ISJ 14: 404-413, 2017 ISSN 1824-307X

RESEARCH REPORT

Molecular characterization and transcriptional analysis of a crustacean heat shock protein 10 gene in shrimp *Litopenaeus vannamei*

M-Q Wang[1], B-J Wang[1], Ke-Y Jiang[1], M Liu[1], Si-Y Han[1,3], L Wang[1, 2]

[1]Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[2]Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China
[3]University of Chinese Academy of Sciences, Beijing 100049, China

Accepted October 20, 2017

Abstract

Heat shock proteins (HSPs) are the most abundant and ubiquitous soluble intracellular proteins which conserved phylogenetically in all living organisms from archaebacteria to humans. Recent research achievements indicated that HSP10s might not only be involved in the responses to environmental stresses, but also play a pivotal role in the host defenses mechanism. In the present study, a cDNA of 715 bp for the Pacific white shrimp *Litopenaeus vannamei* HSP10 (designated as *Lv*HSP10) was cloned via rapid amplification of cDNA ends (RACE) technique. The complete cDNA sequence of *Lv*HSP10 contained an open reading frame (ORF) of 309 bp, which encoded a protein of 102 amino acids. The protein sequence of *Lv*HSP10 shared over 80 % similarity with previously identified HSP10s. There were a CPN10 domain and a chaperonins HSP10/CPN10 signature in the protein sequence of *Lv*HSP10. The mRNA transcripts of *Lv*HSP10 were constitutively expressed in all the tested tissues, including eyestalk, gill, gonad, heart, hemocytes, hepatopancreas, intestine, muscle, nerve and stomach, with the highest expression level in hepatopancreas. The mRNA expression profiles of *Lv*HSP10 in hepatopancreas could be significantly induced by the stimulation of *Vibrio parahaemolyticus*, white spot syndrome virus (WSSV), and low and high pH challenge. These results provided useful information of the potential roles of *Lv*HSP10 in the defense mechanism of shrimp against various biological stimulations and multiple environmental stresses.

Key Words: heat shock protein 10; hepatopancreas; *Litopenaeus vannamei*

Introduction

Heat shock proteins (HSPs) are the most abundant and ubiquitous soluble intracellular proteins which phylogenetically conserved in all living organisms from prokaryotes to eukaryotes (Schlesinger, 1990). In recent years, HSPs have attracted considerable interest among immunologists in the context of transcriptional regulation, evolution and innate immunity (Feder and Hofmann, 1999). According to the molecular mass, HSPs could be classified into several families, such as HSP100s, HSP90s, HSP70s, HSP60s, HSP40s, low molecular mass HSPs (small HSPs) and so on (Sørensen *et al*., 2003). To date, a large number of HSP members have been identified. Among them, HSP10 was first discovered and identified in the serum of pregnant women and displayed immunosuppressive properties (Morton *et al*., 1974). HSP10 is a highly conserved 10 kDa protein (Hartman *et al*., 1992), which co-chaperones with another heat shock protein HSP60 for protein folding as well as the assembly and disassembly of protein complexes to be involved in many biological processes, such as cell apoptosis (Lin *et al*., 2001), cellular differentiation (Cappello *et al*., 2005), cell proliferation (Sasu *et al*., 2001) and innate immunity (Chen *et al*., 1999). For example, mammalian HSP10 exhibited anti-inflammatory activity by inhibiting lipopolysaccharide (LPS) induced Toll-like receptor (TLR) signaling pathway via interactions with HSP60 (Dobbin *et al*., 2005). Moreover, HSP10 alone could be widely involved in protecting cells from stresses caused by infection, inflammation and so on (Jia *et al*., 2011).

Up to the present, most studies of HSP10 are

Corresponding author:
Lei Wang
Key Laboratory of Experimental Marine Biology
Institute of Oceanology
Chinese Academy of Sciences
Qingdao 266071, China
E-mail: wanglei@qdio.ac.cn

focused on typical model organisms (Sun and MacRae, 2005). And relatively little of gene information regarding HSP10 has been obtained from marine animals, such as *Apostichopus japonicas* (Xu *et al*., 2014), *Lutjanus sanguineus* (Zhang *et al*., 2011), *Oryzias latipes* (Hirayama *et al*., 2006), *Penaeus monodon* (Shi *et al*., 2016), *Salmo salar* (Andreassen *et al*., 2009), *Scylla paramamosain* (Ding *et al*., 2013) and *Xenopus tropicalis* (Klein *et al*., 2002). A clearly time-dependent mRNA expression pattern of HSP10 identified in *Lutjanus sanguineus* indicated that HSP10, might co-chaperoned with HSP60, played a pivotal role in the host defenses mechanism of marine animals (Zhang *et al*., 2011). However, information about HSP10s from marine animals is still few and fragmentary, and more research evidences are still needed to illustrate their exact roles in the innate immune system.

The Pacific white shrimp *Litopenaeus vannamei* has been widely cultured in the world as an important commercial species (Li and Xiang, 2013a). However, in the past two decades, outbreaks of infectious disease associated with bacteria, such as *Vibrio parahaemolyticus*, and viruses, such as white spot syndrome virus (WSSV), have become a major constraint, resulting in mass shrimp mortality, reductions in farmed shrimp production and considerable economic losses (Li and Xiang, 2013b; Zhang *et al*., 2016). Moreover, as an aquatic livestock, white shrimp are also suffered from multiple environmental stress during culture, and some of them, such as ammonia/nitrite accumulation, hypoxia stress and pH challenge, could be harmful to the survival of shrimps (Liang *et al*., 2016; Han *et al*., 2017a, b). A better understanding of mechanisms of stress tolerance is necessary for the health management of shrimp farming. In the present study, a novel HSP10 genes have been cloned and investigated in white shrimp (designated as *Lv*HSP10), and the main objectives of the present study were (1) to characterize the molecular feature of *Lv*HSP10 genes, (2) to detect the tissue distribution of its mRNA transcripts and (3) to investigate its temporal mRNA expression pattern after invading microbes and environmental factors stimulation and compare it with the previously identified white shrimp HSP60 (designated as *Lv*HSP60).

Materials and Methods

Shrimp and tissues sample collection

The white shrimps used in the present study were obtained from Ruizi Seafood Development Co. Ltd., Qingdao, China, and all the experiments were conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH). The study protocol and all experimental design were conducted with approval from Experimental Animal Ethics Committee of Institute of Oceanology, Chinese Academy of Sciences. White shrimps, with body weight 8 - 12 g, were cultured placed in 640 L cylindrical tanks with 500 L air-pumped circulating seawater at 20 ± 1 °C for two weeks before processing. Hemolymph was extracted from the ventral sinus of at least three untreated shrimps using a sterile syringe preloaded with equal volume of anticoagulant buffer (NaCl 510 mmol L^{-1}, glucose 100 mmol L^{-1}, citric acid 200 mmol L^{-1}, tri-sodium citrate 30 mmol L^{-1} and EDTA·2Na 10 mmol L^{-1}, pH 7.3). Then the hemocytes were collected by centrifugation at 800*g* for 10 min at 4 °C. Tissues including eyestalk, gill, gonad, heart, hepatopancreas, intestine (mid gut), muscle, nerve and stomach were collected from at least three untreated shrimps, kept in RNA*later* (AM7020, Thermo Fisher Scientific, USA) and stored at -80 °C until RNA isolation.

Table 1 Oligonucleotide primers used in the present study

Primer	Sequence (5`-3`)	Tm (°C)	Brief information
adaptor primer	GGCCACGCGTCGACTAGTAC	60	Anchor primer for 3` RACE
adaptor primer-oligo (dT)	GGCCACGCGTCGACTAGTACT_{17}VN	-	Olido (dT) for cDNA synthetize
*Lv*EF-1α-qRT-F	GTATTGGAACAGTGCCCGT	60	Internal control for real-time PCR
*Lv*EF-1α-qRT-R	CATCTCCACAGACTTTACCTCAG	60	Internal control for real-time PCR
*Lv*HSP10-CDS-F	ATGGCTGGTGCTCTGAAGAGGTTT	64	Gene specific primer for CDS
*Lv*HSP10-CDS-R	TTACTCGGTCTTCATCTTGGCCAAAAG	64	Gene specific primer for CDS
*Lv*HSP10-qRT-F	GGTTGCTGTTGGAGAGGGA	60	Gene specific primer for real-time PCR
*Lv*HSP10-qRT-R	TGACCTTTGTGCCACCGA	60	Gene specific primer for real-time PCR
*Lv*HSP10-RACE-F1	GGCACAAAGGTCACCCTGGAGGAGAAG	64	Gene specific primer for 3` RACE
*Lv*HSP10-RACE-F2	GGGTCAATTCGTAGGCTGATG	64	Gene specific primer for 3` RACE
*Lv*HSP60-qRT-F	ATTGTCCGCAAGGCTATC	60	Gene specific primer for real-time PCR
*Lv*HSP60-qRT-R	ATCTCCAGACGCTTCCAT	60	Gene specific primer for real-time PCR
M13-47	CGCCAGGGTTTTCCCAGTCACGAC	56	Vector primer for sequencing
RV-M	GAGCGGATAACAATTTCACACAGG	56	Vector primer for sequencing

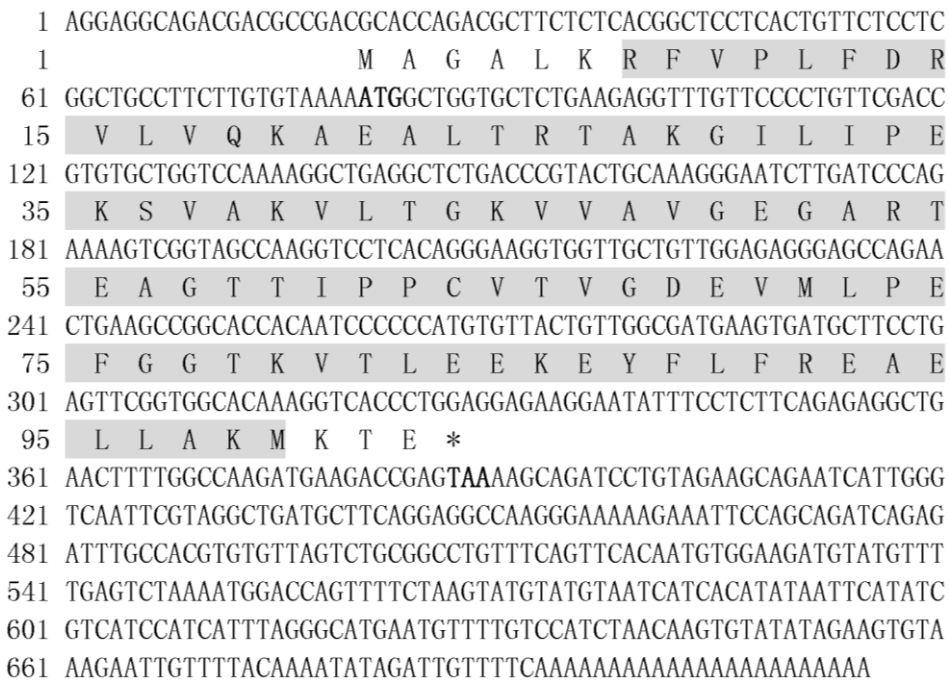

Fig. 1 Nucleotide and deduced amino acid sequences of *Lv*HSP10. The nucleotides and amino acids were numbered along the left margin. The function domain was in shade. The asterisks indicated the stop codon.

Immune stimulation, pH challenge assay and sample collection

Approximately 200 shrimps were employed for microbe stimulation assay. The *V. parahaemolyticus* suspension and WSSV stock were prepared according to previous reports (Yi *et al*., 2014; Xia *et al*., 2015; Sha *et al*., 2016). The shrimps were randomly divided into three groups and each group contained about 60 - 70 individuals. The shrimps were received an injection at the abdominal segment with 100 μL phosphate buffered saline (PBS, pH 7.4, 10010023, Thermo Fisher Scientific, USA), *V. parahaemolyticus* suspension (1×10^5 CFUs μL^{-1}, in PBS) and WSSV stock (1×10^5 copies μL^{-1}, in PBS), respectively. The injected shrimps were returned to seawater tanks immediately and the hepatopancreas of at least three individuals were randomly sampled from each group at 3, 6, 12, 24 and 48 h post injection, kept in RNA*later* and stored at -80 °C until RNA isolation. Approximately 200 shrimps were employed for pH challenge assay. The shrimps were randomly divided into three groups and each group contained about 60 - 70 individuals. Two kind of seawater at pH 6.7 and pH 9.7 were prepared using 4 mol L^{-1} HCl or 4 mol L^{-1} NaOH. Two groups of shrimps were amongst the seawater at pH 6.7 and pH 9.7 after acclimation in normal seawater (pH 8.2), respectively. The pH values were measured daily using a pH meter (PHB-4, Yidian Scientific, China). The hepatopancreas of at least three individuals in the control group and experiment groups were collected at 1, 3, 7, 14 and 28 d after the pH challenge, kept in RNA*later* and stored at -80 °C until RNA isolation.

RNA isolation and cDNA synthesis

Total RNA was isolated from various tissues using TRIzol reagent (15596026, Thermo Fisher Scientific, USA). The synthesis of first strand was carried out with Promega M-MLV using the DNase I (RQ1, M6101, Promega, USA) treated total RNA as template and adaptor primer-oligo (dT) as primer (Table 1). The reaction mixture was incubated at 42 °C for 1 h, terminated by heating to 95 °C for 5 min, and then stored at -80 °C.

Cloning the full-length cDNA of LvHSP10

The partial length sequence of *Lv*HSP10 cDNA was obtained from the transcriptome database of white shrimp (Zhao *et al*., 2017). Two gene-specific primers, *Lv*HSP10-RACE-F1/2, were designed using Primer Premier 5.00 based on this partial length sequence to clone the 3' end of *Lv*HSP10 cDNA by rapid-amplification of cDNA ends (RACE) technique. And the coding sequence (CDS) of *Lv*HSP10 was amplified and confirmed using another two gene-specific primers, *Lv*HSP10-CDS-F/R, which was also designed using Primer Premier 5.00. All PCR amplification was performed in a MJ Mini Personal Thermal Cycler (Bio-Rad, USA), and the PCR products were purified using Monarch DNA Gel Extraction Kit (T1020S, NEB, USA) and cloned into the pMD18-T simple vector (D103A, Takara, Japan). After being transformed into the competent cells *Escherichia coli* strain DH5α (CB101-03, Tiangen, China), the positive recombinants were identified via anti-ampicillin selection and verified by PCR screening using M13-47 and RV-M primers (Table 1). Three of the positive clones were sequenced using a

PRISM 3730XL automated sequencer (Thermo Fisher Scientific, USA).

Bioinformatical analysis of LvHSP10 cDNA and protein sequences

The search for protein sequence similarity was conducted with blastp 2.6.0. The deduced protein sequences of *Lv*HSP10 were analyzed by the EditSeq module in Lasergene program suite 14.0.0.88. The function domains of *Lv*HSP10 were predicted with Simple Modular Architecture Research Tool (SMART) 7.0. Multiple sequence alignments were performed with Clustal Omega 1.2.4 and visualized using multiple alignment show module in Sequence Manipulation Suite 2.0. A Neighbor-Joining (NJ) phylogenic tree was constructed with MEGA 7.0.21. To derive confidence value for the phylogeny analysis, bootstrap trials were replicated 1,000 times.

Expression pattern analysis via real-time quantitative RT-PCR

The mRNA transcripts of *Lv*HSP10 and the previous identified *Lv*HSP60 (Zhou *et al*., 2010) in different tissues or their temporal expression pattern in hepatopancreas of shrimps stimulated with various microbes or pH challenge were investigated by quantitative real-time PCR (qRT-PCR) technique. All qRT-PCR reactions were performed with the SYBR premix ExTaq (Tli RNaseH plus) (RR420, Takara, Japan) using 100 ng cDNA template in a LineGene K FQD-48A (A4) Fluorescence Quantitative PCR Detection System (Bioer, China). All the primers for qRT-PCR were designed using PerlPrimer 1.1.21 and listed in Table 1. The mRNA expression levels of *Lv*HSP10 and *Lv*HSP60 were normalized to those of elongation factor 1 α (EF-1α) for each sample. The relative mRNA expression levels of *Lv*HSP10 and *Lv*HSP60 were generated using comparative C_T method ($2^{-\Delta\Delta Ct}$ method) (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.

Results

Sequence features of LvHSP10

The full-length cDNA sequence of *Lv*HSP10 was obtained by 3' RACE technique, and deposited in GenBank under the accession number MF062460. It comprised 715 bp, containing a 5' untranslated regions (UTR) of 80 bp, a 3' UTR of 326 bp with a poly A tail and an open reading frame (ORF) of 309 bp. The ORF encoded a polypeptide of 102 amino acid residues with a calculated molecular mass of approximately 11.04 kDa and a theoretical isoelectric point (pI) of 8.12. The deduced amino acid sequence of *Lv*HSP10 contained a CPN10 domain (from R^7 to M^{99}, Fig. 1). The deduced protein sequence of *Lv*HSP10 exhibited high similarity with other previously identified HSP10s, such as 93 % identity with that of *P. monodon* (ALS05376) (Shi *et al*., 2016) and 80 % with *S. paramamosain* (AGI74966) (Ding *et al*., 2013). An alignment of the protein sequence of *Lv*HSP10 with those of previously identified HSP10s was shown in Figure 2, and a chaperonins HSP10/CPN10 signature (from F^8 to I^{32}) was revealed. The NJ phylogenetic tree based on protein sequences from multiple HSP10s was positioned separately into two main branches, and *Lv*HSP10 were clustered with its homologue from the black tiger shrimp *P. monodon* (Fig. 3).

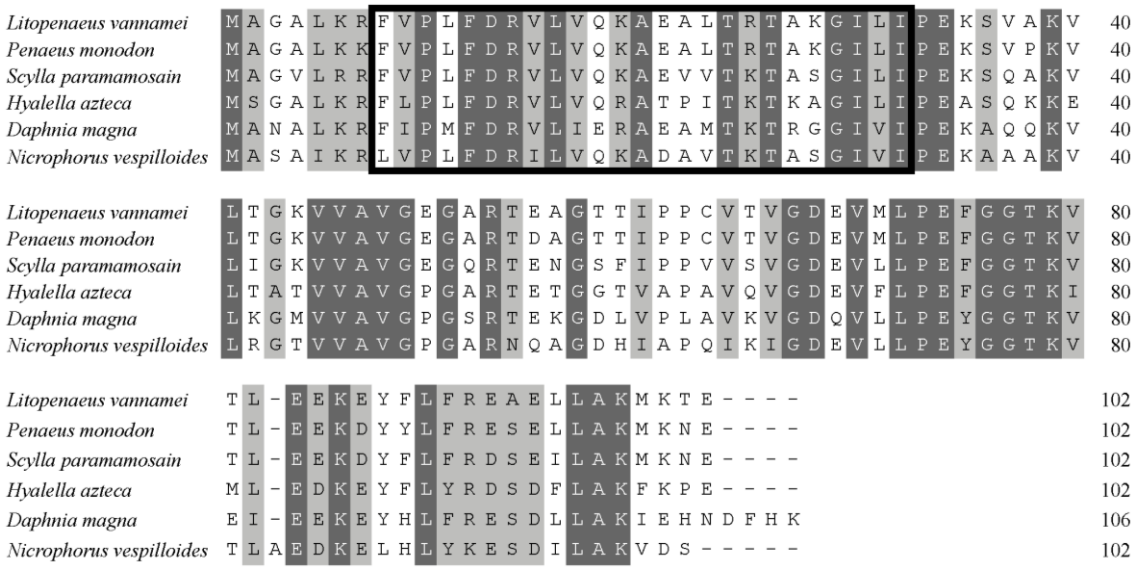

Fig. 2 Multiple alignments of *Lv*HSP10 with previous known HSP10s. The black shadow region indicated positions where all sequences share the same amino acid residue. Similar amino acids were shaded in grey. Gaps were indicated by dashes to improve the alignment. The chaperonins HSP10/CPN10 signatures were boxed. The sequences and their accession numbers are as follows: *Daphnia magna*, KZS03047; *Hyalella azteca*, XP_018022648; *Nicrophorus vespilloides*, XP_017783781; *Penaeus monodon*, ALS05376 and *Scylla paramamosain*, AGI74966.

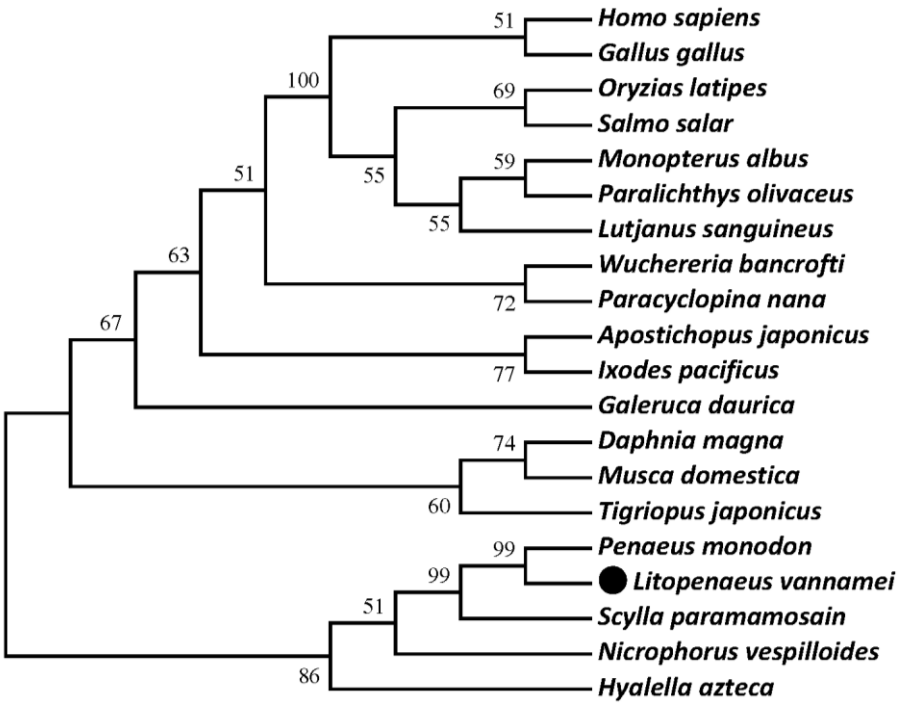

Fig. 3 Consensus neighbor-joining phylogenetic based on the protein sequences of HSP10s from different organisms. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1,000 replicates was taken to represent the evolutionary history of the taxa analyzed. All positions containing gaps and missing data were eliminated. The numbers at the forks indicated the bootstrap value. The sequences and their accession numbers are as follows: *Apostichopus japonicas*, AIF71190; *Galeruca daurica*, ARR95803; *Gallus gallus*, AAB86581; *Homo sapiens*, CAA53455; *Ixodes pacificus*, AAT92186; *Lutjanus sanguineus*, ADM63094; *Monopterus albus*, AAV37068; *Musca domestica*, AQY54358; *Oryzias latipes*, CAB40895; *Paracyclopina nana*, ADV59557; *Paralichthys olivaceus*, ABB76383; *Penaeus monodon*, ALS05376; *Salmo salar*, NP_001133144; *Scylla paramamosain*, AGI74966; *Tigriopus japonicas*, ACA03519 and *Wuchereria bancrofti*, EJW73405.

Tissue distribution of LvHSP10

The qRT-PCR was employed to detect the tissue distribution of *Lv*HSP10 mRNA transcripts with EF-1α as internal control. The *Lv*HSP10 mRNA transcripts could be detected in all the tested tissues, including eyestalk, gill, gonad, heart, hemocytes, hepatopancreas, intestine, muscle, nerve and stomach. The highest mRNA expression level was found in hepatopancreas, which was 9.12-fold ($p < 0.05$) of that in muscle, followed by hemocytes, gill and intestine, which were 5.19-fold, 4.51-fold and 4.27-fold of that in muscle ($p < 0.05$), respectively (Fig. 4).

Expression profiles of LvHSP10 and LvHSP60 post microbe stimulation

The mRNA expression levels of *Lv*HSP10 and *Lv*HSP60 were all up-regulated post the two kinds of microbe stimulation. The mRNA expression level of *Lv*HSP10 was significantly up-regulated at 6 h post *V. parahaemolyticus* stimulation (3.37-fold compared with the origin level, $p < 0.05$), and the highest level was observed at 12 h (5.99-fold, $p < 0.05$, Fig. 5A). While after stimulated with *V. parahaemolyticus*, the mRNA scripts of *Lv*HSP60 significantly increased at 3 h post stimulation (2.34-fold, $p < 0.05$) and reached the peak at 12 h (7.10-fold, $p < 0.05$), kept at a high level at 24 h (2.27-fold, $p < 0.05$) and then decreased to the origin level at 48 h (Fig. 5B). In the WSSV stimulation group, the mRNA transcripts of *Lv*HSP10 significantly increased at 3 h post stimulation (1.97-fold, $p < 0.05$) and reached the maximum level at 12 h (8.15-fold, $p < 0.05$), kept at a high level at 24 h (4.19-fold, $p < 0.05$) and then decreased but still higher than the origin level at 48 h (2.17-fold, $p < 0.05$, Fig. 5C). While the mRNA expression level of *Lv*HSP60 was significantly up-regulated at 6 h post stimulation (6.21-fold, $p < 0.05$) and reached the peak at 12 h (9.12-fold, $p < 0.05$), kept at a high level at 24 h (5.93-fold, $p < 0.05$) and then down-regulated but still higher than the origin level at 48 h (2.21-fold, $p < 0.05$, Fig. 5D).

Expression profiles of LvHSP10 and LvHSP60 post high or low pH change

In the high pH challenge experiments, the mRNA expression level of *Lv*HSP10 was significantly up-regulated at 1 d post high pH challenge (3.97-fold, $p < 0.05$) and reached the peak at 7 d (8.07-fold, $p < 0.05$), kept at a high level at 14 d (7.88-fold, $p < 0.05$) and then down-regulated to a

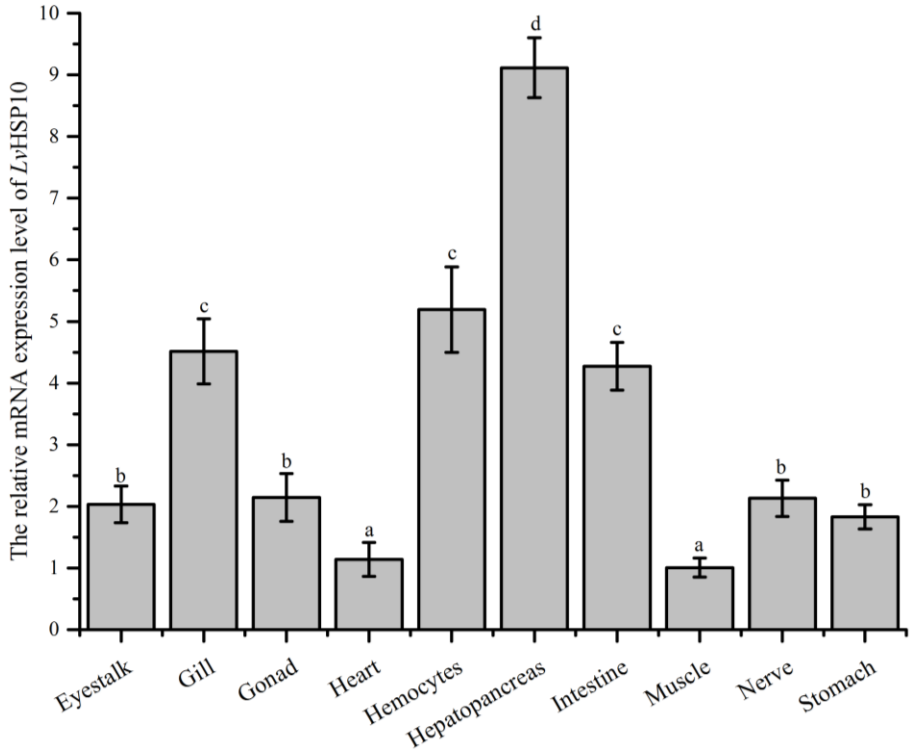

Fig. 4 Tissue distribution of *Lv*HSP10 mRNA transcripts detected by qRT-PCR technique. The mRNA transcripts levels in eyestalk, gill, gonad, heart, hemocytes, hepatopancreas, intestine, muscle, nerve and stomach of three untreated shrimps were normalized to that of muscle. The EF-1α gene was used as an internal control to calibrate the cDNA template for each sample. Vertical bars represent mean ± SD (n = 3), and bars with different characters were significantly different ($p < 0.05$), while bars with same characters were not significantly different.

lower level at 28 d (0.43-fold, $p < 0.05$, Fig. 6A). During the high pH challenge experiments, the mRNA transcripts of *Lv*HSP60 significantly increased at 1 d post high pH challenge (3.39-fold, $p < 0.05$) and reached the peak at 7 d (9.10-fold, $p < 0.05$), kept at a high level at 14 d (8.87-fold, $p < 0.05$) and then decreased to a lower level at 28 d (0.39-fold, $p < 0.05$, Fig. 6B). In the low pH challenge group, the mRNA scripts of *Lv*HSP10 significantly increased at 3 d post low pH challenge (3.87-fold, $p < 0.05$) and reached the peak at 7 d (5.75-fold, $p < 0.05$), kept at a high level at 14 d (3.62-fold, $p < 0.05$) and then decreased to the origin level at 28 d (Fig. 6C). While the mRNA expression level of *Lv*HSP60 was significantly up-regulated at 1 d post low pH challenge (2.47-fold, $p < 0.05$) and reached the peak at 7 d (6.95-fold, $p < 0.05$), kept at a high level at 14 d (2.58-fold, $p < 0.05$) and was then down-regulated to the origin level at 28 d (Fig. 6D).

Discussion

HSPs are the most abundant and ubiquitous soluble intracellular proteins which conserved phylogenetically in all living organisms, including archaebacterial, bacteria and eukaryotes (Schlesinger, 1990). Recent research achievements indicated that HSP10s might not only be involved in the responses to environmental stresses, but also play essential roles in the innate immune defenses mechanism (Jia *et al*., 2011). However, information about HSP10s from marine animals is still few and fragmentary. In the present study, the full-length cDNA of HSP10 was cloned from white shrimp *L. vannamei*. The deduced polypeptide of *Lv*HSP10 consisted of 102 amino acids, and its calculated molecular weight was 11.04 kDa, which was close to those from vertebrate and invertebrate. The protein sequence of *Lv*HSP10 shared over 80 % similarities with other identified HSP10s. Moreover, a CPN10 domain and a chaperonins HSP10/CPN10 signature were revealed from the amino acid sequence of *Lv*HSP10. Additionally, in the NJ phylogenetic tree, *Lv*HSP10 were clustered with its homologue from the black tiger shrimp *P. monodon*. The conserved function domain and signature sequence of *Lv*HSP10, high similarity with other identified HSP10s and the phylogenetic relationship collectively suggested that *Lv*HSP10 was a novel member of invertebrate HSP10 family, and it could have similar functions to those from vertebrates and other invertebrates.

To investigate the potential function of *Lv*HSP10 in shrimp, the distribution of its mRNA transcripts in different tissues was detected by qRT-PCR technique. The mRNA transcripts of *Lv*HSP10 were observed to be constitutively expressed in all the detected tissues, including eyestalk, gill, gonad, heart,

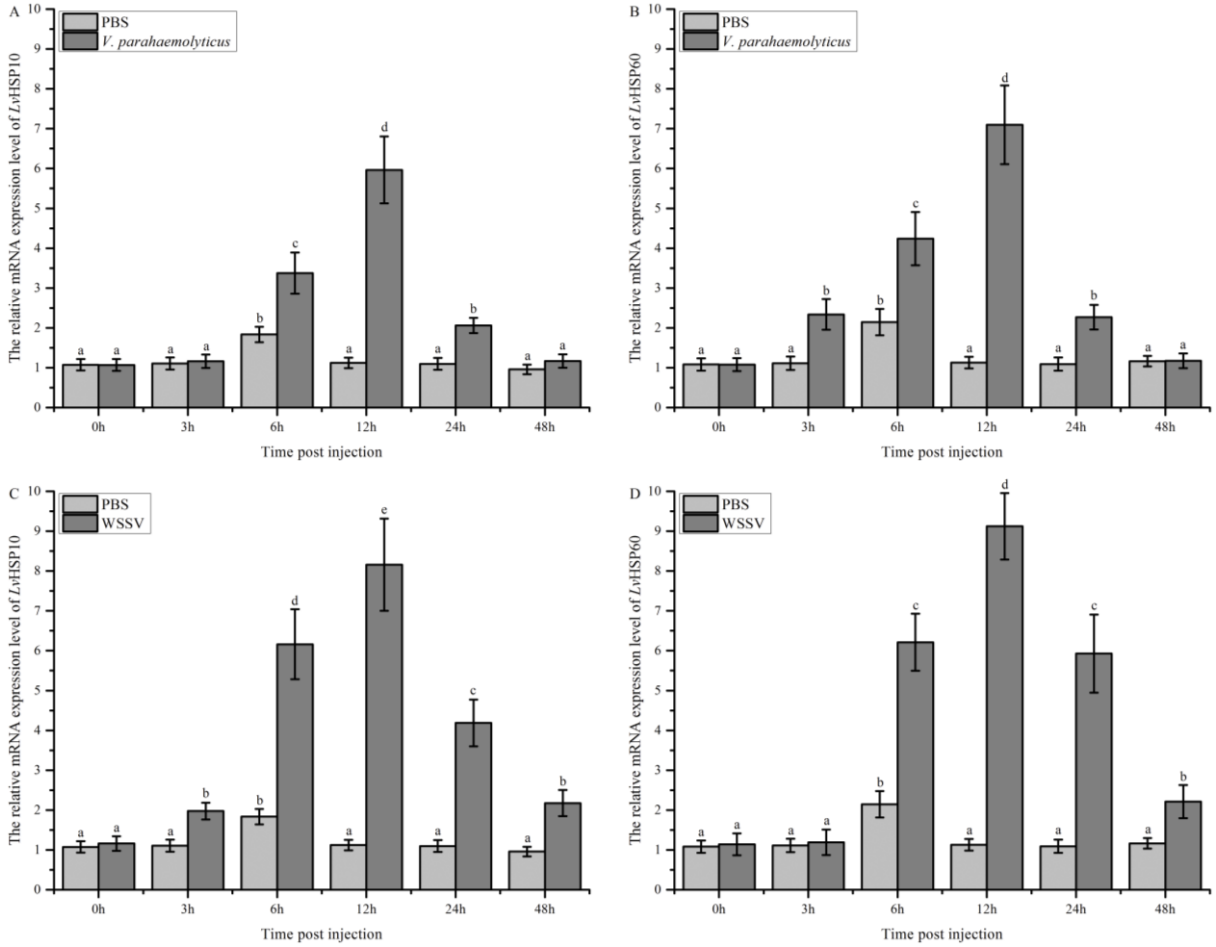

Fig. 5 Temporal mRNA expression profiles of *Lv*HSP10 and *Lv*HSP60 detected via qRT-PCR technique in white shrimp hepatopancreas at 0, 3, 6, 12, 24 and 48 h post microbe stimulation. A: Temporal mRNA expression profiles of *Lv*HSP10 post *V. parahaemolyticus* stimulation; B: Temporal mRNA expression profiles of *Lv*HSP60 post *V. parahaemolyticus* stimulation; C: Temporal mRNA expression profiles of *Lv*HSP10 post WSSV stimulation; D: Temporal mRNA expression profiles of *Lv*HSP60 post WSSV stimulation. The EF-1α gene was used as an internal control to calibrate the cDNA template for each sample. Vertical bars represent mean ± SD (n = 3), and bars with different characters were significantly different ($p < 0.05$), while bars with same characters were not significantly different.

hemocytes, hepatopancreas, intestine, muscle, nerve and stomach. The ubiquity of *Lv*HSP10 mRNA transcripts indicated that it could be involved in many important physiological processes of shrimps. The highest mRNA expression level of *Lv*HSP10 was observed in hepatopancreas, which was as high as 9.12-fold of that in muscle, followed by hemocytes, gill and intestine. Similar mRNA transcripts distribution was also observed in HSP10 from the black tiger shrimp *P. monodon*, whose highest mRNA expression level was also found in the hepatopancreas (Shi *et al*., 2016). The hepatopancreas was considered as the main immune related organ in mollusks and crustaceans (Liu *et al*., 2009; Song *et al*., 2015), hemocytes are the major immune cells and respond to invaders mainly through phagocytosis (Canesi *et al*., 2002), gill has been reported as the first defense line against invading pathogens in lower animals (Ellis, 2001), while intestine was observed to be involve in immune responses with hepatopancreas via the 'liver-gut axis' (Chang *et al*., 2012). The high mRNA expression levels of *Lv*HSP10 in these tissues confirmed the hypothesis that *Lv*HSP10 could be involved in the innate immune system of shrimp.

To further understand the immunological and physiological roles of *Lv*HSP10, its temporal expression profiles in hepatopancreas post microbe stimulation or pH challenge was detected by qRT-PCR technique and compared with those of *Lv*HSP60. In previous researches, a clear time-dependent expression pattern of HSP10 from humphead snapper *L. sanguineus* was observed after bacteria challenge (Zhang *et al*., 2011), while HSP10 from the black tiger shrimp *P. monodon* could be induced after high pH exposure, but exhibited stable expressions at low pH challenge in the hepatopancreas (Shi *et al*., 2016). In the present

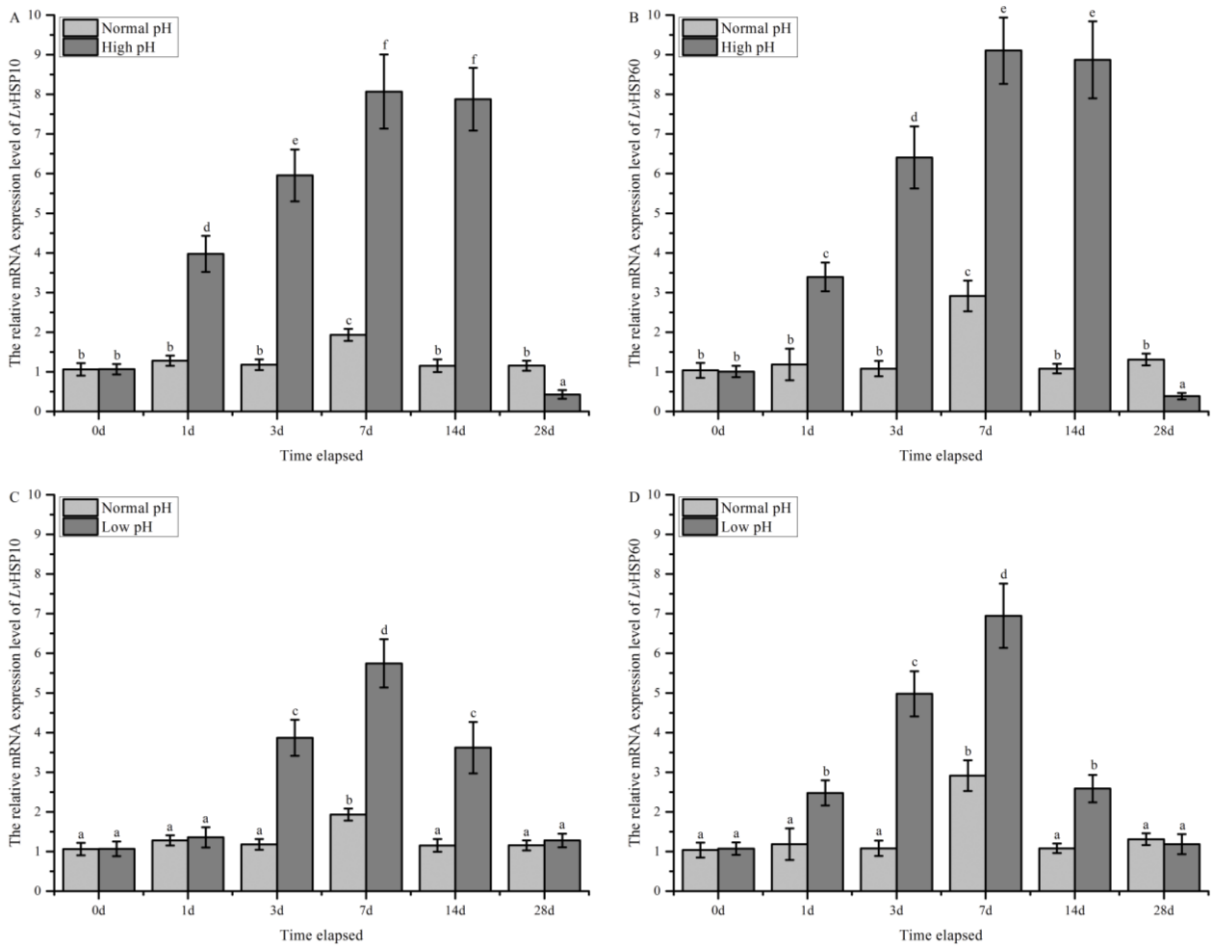

Fig. 6 Temporal mRNA expression profiles of *Lv*HSP10 and *Lv*HSP60 detected via qRT-PCR technique in white shrimp hepatopancreas at 0, 1, 3, 7, 14 and 28 d post high/low pH challenge. A: Temporal mRNA expression profiles of *Lv*HSP10 post high pH challenge; B: Temporal mRNA expression profiles of *Lv*HSP60 post high pH challenge; C: Temporal mRNA expression profiles of *Lv*HSP10 post low pH challenge; D: Temporal mRNA expression profiles of *Lv*HSP60 post low pH challenge. The EF-1α gene was used as an internal control to calibrate the cDNA template for each sample. Vertical bars represent mean ± SD (n = 3), and bars with different characters were significantly different ($p < 0.05$), while bars with same characters were not significantly different.

study, *Lv*HSP10 mRNA transcripts could be significantly induced by the stimulation of *V. parahaemolyticus* and WSSV, and also significantly increased in the high or low pH challenge experiment, suggesting that *Lv*HSP10 play a pivotal role in the host defenses mechanism not only against the cute phase microbe infection but also the long term environmental stresses. Moreover, it has been further observed that the change tendencies of *Lv*HSP10 and *Lv*HSP60 mRNA transcripts were similar to each other in most cases, which is consist with the observation in previous reports (Cappello *et al.*, 2005; Lin *et al.*, 2009; Xu *et al.*, 2014) and further confirmed the hypothesis that HSP10 might co-chaperones with HSP60.

In conclusion, the full-length cDNAs of *Lv*HSP10 were obtained from white shrimp, and its mRNA expression profiles were detected when the shrimps were exposed to microbe stimulation and high/low pH challenge. *Lv*HSP10 was found to be involved in responses to the cute phase microbe infection, as well as the long term environmental stresses. The results obtained from this study would provide useful information of the potential roles of *Lv*HSP10 in the defense mechanism of shrimp against various biological stimulations and multiple environmental stresses.

Acknowledgement

This research was supported by the Key Research Program of the Chinese Academy of Sciences (KFZD-SW-106) and the Major Projects of Shandong Province (2015ZDZX05002). We would like to thank the expert reviewers for their constructive suggestions and enlightening comments during the revision.

Reference

Andreassen R, Lunner S, Høyheim B. Characterization of full-length sequenced cDNA inserts (FLIcs) from Atlantic salmon (*Salmo salar*). BMC Genomics 10: 502, 2009.

Canesi L, Gallo G, Gavioli M, Pruzzo C. Bacteria-hemocyte interactions and phagocytosis in marine bivalves. Microsc. Res. Techniq. 57: 469-476, 2002.

Cappello F, David S, Rappa F, Bucchieri F, Marasà L, Bartolotta TE, *et al.* The expression of HSP60 and HSP10 in large bowel carcinomas with lymph node metastase. BMC Cancer 5: 139, 2005.

Chang ZQ, Li J, Liu P, Kuo MMC, He YY, Chen P, *et al*. cDNA cloning and expression profile analysis of an ATP-binding cassette transporter in the hepatopancreas and intestine of shrimp *Fenneropenaeus chinensis*. Aquaculture 356: 250-255, 2012.

Chen W, Syldath U, Bellmann K, Burkart V, Kolb H. Human 60-kDa heat-shock protein: a danger signal to the innate immune system. J. Immunol. 162: 3212-3219, 1999.

Ding J, Chen FY, Ren SY, Qiao K, Chen B, Wang KJ. Molecular characterization and promoter analysis of crustacean heat shock protein 10 in *Scylla paramemosain*. Genome 56: 273-281, 2013.

Dobbin C, Gray C, Naylor D, James A, Flores F, Flesch I, *et al.* Heat shock protein 10 modulates innate immunity through interaction with multiple Toll-like receptor family members. Tissue Antigens 66: 433-434. 2005.

Ellis A. Innate host defense mechanisms of fish against viruses and bacteria. Dev. Comp. Immunol. 25: 827-839, 2001.

Feder ME, Hofmann GE, Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61: 243-282, 1999.

Han SY, Wang BJ, Liu M, Wang MQ, Jiang KY, Qi CC, *et al.* Effect of cyclic serious/medium hypoxia stress on the survival, growth performance and resistance against *Vibrio parahemolyticus* of white shrimp *Litopenaeus vannamei*. Inv. Surv. J. 14: 259-270, 2017a.

Han SY, Wang BJ, Wang MQ, Liu QB, Zhao W, Wang L. Effects of ammonia and nitrite accumulation on the survival and growth performance of white shrimp *Litopenaeus vannamei*. Inv. Surv. J. 14: 221-232, 2017b.

Hartman DJ, Hoogenraad NJ, Condron R, Høj P. Identification of a mammalian 10-kDa heat shock protein, a mitochondrial chaperonin 10 homologue essential for assisted folding of trimeric ornithine transcarbamoylase *in vitro*. Proc. Natl. Acad. Sci. USA 89: 3394-3398, 1992.

Hirayama M, Mitani H, Watabe S. Temperature-dependent growth rates and gene expression patterns of various medaka *Oryzias latipes* cell lines derived from different populations. J. Comp. Physiol. 176B: 311-320, 2006.

Jia HB, Halilou AI, Hu L, Cai WQ, Liu J, Huang B. Heat shock protein 10 (Hsp10) in immune-related diseases: one coin, two sides. Int. J. Biochem. Mol. Biol. 2: 47-57, 2011.

Klein SL, Strausberg RL, Wagner L, Pontius J, Clifton SW, Richardson P. Genetic and genomic tools for *Xenopus* research: The NIH *Xenopus* initiative. Dev. Dynam. 225: 384-391, 2002.

Li FH, Xiang JH. Recent advances in researches on the innate immunity of shrimp in China. Dev. Comp. Immunol. 39: 11-26, 2013a.

Li FH, Xiang JH. Signaling pathways regulating innate immune responses in shrimp. Fish Shellfish Immunol. 34: 973-980, 2013b.

Liang ZX, Liu R, Zhao DP, Wang LL, Sun MZ, Wang MQ, *et al*. Ammonia exposure induces oxidative stress, endoplasmic reticulum stress and apoptosis in hepatopancreas of pacific white shrimp (*Litopenaeus vannamei*). Fish Shellfish Immunol. 54: 523-528, 2016.

Lin KM, Lin B, Lian IY, Mestril R, Scheffler IE, Dillmann WH. Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochondrial function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation. Circulation 103: 1787-1792, 2001.

Liu HP, Söderhäll K, Jiravanichpaisal P. Antiviral immunity in crustaceans. Fish Shellfish Immunol. 27: 79-88, 2009.

Morton H, Hegh V, Clunie G. Immunosuppression detected in pregnant mice by rosette inhibition test. Nature 249: 459-460, 1974.

Sasu S, LaVerda D, Qureshi N, Golenbock DT, Beasley D. *Chlamydia pneumoniae* and chlamydial heat shock protein 60 stimulate proliferation of human vascular smooth muscle cells via Toll-like receptor 4 and p44/p42 mitogen-activated protein kinase activation. Circ. Res. 89: 244-250, 2001.

Schlesinger MJ. Heat shock proteins. J. Biol. Chem. 265: 12111-12114, 1990.

Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C_T method. Nat. Protoc. 3: 1101-1108, 2008.

Sha YJ, Wang BJ, Liu M, Jiang KY, Wang L. Interaction between *Lactobacillus pentosus* HC-2 and *Vibrio parahaemolyticus* E1 in *Litopenaeus vannamei in vivo* and *in vitro*. Aquaculture 465: 117-123, 2016.

Shi JX, Fu MJ, Zhao C, Zhou FL, Yang QB, Qiu LH. Characterization and function analysis of Hsp60 and Hsp10 under different acute stresses in black tiger shrimp, *Penaeus monodon*. Cell Stress Chaperon 21: 295-312, 2016.

Song LS, Wang LL, Zhang H, Wang MQ. The immune system and its modulation mechanism in scallop. Fish Shellfish Immunol. 46: 65-78, 2015.

Sørensen JG, Kristensen TN, Loeschcke V. The evolutionary and ecological role of heat shock proteins. Ecol. Lett. 6: 1025-1037, 2003.

Sun Y, MacRae TH. Small heat shock proteins: molecular structure and chaperone function. Cell. Mol. Life Sci. 62: 2460-2476, 2005.

Xia Q, Wang BJ, Liu M, Jiang K, Wang L. A new method to evaluate the effects of bacterial dosage, infection route and *Vibrio* strain in experimental challenges of *Litopenaeus vannamei*, based on the Cox proportional hazard model. Fish Shellfish Immunol. 46: 686-692, 2015.

Xu DX, Sun LN, Liu SL, Zhang LB, Ru XS, Zhao Y, *et al.* Molecular cloning of heat shock protein 10 (Hsp10) and 60 (Hsp60) cDNAs and their

expression analysis under thermal stress in the sea cucumber *Apostichopus japonicus*. Comp. Biochem. Physiol. 171B: 49-57. 2014.

Yi QL, Liu R, Sun R, Wang LL, Zhou Z, Wang MQ, *et al*. The protection of CpG ODNs and *Yarrowia lipolytica* harboring VP28 for shrimp *Litopenaeus vannamei* against White spot syndrome virus infection. Inv. Surv. J. 11: 119-131, 2014.

Zhang XZ, Dai LP, Wu ZH, Jian JC, Lu YS. Molecular cloning, mRNA expression, and characterization of heat shock protein 10 gene from humphead snapper *Lutjanus sanguineus*. Mar. Genom. 4: 143-150, 2011.

Zhang XJ, Song XL, Huang J. Impact of *Vibrio parahaemolyticus* and white spot syndrome virus (WSSV) co-infection on survival of penaeid shrimp *Litopenaeus vannamei*. Chin. J. Oceanol. Limnol. 34: 1278-1286, 2016.

Zhao W, Wang L, Liu M, Jiang KY, Wang MQ, Yang G, *et al.* Transcriptome, antioxidant enzyme activity and histopathology analysis of hepatopancreas from the white shrimp *Litopenaeus vannamei* fed with aflatoxin B1 (AFB1). Dev. Comp. Immunol. 74: 69-81, 2017.

Zhou J, Wang WN, He WY, Zheng Y, Wang L, Xin Y, *et al.* Expression of HSP60 and HSP70 in white shrimp, *Litopenaeus vannamei* in response to bacterial challenge. J. Invertebr. Pathol. 103: 170-178, 2010.