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Full Length Research Paper

Molecular cloning, characterization and expression analysis of heat shock protein 90 (HSP90) from the mud crab *Scylla paramamosain*

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Heat shock protein 90 (HSP90) is a highly conserved protein and plays an important role in maintaining the structure of protein, participating in the immunity and regulating the cell cycle. Using the rapid amplification of cDNA ends (RACE) techniques, the cDNA sequence of HSP90 gene (designated *Sp-HSP90*) was cloned and characterized from the mud crab *Scylla paramamosain*. The full-length cDNA of *Sp-HSP90* is 2677 bp with a complete open reading frame (ORF) of 2166 bp, which encodes a polypeptide of 721 amino acids. Five conserved blocks defining HSP90 protein family were found in the deduced amino acid sequence of *Sp-HSP90.* It contains an adenosine-5'-triphosphatase (ATPase) domain in the N-terminal and a conserved signature sequence MEEVD in the C-terminal. Quantitative real-time polymerase chain reaction (PCR) (qRT-PCR) analyses revealed the distribution of *Sp-HSP90* mRNA in different tissues and its temporal expression in haemocytes of the crabs challenged with *Vibrio parahaemolyticus*. Different levels of *Sp-HSP90* mRNA were detected in heart, hepatopancreas, muscle, haemocytes, testis and ovary except eyestalk. The expression level of *Sp-Hsp90* mRNA in hemocytes was found to be obviously up-regulated after live and heat-killed bacterial challenge and significantly higher in live bacteria group than that in heat-killed bacteria group. These results suggest that *Sp-HSP90* gene might act on immunity and resistance to infection in *S. paramamosain*.

Key words: Heat shock protein 90, quantitative real-time polymerase chain reaction (PCR), *Scylla paramamosain*, *Vibrio parahaemolyticus.*

INTRODUCTION

Heat shock proteins (HSPs), also known as stress proteins and extrinsic chaperones, are a suite of highly conserved proteins of varying molecular weight produced in all cellular organisms when they are exposed to stress (Welch, 1993; Roberts et al., 2010). It is now found that HSPs are also up-regulated when exposed to heat, anoxia ischaemia, toxins, protein degradation, hypoxia, acidosis and microbial damage (Chiang et al., 1989; Welch, 1993; Zhu et al., 2011; Quintana and Cohen, 2011). In eukaryotes, HSPs are categorized into several families and named according to their function, sequence homology and molecular weight, including HSP100, HSP90, HSP70, HSP60, HSP40 and several smaller HSP groups (Roberts et al., 2010). Many HSP genes are also expressed in cells under normal non-stress and play a fundamental role in the regulation of normal biological function. Among them HSP90 proteins are ubiquitously expressed chaperones accounting for 1-2% of all cellular proteins in most cells (Csermely et al., 1998). They play crucial roles in the folding and assembly of other cellular proteins (Gething and Sambrook, 1992; Du et al., 2008; Picard, 2002; Wiech et al., 1992), and are also involved in regulation of kinetic partitioning between folding, translocation and aggregation as well as having a wider role in relation to the innate immunity, apoptotic and inflammatory processes or other stressing conditions (Ellis, 1990; Moseley, 2000; Srivastava, 2002; Pockley,

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Table 1. Oligonucleotide primers used in the study.

Primer	Sequence (5'-3')
Sp-HSP90-3GSP1	TTGTCAAGAAGCACTCTCAGTTTATT
Sp-HSP90-3GSP2	AAGGAAGGTGACAAGAAGAAAAAGAA
Sp-HSP90-5GSP1	AGATATTCGGGAATCAACTCCTCAC
Sp-HSP90-5GSP2	GAGCCTGATGGGGTAGCCAATAAAC
Sp-HSP90-RTF	CTGAAGGAGGACCAGACGGA
Sp-HSP90-RTR	CCTGATGGGGTAGCCAATAAA
18S-RT-F	GGGGTTTGCAATTGTCTCCC
18S-RT-R	GGTGTGTACAAAGGGCAGGG

2003; Miyata and Yahara, 1992, 1995; Schumacher et al., 1994; Nathan et al., 1997; Freeman and Morimoto, 1996; Yeyati et al., 2007; Vabulas et al., 2010). Besides these mentioned functions, HSP90 is also involved in regulating ovarian developmental process by binding to estrogen receptor to increase vitellogenin secretion (Zhao et al., 2011).

Aquatic animals are usually in complex and sophisticated environment which varies greatly depending on the season, weather condition, or human activity. Variations in the aquatic environment will have a great effect on many biological processes of the organism including development, growth, and reproduction (Li et al., 2009). Crustacean lack an acquired adaptive immune system and host defense is believed to depend entirely on innate, non-adaptive mechanisms to resist invasion by pathogens (Gross et al., 2001). Discovery of immune-related factors are helpful for understanding the molecular response of crustaceans to pathogens. In recent years some genes related to immunity have been reported, such as anti-lipopolysaccharide factor (ALF) (Liu et al., 2005; Yedery and Reddy, 2009), penaeidin-like antimicrobial peptide (Chiou et al., 2005), kazal-type serine proteinase inhibitor (Jarasrassamee et al., 2005), and glutathione transferase (Zhao et al., 2010).

Crabs of the genus *Scylla* are strongly associated with mangrove areas throughout the Pacific and Indian oceans and form the basis of substantial fishery and aquaculture operations (Keenan, 1999). Four non-hybridizing species, such as *S. serrata*, *S. paramamosain*, *S. olivacea* and *S. tranquebarica*, have been identified based on morphometric and genetic analysis (Keenan et al., 1998). Among four mud crab species, *S. paramamosain* is abundantly found in Taiwan, Philippines, Indonesia and the Bay of Bengal (Keenan et al., 1998), and is also the most common species in the southeast coast of China (Ma et al., 2006). The crab is an important marine species for aquaculture in China because of its high nutritional value to humans. In recent years, the crab is prone to been infected by microbes with the enlargement of breeding scale. In this case, more studies should be addressed on the obtainment and understanding of immunity-related proteins in the mud crab. For example, a serine proteinase (Liu et al., 2010), crustin (Imjongjirak et al., 2009), an ALF (Imjongjirak et al., 2007) have been isolated and cloned from the mud crab *S. paramamosain*. As an important chaperone, however, HSP90 have not been reported in *S. paramamosain*. In this study, we describe the molecular cloning and characterization of the full-length cDNA of the HSP90 gene from *S. paramamosain* by the rapid amplification of cDNA ends (RACE) technique. The expression profile in different tissues of *S. paramamosain* was investigated. Moreover the change of HSP90 mRNA expression was also examined in haemocytes after live and heat-killed pathogenic bacteria challenge.

MATERIALS AND METHODS

Materials, reagents and isolation of RNA

Healthy *S. paramamosain* crabs averaging 300 g in weight were collected from Hainan Island, China. Different tissues such as hepatopancreas, testis, muscles, ovary, heart, and eyestalk were excised and preserved in liquid nitrogen until RNA extraction. Each tissue from three individuals was isolated for detection. Total RNA was extracted from different tissues of *S. paramamosain* using TRIzol reagent (TaKaRa, Shiga, Japan) according to the manufacturer's protocol. The isolated total RNA was treated with RNase-free DNase I (Sigma) to eliminate possible genomic DNA contamination. All the RNA was stored at -80℃ until further experiment.

Cloning the full-length cDNA of HSP90

The fragment of HSP90 was identified in the cDNA library constructed with the hepatopancreas tissue of *S. paramamosain* in our laboratory. The full-length HSP90 cDNA was obtained by the reverse-transcription polymerase chain reaction (RT-PCR) and RACE methods. The RNA from hepatopancreas was used for the construction of the cDNA library using the SMART PCR cDNA Synthesis Kit (Clontech, USA) according to the manufacturer's instructions. All primers used in this study are shown in Table 1. AP

(as the RT primer), AUAP (as the universal amplification primer) and the two gene-specific primers of *Sp-HSP90*-3GSP1 (as the 3'-RACE first primer) and *Sp-HSP90*-3GSP2 (as the nested primer) were used for 3'-RACE. 5'-RACE including RT, dC tailing and PCR amplifications was carried out following the protocol provided by the manufacturer. The primers, UPM, NUP and primer *Sp-HSP90*-5GSP1 and *Sp-HSP90*-5GSP2 were, respectively used for 5'-RACE. The PCR fragments were analyzed by electrophoresis on 1.5% agarose gels to determine length differences. Amplified cDNA fragments were cloned into the pMD18-T vector (TaKaRa) following the instructions provided. Recombinant bacteria were identified by blue/white screening and confirmed by PCR. Plasmids containing the inserted HSP90 fragment were used as a template for DNA sequencing.

Sequence analysis of HSP90

Sequences were analyzed based on nucleotide and protein databases using the BLASTX and BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/). The protein prediction was performed using the open reading frame (ORF) Finder tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Amino acid sequences of HSP90 from various species in crustaceans and insects were retrieved from the NCBI GenBank database and analyzed using the ClustalW Multiple Alignment program (http://www.ebi.ac.uk/clustalw/). Multiple sequence alignments of HSP90 were carried out online (http://www.ch.embnet.org/software/BOX_form.html). Motif scan program (http://hits.isb-sib.ch/cgi-bin/motif_scan) and PROSITE program (http://kr.expasy.org/ prosite/) were used to predict the functional sites or domains in the amino acid sequence. Phylogenetic relationships were deduced and dendrograms were produced by using MEGA 4.0 program (http://www.megasoftware.net/). An unrooted phylogenetic tree was determined using the neighbour-joining method with the Kirumma two parameters. The relative importance of branching order was evaluated by the bootstrapping method (1000 replications).

Expression of HSP90 gene in different tissues

The expression of HSP90 mRNA in haemocytes, hepatopancreas, testis, muscles, ovary, heart, and eyestalk was evaluated by quantitative real-time RT-PCR (qRT-PCR) analysis which was performed using SYBR Premix Ex Taq (TaKaRa, Dalian, China) on an ABI StepOne Real-Time PCR System (Applied Biosystems, USA). The first-strand cDNA was synthesized using MMLV reverse transcriptase with 5 µg of total RNA. The cDNA was maintained at -20°C for qRT-PCR. The gene-specific primers of *Sp-Hsp90*-RTF and *Sp-Hsp90*-RTR were used to amplify the HSP90 transcript, and the primers, 18S-RT-F and 18S-RT-R, were used to amplify the 18S rRNA fragment as an internal control because of its steady expression (Zhang et al., 2011). The mentioned primers are shown in Table 1. Amplifications were performed in a 96-well plate with a 20 µL reaction volume containing 10 µL of 2× SYBR Premix Taq™ (TaKaRa) , 0.8 µL of PCR Forward Primer (10 µM), 0.8 µL of PCR Reverse Primer (10 µM), 0.4 µL of ROX Reference Dye II (50×), 2.0 µL of cDNA temple and 6.0 µL of DEPC-water. The thermal profile for SYBR Green qRT-PCR was 30 s at 95°C, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s. DEPC-water was used to replace the template in the negative control. The standard curve and the gene expression levels were analyzed automatically by the system. A melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm that only one product was amplified and detected. From the standard curve of HSP90, the slope was -4.00, R^2 was 0.999, and amplification efficiency was 77.77%. The standard curve of 18S rRNA indicated that the slope was -4.21, R^2 was 0.999, and amplification efficiency was 72.73%.

HSP90 expression in haemocytes after *Vibrio parahaemolyticus* challenge

Healthy crabs averaging 300 g in weight were collected from Qinglan, Hainan Island, China, acclimated for three days before experiments. In this experiment, the preliminary trial showed that the expressions of *Sp-Hsp90* were basically steady and the differences were insignificant as time went on. Totally, 90 crabs were employed in the test. The crabs were randomly divided into three groups (two challenge groups and a control group) and each treatment was applied in triplicate. The heat-killed bacteria (2×10^6 CFU/mL) were obtained by boiling water under 100°C for 10 min. Each crab in the two challenge groups, respectively received injection of 100 µL live and heat-killed *V. parahaemolyticus* suspended in saline (2×10^6 CFU/mL), while the crabs in the control group received the same volume of normal saline only. After treatment, three individuals of each replicate were randomly sampled at 0, 1, 3, 6, 12, and 24 h post-injection. Hemolymph sampling and collection of haemocytes was performed from three samples and performed according to a method previously reported (Zhao et al., 2009). Gene-specific primers of *Sp-Hsp90*-RTF and *Sp-Hsp90*-RTR and the internal primers of 18S-RT-F and 18S-RT-R described above were used for the reaction according to the manufacturer's instructions. In a 96-well plate, each sample was run in triplicate along with the internal control gene. DEPC-water for the replacement of template was used as a negative control. The process was the same as the expression in different tissues. From the standard curve of HSP90, the slope was -3.78, R^2 was 0.994, and amplification efficiency was 84.01%. The standard curve of 18S rRNA indicated that the slope was -4.21, R^2 was 0.999, and amplification efficiency was 72.73%.

Statistical analysis

The results of qRT-PCR at different tissues and each post-injection stage were calculated to derive the mean and standard deviation (SD). All data obtained from the qRT-PCR analysis were analyzed with STATISTICA 6.0 software depending on one-way analysis of variance (ANOVA) method. When the treatment difference was significant, a post hoc test was used for multiple comparisons (Duncan test). Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

RESULTS

cDNA cloning and sequence analysis of *Sp-HSP90*

The full-length cDNA fragment of *Sp-HSP90* was obtained and deposited in GenBank (Accession No. JF265066). The *Sp-HSP90* cDNA was 2677 bp in length containing an ORF of 2166 bp, 83-bp 5' untranslated region (5'-UTR) and 428-bp 3'-UTR, with a poly (A) signal. Based on the deduced polypeptide sequence, the ORF encodes a putative protein of 721 amino acids with a predicted molecular weight of 82.71 kDa and a theoretical isoelectric point of 4.62. The putative HSP90 proteins family signature (YSNKSIFLRE) (31-40) located in the N-terminal of *Sp-HSP90* amino acid sequence. Five conserved blocks defining HSP90 protein family

were found in the deduced amino acid sequence of *Sp-HSP90*: NKEIFLRELISNSSDALDKIR (33–53), LGTIAKSGT (100–108), and IGQFGVGFYSAYLVAD (124–139) were located at N-terminal domain; IELYVRRVFI (351–360) and GVADSEDLPLNISREM (377–391) were located in the middle domain. A typical histidine kinase-like adenosine-5'-triphosphatase (ATPase) domain was located at 33–187 of *Sp-HSP90*. The conserved "GxxGxG" motif essential for adenosine-5'-triphosphate (ATP) binding (Prodromou et al., 1997) was also found in the amino acids of *Sp-HSP90*. The C-terminal conserved MEEVD motif was found in the *Sp-HSP90* amino acid sequence. The full-length nucleotide sequence and the deduced amino acid sequence are shown in Figure 1.

Homology analysis of *Sp-HSP90*

BLAST analysis indicated that ORF of *Sp-HSP90* shared high degree of sequence homology with HSP90s from other species, for example, 95% of *Portunus trituberculatus* HSP90-1 (2037/2167), 89% of *Chiromantes haematocheir* HSP90 (1923/2173), 89% of *Eriocheir sinensis* HSP90 (1921/2174), and 85% of *Penaeus monodon* HSP90 (1828/2162). Similarly, the deduced amino acid sequence of the *Sp-Hsp90* shows very high homology (83-98%) with HSP90 of the other crustacean: *P. trituberculatus* (98%), *E. sinensis* (93%), *C. haematocheir* (93%), and *Exopalaemon carinicauda* (83%).

The alignment of HSP90 showed that some amino acid residues were highly conserved in different species. Two major groups clustered in the phylogeny tree: crustacean and insect groups. *Sp-HSP90* was located in the crab subgroup separated from shrimp subgroup and was observed to be closest to HSP90s of *P. trituberculatus* (Figure 2). The relationship is in agreement with the concept of traditional taxonomy.

Expression of *Sp-HSP90* gene in tissues

To better understand the biological function of *Sp-Hsp90*, we examined tissue distribution of *Sp-Hsp90* mRNA by qRT-PCR with 18S RNA as an internal control. The mRNA transcripts of *Sp-HSP90* were detected in examined tissues except eyestalk at different expression levels (Figure 3). The highest expression was observed in heart, the middle level in hepatopancreas, muscle and haemocytes.

Sp-HSP90 expression in haemocytes after *Vibrio parahaemolyticus* challenge

To study the response to resisting the microbial invasion, the expression of *Sp-Hsp90* mRNA in haemocytes after bacterial challenge was measured by qRT-PCR as described above. In this study, live and heat-killed *V. parahaemolyticus* were injected to the challenge crab to understand the response ability to bacterial infection. The expression level of *Sp-Hsp90* mRNA was found to be up-regulated after live and heat-killed bacterial challenge and significantly higher in live bacteria group than that in heat-killed bacteria group. In the early hours after injection, the expression of *Sp-HSP90* mRNA in two challenge groups concurrently increased and obvious enhancement appeared at 3 h post-infection. As time progressed, the expression of *Sp-HSP90* mRNA gradually dropped and reached the lowest level at 12 h post-infection in heat-killed-bacteria group. However, the level of *Sp-HSP90* mRNA maintained to rise in live bacteria group, and peaked at 6 h, then decreased at 12 h and 24 h post-infection.

DISCUSSION

In addition to serving as molecular chaperones, HSPs have been implicated in autoimmune diseases, antigen presentation and tumor immunity (Tsan and Gao, 2004). HSP90, co-chaperoned with other proteins such as the HSP70 family proteins (Pratt and Toft, 2003), is a highly conserved and abundant protein involved in protein folding, cytoprotection, proteosomic degradation and a number of cellular regulatory pathways (Minami et al., 2000; Hartl and Hayer-Hart, 2002; Zhang and Burrows, 2004; Brown et al., 2007). In this study, the full-length cDNA sequence of HSP90 (*Sp-HSP90*) was cloned from the mud crab *S. paramamosain*. Five conserved amino acid sequences, a characteristic motif of cytosolic HSP members, were present in the deduced amino acid sequence of *Sp-HSP90*. But two variation sites were found in the two motifs of IE^{352}LYVRRVFI and GVA^{379}DSEDLPLNISRE respectively, in which Glu $(E)^{352}$ and Ala $(A)^{379}$ replace the Lys (K) and Val (V) exiting in most organisms. The presence of sequence MEEVD on the C-terminus is also a character shared by all of the cytosolic HSP90 proteins (Gao et al., 2008). These aforementioned characteristic motifs in the deduced *Sp-HSP90* amino acid sequence suggested that *Sp-HSP90* protein have the same physiological function as other animals. BLAST analysis revealed that the deduced amino acid sequence of *Sp-HSP90* shared high similarity with other known HSP90s (more than 79% similarity in all the matches), especially with those from crustacean such as *P. trituberculatus* (GenBank No. ACQ90225), *E. sinensis* (No. ACJ01642) and *C. haematocheir* (No. AAS19788). According to characteristic of its protein and BLAST analysis, *Sp-HSP90* was concluded to be a cytosolic member of HSP90 family. The phylogenetic tree revealed that *Sp-HSP90* belonged to the crustacean group and was located in the crab branch, which was in

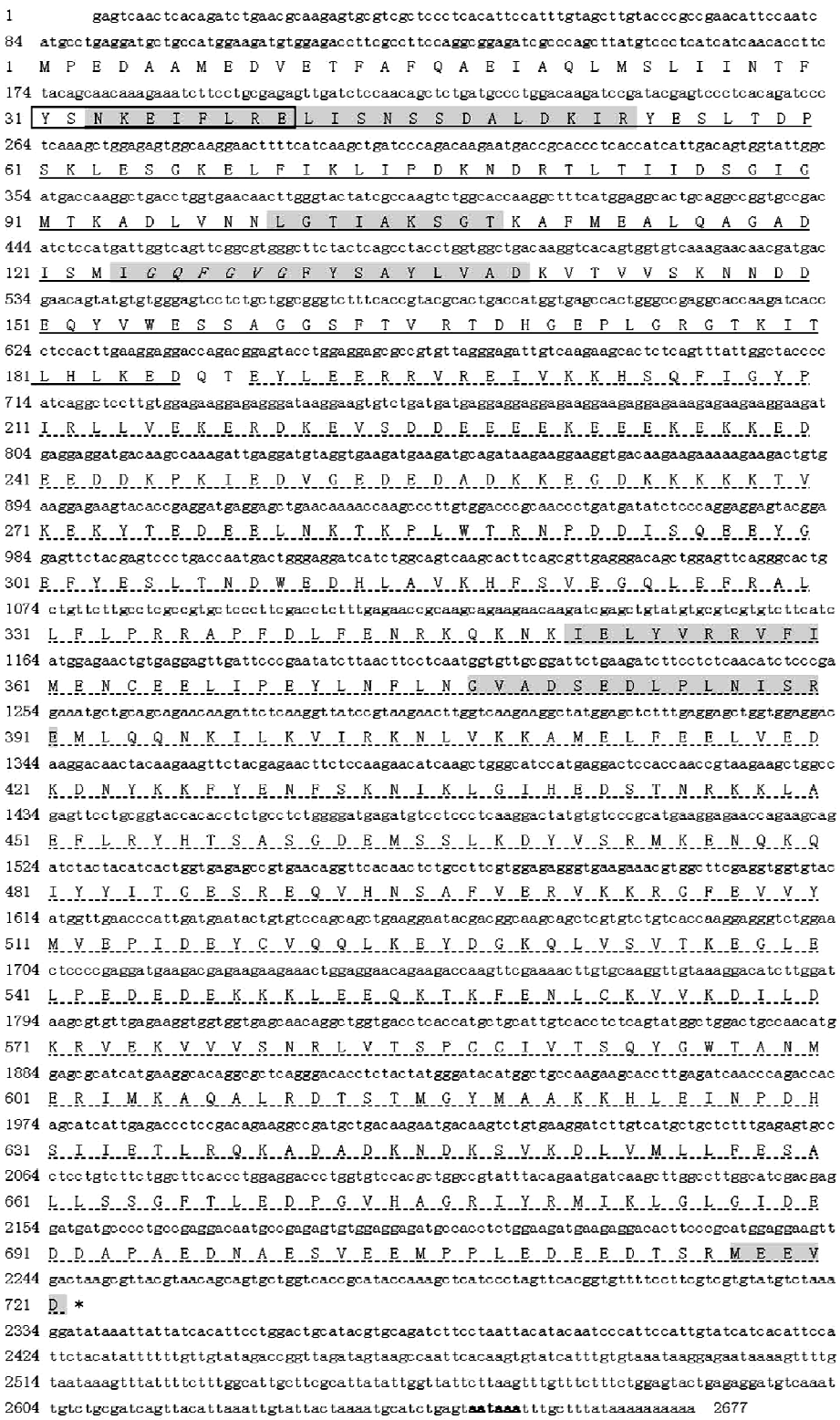

Figure 1. Nucleotide and deduced amino acid sequences of *Sp-HSP90* from *Scylla paramamosain*. Five amino acid blocks defining HSP90 protein family and consensus sequence MEEVD are highlighted as shaded regions. Conserved "GxxGxG" motif is shown in italic. The putative HSP90 proteins family signature is shown in the open box. The ATPase domain of *Sp-HSP90* is underlined. The HSP90 protein domain is underlined by a dotted line. The termination signals (AATAAA) in the 3'-untranslated region (UTR) are in bold. The stop codon is indicated by an asterisk.

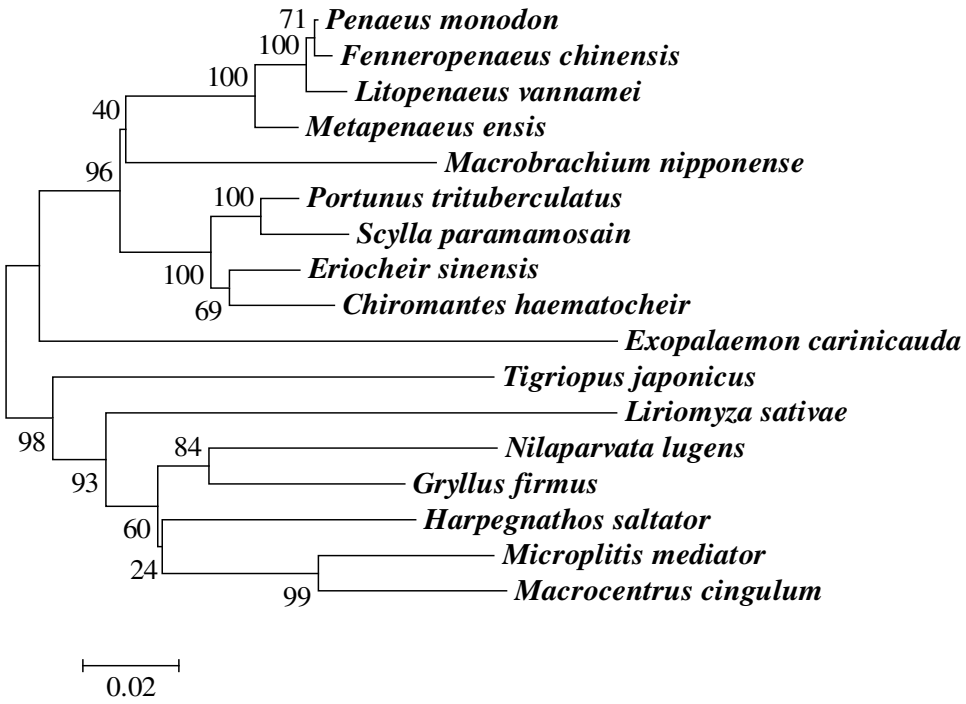

Figure 2. Phylogenetic tree of HSP90 amino acid sequences constructed with the neighbour-joining method. The species names and the GenBank accession numbers are as follows: *Penaeus monodon*, ABM54577; *Fenneropenaeus chinensis*, ABM92446; *Litopenaeus vannamei*, ADU03767; *Metapenaeus ensis*, ABR66910; *Macrobrachium nipponense*, ADK66920; *Portunus trituberculatus*, ACQ90225; *Eriocheir sinensis*, ACJ01642; *Chiromantes haematocheir*, AAS19788; *Exopalaemon carinicauda*, ADM88040; *Tigriopus japonicus*, ACA03524; *Liriomyza sativae*, AAW49253; *Nilaparvata lugens*, ADE34169; *Gryllus firmus*, ADK64952; *Harpegnathos saltator*, EFN88374; *Microplitis mediator*, ABV55506; *Macrocentrus cingulum*, ACE77780.

accordance with traditional taxonomy.

HSP90s play a fundamental role in the regulation of normal protein synthesis within the cell (Roberts et al., 2010). The qRT-PCR analysis showed that *Sp-HSP90* mRNA was mostly expressed in the tested tissues of *S. paramamosain* except eyestalk. This universal distribution suggested that *Sp-HSP90* plays an essential role as a molecular chaperone in the crab.

The hemocyte was one of the important immune sites involved in recognition, phagocytosis, melanization and cytotoxicity to control to combat bacterial infections (Gross et al., 2001). The relative expression level of HSP90 transcript in haemocytes was obviously up-regulated after bacterial challenge in *Argopecten irradians* (Gao et al., 2008). HSP90s also played different roles in physiological and stressful conditions. For example, two HSP90s exhibited diverse expression levels under different stressful conditions in crab *P. trituberculatus* (Zhang et al., 2009). Moreover, HSP90 was up-regulated under optimal concentration of zinc in *Spodoptera litura* (Shu et al., 2011) and under optimal concentration of selenium in Pacific abalone *Haliotis discus hannai* (Zhang et al., 2011), induced by heat shock and hypoxia stresses in *Fenneropenaeus chinensis* (Li et al., 2009), and also markedly enhanced after heat treatment in black tiger shrimp *P. monodon* (Jiang et al., 2009). We studied the gene expression profile of *Sp-HSP90* in haemocytes in response to live and heat-killed *V. parahaemolyticus.* In heat-killed group, a significant enhancement of *Sp-HSP90* transcription appeared at 3 h post-infection and dropped at 6 h and kept a low level from 12-24 h. However, the expression level of *Sp-HSP90* changed more remarkably in live group than heat-killed group. The expression level of *Sp-HSP90* rose gradually at 1 h and reached the highest at 6 h, then decreased from 12 h. The expression level of *Sp-HSP90* in live group was 1.9 fold than heat-killed group at 3 h, increased to 11 fold at 6 h and 33 fold at 12 h. The data suggest that the live bacteria were probably stronger inducer for *Sp-HSP90* than heat-killed bacteria. In mouse, when RAW264.7 cells were exposed to live bacteria, the bacteria were taken up and apoptosis occurred; when bacteria were heat inactivated, uptake was significantly reduced and almost no apoptosis was observed (Häcker et al., 2002). The result that the expression level of *Sp-HSP90* in heat-killed group shown is obviously lower than live group and also validated the fact that live bacteria possess stronger pathogenicity than

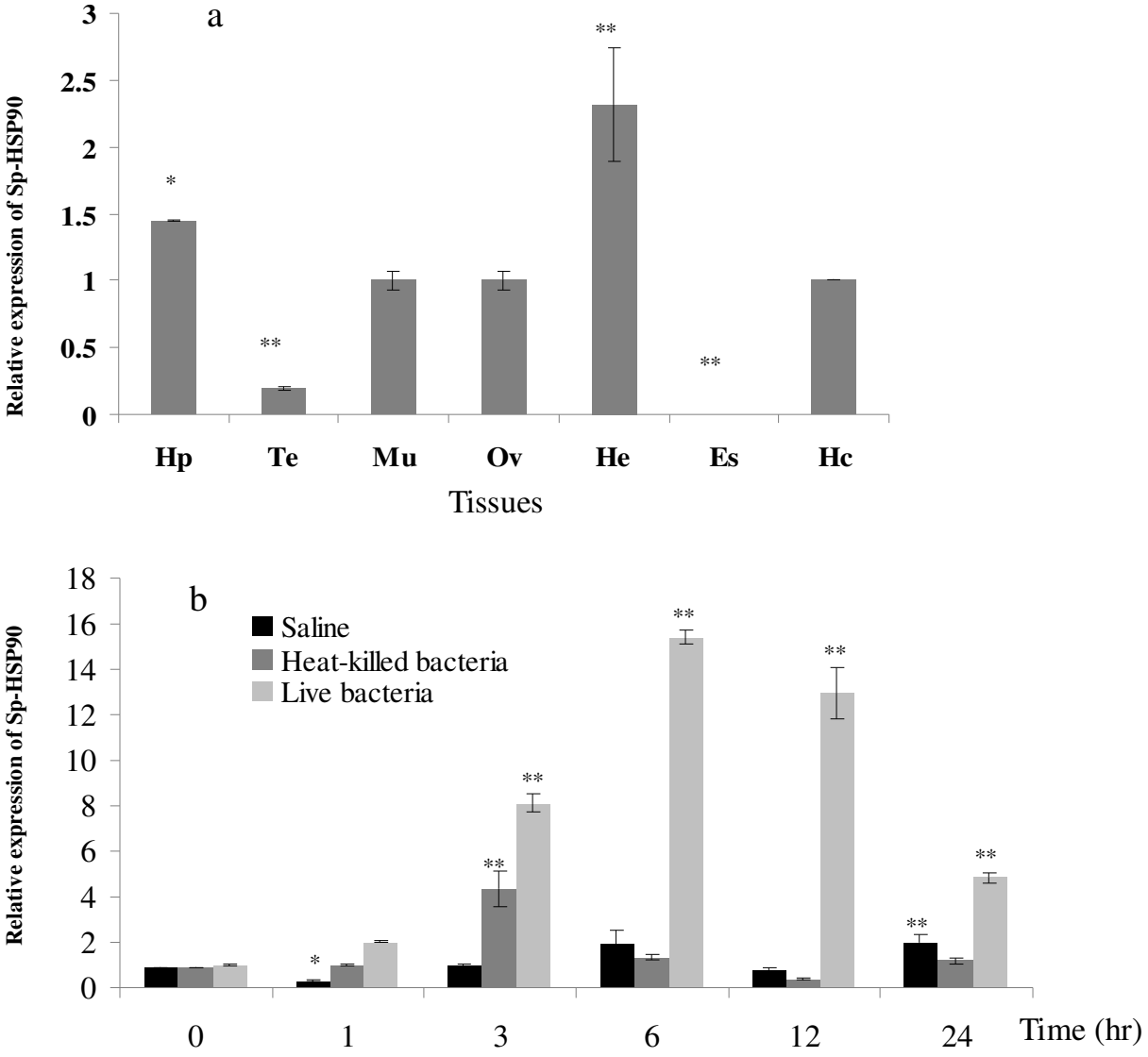

Figure 3. Expression and induction of *Sp-HSP90* mRNA. The amount of *Sp-HSP90* mRNA was normalized to the 18S rRNA transcript level. Data are shown as means ± SD of three repeated experiments. The "Y" axis represents the relative ratio of expression levels of *Sp-HSP90*/18SrRNA mRNA. **a**, Tissue expression of the *Sp-HSP90* mRNA relative to 18S RNA by qRT-PCR. The ratio of expression levels of *Sp-HSP90*/*18S* in muscles was initiated as "1.0", so the relative ratios of expression levels of *Sp-HSP90*/*18S* in other tissues were determined by comparing with which in muscles. Significant differences of hepatopancreas (Hp), testis (Te), muscle (Mu), Ovary (Ov), heart (He), eyestalk (Es) compared with haemocytes (Hc) are indicated by an asterisk ($P < 0.05$) and two asterisks ($P < 0.01$), respectively; **b**, temporal expression of *Sp-HSP90* mRNA relative to 18S RNA by qRT-PCR in haemocytes after live and heat-killed *V. parahaemolyticus* challenge. A significant difference from samples taken post-infection compared to that at 0 h are indicated with an asterisk ($P < 0.05$) and two asterisks ($P < 0.01$), respectively.

heat-killed bacteria. So, more *Sp-HSP90* transcripts were produced in live group than heat-killed group in order to modulate the cellular immune responses in the host cells. Thus, *Sp-HSP90* is likely to function as an essential chaperone involved in immune response by hydrolyzing ATP (Pearl and Prodromou, 2006) to protect organism from damage of bacteria. However, the function mechanism of *Sp-HSP90* on immunity needs further investigation.

In this study, the regular change after bacterium challenge showed that *Sp-HSP90* gene can be induced and potentially plays a critical role during acute-phase bacterial pathogenesis. This is the first time that response of HSP90 has been observed in the mud crab, indicating that *Sp-HSP90* is potentially involved in the crab immune responses to bacterial infection. These data would be helpful to understand the significance of HSP90 to immune defense in the crab.

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Abbreviations:

HSP90, Heat shock protein 90; **RACE**, rapid amplification of cDNA ends; **PCR**, polymerase chain reaction; **qRT-PCR**, quantitative real-time PCR; **ALF**, anti-lipopolysaccharide factor; **RT-PCR**, reverse-transcription polymerase chain reaction; **ORF**, open reading frame; **UTR**, untranslated region; ***Sp-HSP90***, the cDNA sequence of HSP90 gene.

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