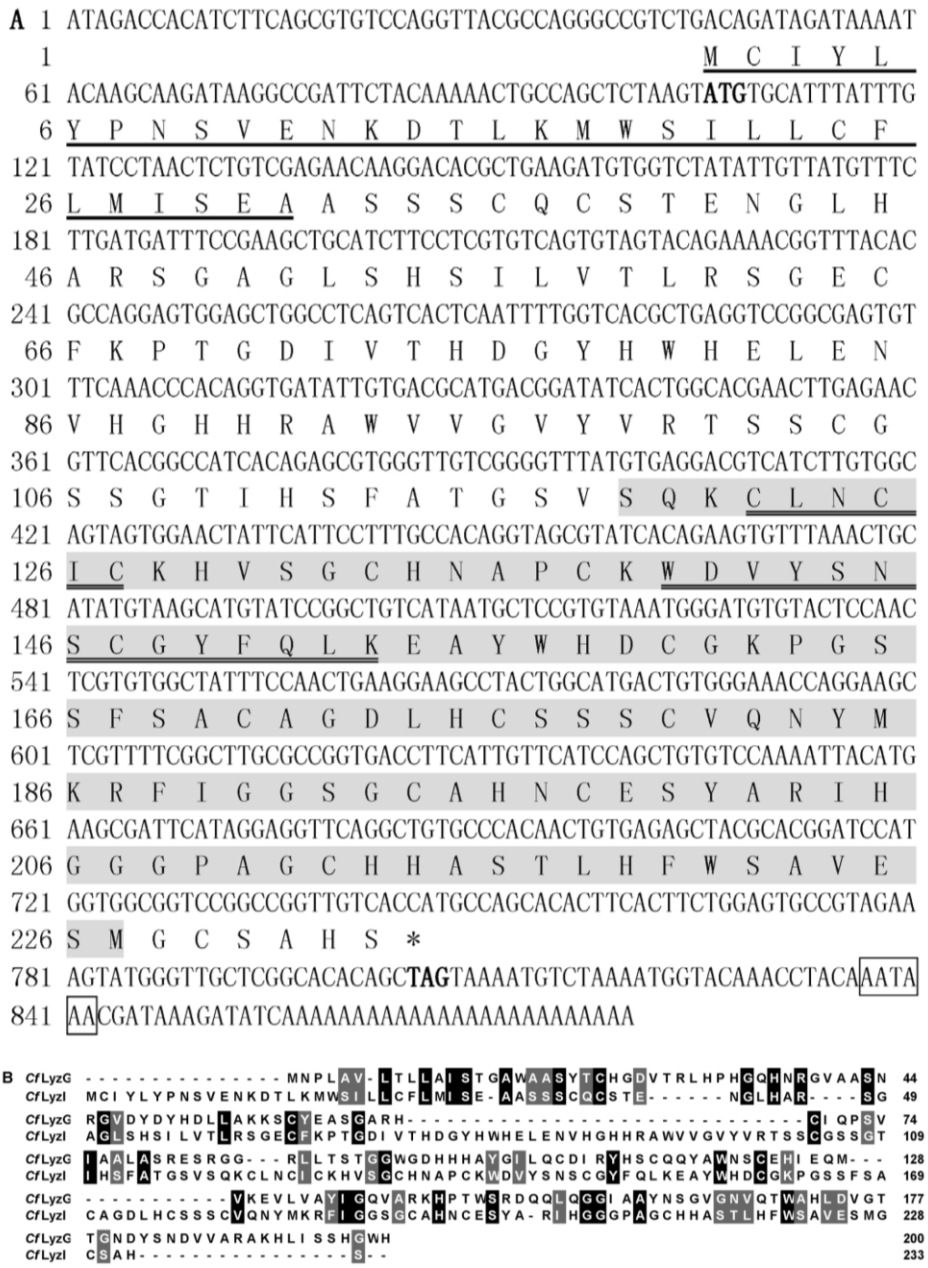

Fig. 1 Sequence features and multiple alignments of *Cf*LyzI. A: Sequence features. The nucleotides and amino acids were numbered along the left margin. The putative signal peptide was underline. The typical function domain was in shade. The specific motifs were double underlined. The stop codon was indicated by the asterisk. The polyadenylation signal (AATAAA) site was boxed. B: Multiple alignments of *Cf*LyzI with *Cf*LyzG. The black shadow region stood for positions with the same amino acids. Similar sites were in grey. Gaps were indicated by dashes

The tissue and temporal mRNA expression patterns of Cf*LyzI*

The qRT-PCR was employed to detect the tissue and temporal mRNA expression patterns of *Cf*LyzI. The *Cf*LyzI mRNA could be detected in all the investigated tissues. The peak level of *Cf*LyzI mRNA was found in hemocytes, followed by gill, which were 9.18-fold and 3.39-fold of that in gonad, respectively ($p < 0.05$, Figure 2A). Hemocytes were selected to test the temporal mRNA expression patterns of *Cf*LyzI post *Vibrio* stimulation. The mRNA expression levels of *Cf*LyzI were significantly up-regulated at 3 h post *Vibrio* stimulation (4.55-fold compared with the origin level, $p < 0.05$), reached to the peak level at 12 h (9.09-fold, $p < 0.05$), and then down-regulated to the origin level at 48 h (Figure 2B). Additionally, *Cf*LyzI mRNA expression levels in hemocytes were temperature-dependent. Compared with the control group, the *Cf*LyzI mRNA expression levels were stable at 15 °C and 25 °C, but significantly decreased at 10 °C and 30 °C, which was 0.53-fold and 0.22-fold compared with the origin level ($p < 0.05$), respectively (Figure 2C).

Cumulative mortality of Cf*LyzI-suppressed scallops*

The effect of RNAi for *Cf*LyzI was confirmed by qRT-PCR. Generally, 70% inhibition of mRNA expression post dsRNA injection was considered as a threshold for an effective RNAi experiment (Krueger *et al.*, 2007). In the present study, the mRNA abundance of *Cf*LyzI gene started to decrease at 24 h post dsRNA injection and maintained rather low (less than 0.3-fold of the origin expression level) from 36 h to 84 h (Figure 3A). So, 36 h post-dsRNA injection was selected as the optimum time point for the following *Vibrio* stimulation and cumulative mortality assay. Without *Vibrio* stimulation, the final mortality rates were 9.47%, 8.92%, 8.83% and 9.29% for normal, PBS injected, EGFP-dsRNA injected and *Cf*LyzI-suppressed scallops, respectively (Figure 3B). Being stimulated with *Vibrio*, the cumulative mortality of *Cf*LyzI-suppressed scallops was significantly higher than those of control groups from 12 h post stimulation. The *Cf*LyzI-suppressed scallops died out at 72 h, while the cumulative mortalities were 51.35%, 58.15% and 57.69% for normal, PBS injected and EGFP-dsRNA injected scallops at the same time, respectively. The semi-lethal time for *Cf*LyzI-suppressed scallops was less than 48 h, while those of other groups were about 72 h (Figure 3C).

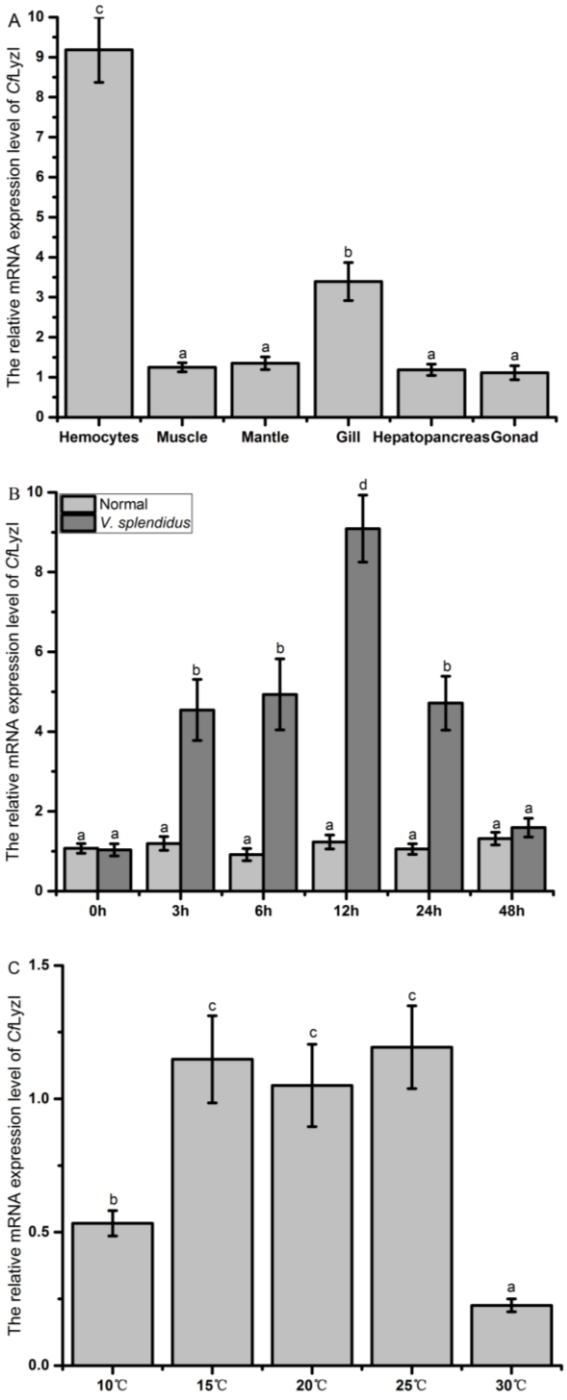

Fig. 2 Tissue and temporal expression patterns of *Cf*LyzI. A: Tissue distribution of *Cf*LyzI. B: Temporal mRNA expression patterns of *Cf*LyzI post *Vibrio* stimulation. C: Temporal mRNA expression patterns of *Cf*LyzI under temperatures stress. Bars with different characters stood for significant difference ($p < 0.05$)

Discussion

I-type lysozymes play pivotal roles in invertebrate innate immunity and are considered to be the first barrier against invading microbes (Saurabh and Sahoo, 2008). In invertebrates, especially in marine invertebrates, recent research achievements indicated that i-type lysozymes were extensively involved in innate immune responses and exhibit more extensive activities than those of terrestrial invertebrates, due to various invading microbes in the aquatic environment (Jielian *et al.*, 2017). For examples, an i-type lysozyme from the Asiatic hard clam *Meretrix meretrix* expressing along with larval development showed typical lysozyme activity and strong antibacterial activity against Gram negative and Gram positive bacteria, and its SNPs were correlated with the resistance to *Vibrio*

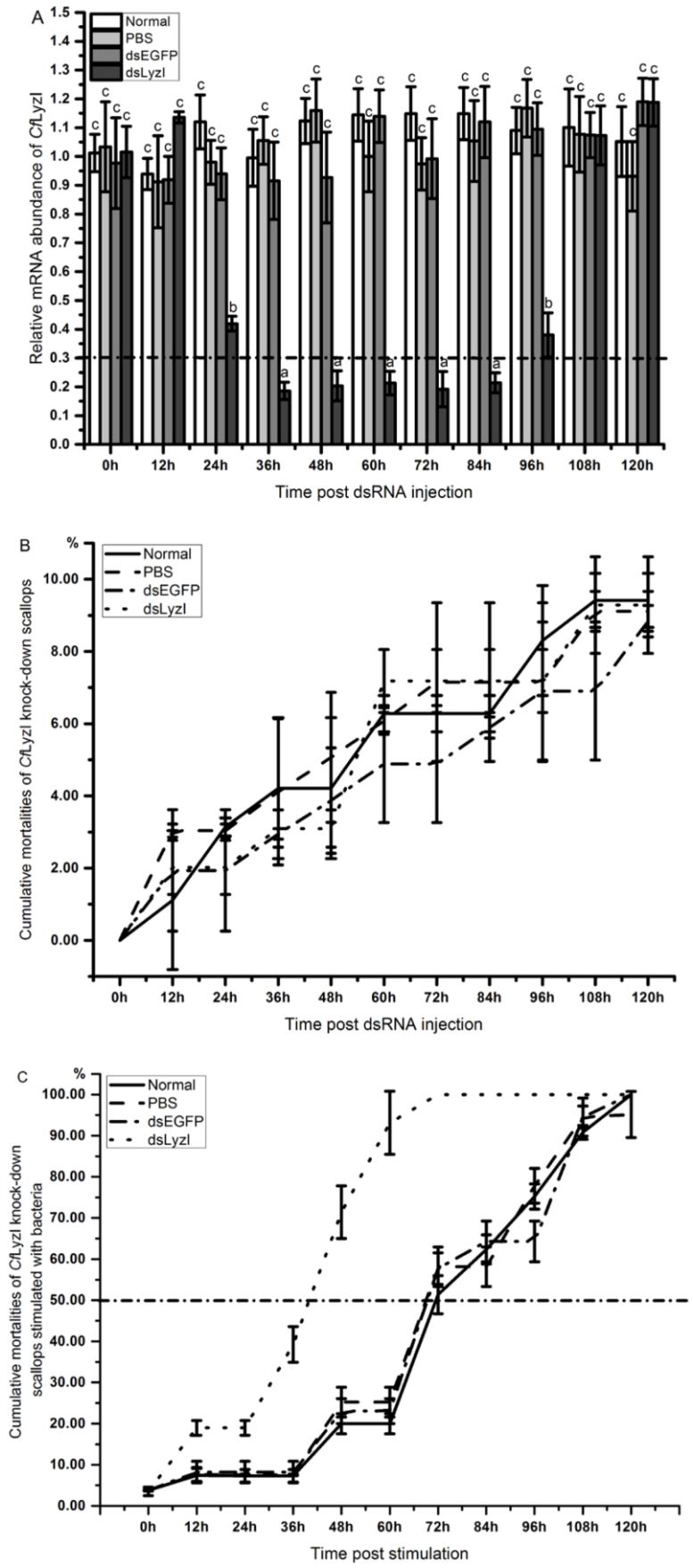

Fig. 3 RNAi of *Cf*LyzI and its effect. A: The relative abundance of *Cf*LyzI mRNA in scallops hemocytes. Bars with different characters stood for significant difference ($p < 0.05$). B: Mortalities without *Vibrio* stimulation. C: Mortalities post *Vibrio* stimulation

and growth of hosts (Yue *et al*., 2011; 2012; 2013). The intensive expression profiles and strong antimicrobial activities against bacteria of recombinant i-type lysozyme indicated its potential antibacterial roles in the sea cucumber *Apostichopus japonicas* (Wang *et al*., 2008; 2009; Yang *et al*., 2010). A recombinant i-type lysozyme from the white shrimp *Litopenaeus vannamei* showed a broad spectrum of antimicrobial properties with high antibacterial activities against *Vibrio* species (Chen *et al*., 2016), while an i-type lysozyme was potentially involved in the ontogenesis and immune defense in Kuruma shrimp *Marsupenaeus japonicus* (Liu *et al*., 2016). Additionally, the research achievements on i-type lysozymes in the eastern oyster *Crassostrea virginica* indicated a possible adaptive evolutionary pathway for i-type lysozymes from host defense to digestion in bivalves (Xue *et al*., 2004; 2007; 2010). Although many i-type lysozyme genes have been identified from marine bivalves, to our best knowledge, no i-type lysozymes have been studied in Zhikong scallop yet (Jielian *et al*., 2017). In the present study, a novel i-type lysozyme, *Cf*LyzI, was identified and characterized from *C. farreri*. Its molecular feathers, tissue and temporal expression patterns and potential function were also investigated.

Bioinformatics analysis revealed that *Cf*LyzI contained a typical destabilase domain as the same as previously identified i-type lysozymes, and exhibited high similarity with its invertebrate counterparts. Two specific motifs were revealed, which was as same as the observation in razor clam *Sinonovacula constricta* and Manila clams *Venerupis* (*Ruditapes*) *philippinarum* (Zhao *et al*., 2010; Chen *et al*., 2018). Moreover, similar with its counterpart from *S. constricta*, the deduced protein sequence of *Cf*LyzI contained high amount of cysteine residues (8.59%, 20 among 233 residues), contributing to its stability in the high osmolality seawater (Chen *et al*., 2018). Its sequence characteristics, high similarities with other previously identified i-type lysozymes collectively suggested that *Cf*LyzI is a novel member of i-type lysozymes and may have similar functions with its homologues from other invertebrates.

The i-type lysozyme functions as the essential defender against invading microbes, and its mRNA transcripts have been reported to be ubiquitously found in various tissues in marine invertebrates (Jielian *et al*., 2017). In the present study, the *Cf*LyzI mRNA transcripts were detectable in all the investigated tissues and such ubiquity indicated that it would process many important physiological functions, especially as the first barrier against invading microbes in innate immunity. The peak level of *Cf*LyzI mRNA expression was found in hemocytes, followed by gill, and the variable tissue distribution of *Cf*LyzI mRNA transcripts would be related with tissue function. Mollusk hemocytes have been reported to be responsible for bactericidal activity by mediating numerous toxic compounds, such as lysozyme, lysosomal enzymes, nitric oxide, phenol oxidase and superoxide (Li *et al*., 2008). Gill was reported to be the first line against invading microbes in fish or lower animals, and a recent report demonstrated that tubules of gill filaments might be one of the potential hematopoietic positions in mollusk (Li *et al*., 2017a). The abundance of *Cf*LyzI mRNA in hemocytes and gill implied its pivotal roles in the innate immunity of scallops.

Hemocytes are the major immune cells and respond to invading microbes mainly via phagocytosis in mollusks, and this tissue is usually selected to investigate the fluctuation of immune related genes (Canesi *et al*., 2002). In the present study, hemocytes were also selected to test the temporal expression patterns of *Cf*LyzI post *Vibrio* stimulation and during temperature stress. It has been reported that the mRNA scripts of i-type lysozyme could be induced by various invading microbes (Jielian *et al*., 2017). After the *Vibrio* stimulation, the expression levels of *Cf*LyzI mRNA in hemocytes sharply increased and reached the peak at 12 h, which was consist with previous observation on other mollusks species (Chen *et al*., 2018). Hence, *Cf*LyzI could participate in innate immunity via acting as an important innate immune effector to kill or eliminate invading microbes. Additionally, the mRNA expression of *Cf*LyzI were significantly depressed at 10 °C and 30 °C, and high temperature could inhibit the mRNA expression of *Cf*LyzI more efficiently, compared with cold shock. Such susceptible to temperature, especially to heat shock, might provide valuable insights and potential clue to a possible treatment for large scale mortalities of cultured scallops in summer.

As a major pathogen, the Gram negative bacteria *Vibrio* was believed to cause mass mortality of cultured scallop (Zhao *et al*., 2007a). A previous study revealed that SNPs of *Cf*LyzG was speculated to be associated with the resistance or susceptibility of *C. farreri* to *Vibrio* (Zhao *et al*., 2007b; Li *et al*., 2009; 2013). In the present study, the role of *Cf*LyzI against *Vibrio* infection in scallop has been evaluated via *Vibrio* stimulation and RNAi technique. After *Vibrio* stimulation, the cumulative mortality of *Cf*LyzI-suppressed scallops significantly increased and the semi-lethal time for *Cf*LyzI-suppressed scallops significantly shortened. These results suggested that the *Cf*LyzI was involved in innate immunity and essential for hosts' survival during *Vibrio* stimulation in *C. farreri*.

In conclusion, the complete cDNA sequence of a novel i-type lysozyme was identified and characterized in *C. farreri*. Its mRNA transcripts could be significantly induced by *Vibrio* stimulation. The *Cf*LyzI-suppressed scallops turn to be more susceptible to *Vibrio*. All these results indicated that *Cf*LyzI was an efficient effector involved in the innate immunity and also essential for hosts' survival during *Vibrio* stimulation in *C. farreri*.

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