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RESEARCH REPORT

Molecular cloning of the heat shock protein 90 gene in scallop *Mizuhopecten yessoensis* and the effects of temperature stress on gene expression

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Abstract

Mizuhopecten yessoensis, one of the most highly favored scallops on the international market, has suffered from massive summer mortalities. Water temperature is an important environmental stressor which has significant effects on the physiological and biochemical response of scallops. Heat shock protein 90 (HSP90) plays a key role in defense against various environmental stresses that could damage the cellular and molecular structure of cells. In this study, molecular characterization and expression of HSP90 in *M. yessoensis* (designated MyHSP90) were analyzed as an indicator to understand the mechanisms of heat shock response in *M. yessoensis* under temperature stress. The full-length sequence of MyHSP90 cDNA (GenBank accession no. MF196912) is composed of 2639 base pair encoding a 726-amino acid polypeptide with a predicted molecular mass of 83.26 kDa. Tissue expression analysis of MyHSP90 genes revealed ubiquitous expression in each tissue examined, and showed a temperature-dependent response, and the expression level was up-regulated significantly in all the six tissues tested ($p < 0.05$) except gonad. The results will be useful in furthering the understanding of the massive summer mortalities in *M. yessoensis* under temperature stress. Additionally, MyHSP90 could be used as a potential biomarker in practice to monitor environmental changes in future studies.

Key Words: *Mizuhopecten yessoensis*; scallop; heat shock protein 90; cloning and expression; temperature stress

Introduction

Scallops are one of the most valuable marine resources in China. The scallop *Mizuhopecten yessoensis* (or *Patinopecten yessoensis*) is a cold-water species and found around the northwestern Pacific coast of China, Korea, Japan and Russia (Hou *et al*., 2011). It is one of the most highly favored scallops on the international market, and the global capture production of *M. yessoensis* is 366 kilotons with a total value over US $1.5 billion in 2014 (FAO, 2014) while the aquaculture production for this species is195 kt. *M. yessoensis* was introduced from Japan to China by Liaoning Ocean and Fisheries Research Institute in 1982 (Wang, 1984), and aquaculture of this species has been growing rapidly since its introduction because it has a larger size and a higher market price than other native scallops. It is cultured only in the northern province, Liaoning, and the north side of the Shandong peninsula (Shumway and Parsons, 2015), and it has become a major aquaculture industry in these areas. However, this species has difficulties with the high summer temperatures found in the Bohai Sea of China, which often reaches 25 °C – 28 °C, and it has been suffering massive summer mortalities since 1998, which causes a 37 % decline in scallop production (MAC, 1999).

Water temperature is an important environmental stressor which could have significant effects on the survival, growth and reproduction of marine organisms (Bruc et *et al*., 2012; Bian *et al*., 2014), as well as effect relevant gene expression in molluscs (Farcy *et al*., 2007; Fearman and Moltschaniwskyj, 2010; Jiang *et al*., 2016). High temperatures, especially, affects the mortalities of cold-water scallop species *M. yessoensis* as

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described previously. Therefore, it is important to investigate the mechanisms of the heat shock response in *M*. *yessoensis* under temperature stress, and the current study focuses on heat shock proteins.

Heat shock proteins(HSPs) are a family of proteins that were discovered in *Drosophila* after exposure to high temperatures caused the genes to appear as chromosome puffs, hence the name heat shock in 1962 (Ritossa, 1962). Subsequently, many HSPs and their genes have been characterized (Fehrenbach and Niess, 1999; Lindquist 2003; Sørensen *et al*., 2003). HSPs are highly conserved proteins which are found in all organisms from bacteria to plants and animals (Kiang and Tsokos, 1998; Sørensen *et al*., 2003). As molecular chaperones, HSPs are evolutionary important and play a role in the protection of various environmental stresses which could damage cellular and molecular structures in cells (Sørensen *et al*., 2003; Peng *et al*., 2016). HSPs are a superfamily that are often classified according to their molecular weight, such as the HSP70 family (molecular weight approximately 66 - 78 kDa), the HSP90 family (molecular weight 83 - 110 kDa), the HSP33 family, the HSP60 family, and the small molecular weight family (molecular weight 12 - 43 kDa) (Sørensen *et al*., 2003; Li *et al*., 2004).

Among the HSP family, HSP70has been shown to be one of the most sensitive to thermal stress (Uhlinger *et al*., 1998; Hamdoun *et al*., 2003). In invertebrates, HSP70 has been cloned from different species of molluscs (Boutet *et al*., 2003; Franzellitti and Fabbri, 2005; Farcy *et al*., 2007; Gunter and Degnan, 2007; Zhang and Zhang, 2012; Liu *et al*., 2015), and in scallops, such as *Argopecten irradians*, *Placopecten magellanicus* and *Patinopecten yessoensis*, heat stress induces the over-expression of *hsp70*, (Brun*et al*., 2008, Jiang *et al*., 2016). HSP90 is another highly conserved member (Csermely *et al*., 1998), weighing roughly 90 kDa. HSP90 is highly conserved and expressed in a variety of different organisms from bacteria to all branches of eukarya (Chen *et al*., 2006). Unlike with HSP70, only a few studies have demonstrated that thermal stressors stimulate HSP90 expression in molluscs (Choi *et al*., 2008; Liu *et al*., 2015), including two species of scallop *Azumapecten* (*Chlamys*) *farreri* and *A. irradians* (Gao *et al*., 2007, 2008). However, the HSP90 response has not been investigated in *M*. *yessoensis* under high-temperature stress.

To investigate the mechanisms of heat shock response in *M*. *yessoensis* under temperature stress, it is important to realize the existence of HSP90 in this species first. Therefore we cloned and characterized the full-length sequence of HSP90cDNA in *M*. *yessoensis* (designated MyHSP90), and studied the expression pattern of MyHSP90 in each tissue, as well as defining the molecular regulation of Myhsp90 mRNA expression under temperature stress.

Materials and Methods

Animals and rearing conditions

The scallops *M. yessoensis* were bred at Dalian Ocean University, China. They were grown around Zhangzi Island for two years. Healthy *M. yessoensis*, averaging 8.0 ± 2.0cm in shell length, were transported back to the laboratory of Dalian Ocean University and acclimated in culture tanks containing aerated sand-filtered seawater (salinity: 30 ‰, pH 8.2) at 15 ± 1 °C for 7 days. Before the test, the scallops were fed a powder of spirulina and *Sargassum thunbergii*, and seawater was changed completely twice a day.

Temperature stress test and sample collection

The scallops were randomly divided into five groups of 10 individuals, and each group was treated at 15 (as control), 20 °C, 22 °C, 24 °C and 26 °C for 7 days, respectively, while each temperature was replicated three times (n = 3). Three scallops from each replicated group were sampled at each test temperature. Then the tissues (including gonad, adductor muscle, mantle, blood/hemocytes, gill, and kidney) were excised and stored at −80°C until use. Scallop blood/hemocytes (hemolymph) was collected from the adductor muscle using a 20 G-needle and syringe, and then centrifuged at 800*g*, 4 °C for 10 min to obtain the hemocytes. The blood/hemocytes pellets were immediately stored at −80 °C until use.

Table 1 Nucleotide sequences of primers used in this study

Primers	Sequence (5'→3')	Usage
3', 5' RACE		
MyHSP90-F1	ACATGGCTGCCAAGAAACATCTAGAGA	3' RACE primer
MyHSP90-R1	GAGCCACCAGCAGATGATTCCCAGAT	5' RACE primer
UMP-1	TAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	3'/5' RACE primer
UMP-2	CTAATACGACTCACTATAGGGC	3'/5' RACE nested primer
M13F	TGTAAAACGACGGCCAGT	Colony PCR
M13R	CAGGAAACAGCTATGACC	Colony PCR
qPCR		
MyHSP90-F2	TCTGGGAATCATCTGCTGG	qPCR primer
MyHSP90-R2	GATTGGGTAACCGATGAACTG	qPCR primer
Cytb-F	ATTCGGATTCAGGAAGTGGC	Reference primer
Cytb-R	ATTTGGTCCAGCATGTC	Reference primer

RACE: rapid amplification of cDNA ends; qPCR: quantitative real-time polymerase chain reaction; Cytb: Cytochrome b.

Table 2 The homology analysis between *Mizuhopecten yessoensis* and other representative species for HSP90

Species	GenBank accession no.	Identity (%)
Molluscs (14)		
Mizuhopecten yessoensis	MF196912	-
Azumapecten farreri	AAR11781.1	97.66
Argopecten irradians	ABS50431.1	94.75
Crassostrea gigas	EKC25687.1	85.87
Crassostrea hongkongensis	ADL59936.1	85.46
Scapharca broughtonii	ALZ42089.1	83.75
Ruditapes philippinarum	AHY27548.1	83.33
Cellana toreuma	AGH32328.1	80.47
Hyriopsis cumingii	ALM87690.1	80.17
Laternula elliptica	ACF35426.1	80.72
Mytilus coruscus	ALL27016.1	83.37
Mytilus galloprovincialis	CAJ85741.1	82.96
Paphia undulata	AFZ93093.1	82.37
Corbicula fluminea	AMM04544.1	81.96
Insects (3)		
Plutella xylostella	AHA36864.1	77.27
Mythimna separata	ABY55234.1	65.27
Bemisia tabaci	ADO14474.1	75.56
Nematodes (2)		
Bursaphelenchus mucronatus	ADK26462.1	76.41
Heterodera glycines	AAO14563.2	73.37
Crustaceans (3)		
Penaeus monodon	ABM54577.1	78.06
Eriocheir sinensis	AHA61463.1	77.99
Macrobrachium nipponense	ADK66920.1	76.31
Teleosteans (4)		
Danio rerio	NP_571385.2	75.17
Salmo salar	AAD30275.1	75.48
Oncorhynchus mykiss	CDQ59193.1	76.11
Megalobrama amblycephala	AGI97008.1	75.76
Reptiles (2)		
Pelodiscussinensis	XP_006120052.1	76.72
Alligator mississippiensis	NP_001274200.1	76.58
Aves (1)		
Gallus gallus	NP_996842.1	77.24
Mammals (3)		
Homo sapiens	NP_031381.2	77.21
Mus musculus	NP_032328.2	77.21
Bos taurus	NP_001073105.1	77.49

Total RNA extraction and reverse transcription

Total RNA was extracted from six tissues of *M. yessoensis* with a RNAprep Pure Kit (For Tissue) (Tiangen Biotech, Beijing, China) according to the manufacturer's protocol. RNA quantity and purity were performed at 260/280 nm and 260/230 nm absorbance ratios using a NV3000Micro-spectrophotometer (Vastech Inc., Wilmington, DE, USA), while the integrity was verified with electrophoresis on 1 % agarose gels. Sample concentrations were determined to ensure sufficient RNA for complementary DNA (cDNA) synthesis. The cDNA was synthesized from total RNA from each sample using SMARTer RACE 5′/3′ Kit (Clontech Laboratories, Inc., Palo Alto, CA, USA). The cDNA was diluted to 1:50 and stored at −20°C for subsequent cloning and expression analysis.

Cloning the full-length cDNA of MyHSP90 using rapid-amplification of cDNA ends (RACE) PCR

Partial sequences of MyHSP90 cDNA were obtained from the splicing transcriptome of the scallop *M. yessoensis* established by our laboratory (Ding *et al*., 2015), and the gene-specific primers were designed according to the partial sequences. The nucleotide sequences of all the primers used in this study are shown in Table 1, and the primers were obtained from Sangon Biotech (Shanghai, China). The MyHSP90 cDNA from the gill of *M. yessoensis* was used as a template for cloning. The

PCR was performed using a Gradient Mastercycler (Eppendorf AG, Hamburg, Germany) in a total volume of 50 μL, comprising 25 μL of 2 ×SeqAmp buffer, 2.5 μL of cDNA template, 5 μL of 10×UPM, 1μL of MyHSP90-F1 or MyHSP90-R1 (10 μM), 1μL of SeqAmp DNA polymerase and 15.5 μL distilled water. The PCR program was as follow: 30 cycles of 94°C for 30s, 65 °C for 30 s, and 72 °C for 1 min; The nested PCR program was as follow: 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. The PCR products were analyzed with electrophoresis on 1.0 % agarose gel, and the expected bands were purified using EasyPure Quick Gel Extraction Kit (TransGen Biotech, Beijing, China). The target amplified cDNA fragments were subcloned into the pEASY-T1 cloning vector (TransGen Biotech) and transformed into Trans-T1 phage resistant chemically competent cells (TransGen Biotech) following the manufacturer's protocol. The positive clones were identified with colony PCR with M13 primers (M13F and M13R, Table 1), and sequenced at Sangon Biotech (Shanghai, China).

Bioinformatic analysis

The nucleotide sequence of the full-length MyHSP90 cDNA sequence and deduced amino acid (aa) sequence were analyzed in the Basic Local Alignment Search Tool (BLAST) program at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/blast/). The open reading frame (ORF) was identified with ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/). The deduced aa sequence information was analyzed with the Expert Protein Analysis System (http://www.expasy.org/). The physical and chemical parameters of the deduced protein were computed using the ProtParam tool (http://web.expasy.org/protparam/), and the computed parameters included the molecular weight, theoretical isoelectric points (pI), instability index, and grand average of hydropathicity (GRAVY) (Gasteiger *et al*., 2005). The presence and location of the signal peptide cleavage site in the aa sequence were predicted with SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/) (Petersen *et al*., 2011). The functional domain and important sites of the protein were predicted by InterPro (http://www.ebi.ac.uk/interpro/).Due to the absence of the X-ray crystal structure of scallop HSP90 in the Protein Data Bank (PDB, http://www.rcsb.org/pdb/home/home.do), homology modeling was performed to find the three-dimensional (3D) structure of the protein from its primary sequence. The template protein was human HSP90 (PDB code: 5FWP chain A (Verba *et al*., 2016) with a high resolution (3.9 Å). The homology structure models of the scallop HSP90 were generated using the program SWISS-MODEL, automated protein structure homology-modeling server (https://www.swissmodel.expasy.org/) with all default parameters (Arnold *et al*., 2006; Guex *et al*., 2009; Biasini *et al*., 2014).

Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment of the deduced aa sequence of MyHSP90 was performed using the ClustalW program (http://www.ch.embnet.org/software/ClustalW.html). The phylogenetic tree was constructed according to aa sequences of the selected HSP90 (Table 2) using the neighbor-joining (NJ) method in MEGA 6.0 software and bootstrap analysis (1,000 times) to evaluate the reliability of the phylogenetic trees (Saitou and Nei, 1987).

Quantitative analysis of MyHSP90 mRNA expression

Total RNAs were pooled from three *M. yessoensis* scallops, and the relative levels of MyHSP90 mRNA transcripts in different tissues of the scallop were analyzed with quantitative real-time PCR (qPCR), which was conducted with the Applied Biosystems 7500 Real-time System (Applied Biosystems, Foster City, CA, CA, USA). The reaction conditions were 94 °C for 5 min, followed by 28 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. The final extension step was at 72 °C for 5 min. Three independent biological replicates were carried out, and the dissociation curve of the amplicon was analyzed to confirm that there was only PCR product in each reaction. The cytochrome b gene (*cytb*) was used as the internal reference gene. After being normalized to the *cytb* gene, the relative expressions of MyHSP90 mRNA were calculated with the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Statistical analysis

All data were expressed as the mean± SD. All analyses were performed using the analysis of variance (ANOVA) of the SPSS software (version 16.0). $p < 0.05$ was considered statistically significant.

Results

Molecular cloning and characterization of MyHSP90 cDNA in M. yessoensis

The nucleotide and deduced aa sequence of MyHSP90 cDNA are shown in Figure 1. The cDNA sequence of MyHSP90 was submitted to GenBank (accession no. MF196912). The full-length of MyHSP90cDNAwas 2639 base pair (bp), containing a 54-bp 5′-terminal untranslated region (UTR), a 2178-bp ORF, and a 406-bp 3′-UTRwith a poly(A) tail. The ORF encoded a 726-aa polypeptide with a predicted molecular mass of 83.26kDaand a theoretical pI of 4.81. The instability index was computed to be 39.78, which classified the protein as stable. The grand average of hydropathicity of this protein was −0.698. The deduced aa sequence of MyHSP90 lacked a typical signal peptide as predicted by SignalP 4.1 Server.

InterPro program analysis revealed that there was a highly conserved HSP90 site (YSNKEIFLRE) and HSP90 family signature motifs (MEEVD) at the C-terminus in the MyHSP90 sequence. A typical histidine kinase-like ATPase domain was located from position 14-228 aa. A ribosomal protein S5 domain and HSP90 C-terminal domain was located from position 258-540 aa and 564-685 aa, respectively (Fig. 1).

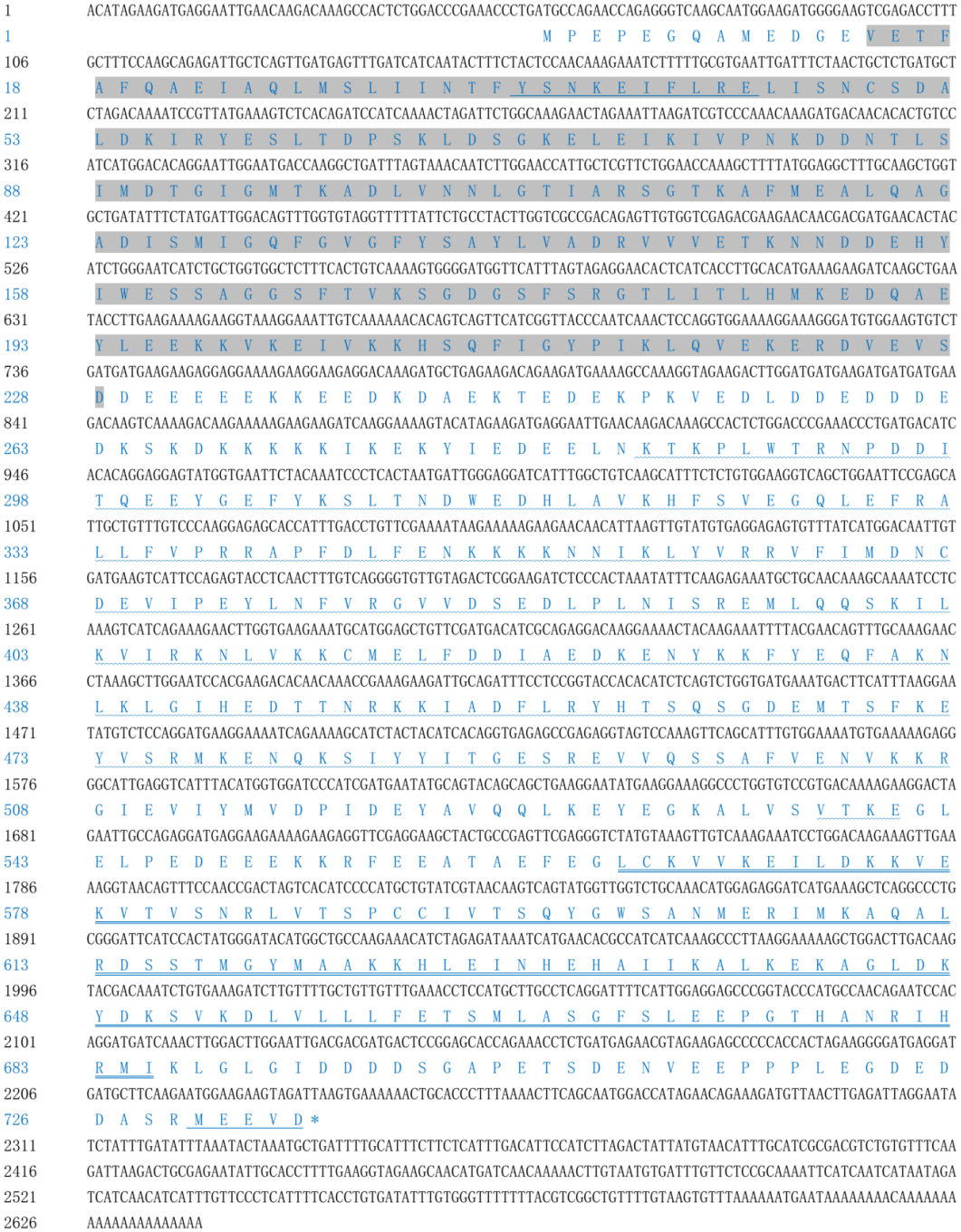

Fig. 1 Nucleotide sequence of the MyHSP90 gene and its deduced amino acid sequence from *Mizuhopecten yessoensis* (GenBank accession no. MF196912). The amino acid sequence is shown with one letter codes below the nucleotide sequence in blue. HSP90 conserved site (YSNKEIFLRE) and HSP90 family signature motifs (MEEVD) are indicated in underline. Histidine kinase-like ATPase, ATP-binding and HSP 90 N-terminal domain are shaded in gray. Ribosomal protein S5 domain and HSP90 C-terminal domain are indicated in wavy lines and double underlines, respectively.

The primary sequence alignment of the aa showed that the rate of consistency between the target (MyHSP90) and the template (human HSP90β) protein was 77.21 % (Fig. 2A). The 3D structure ofMyHSP90 was modeled using SWISS-MODEL workspace at the ExPASy server (Fig. 2B).

Multiple sequence alignment and phylogenetic analysis

The deduced aa sequence of MyHSP90 shared high homology with those of other representative speciesincluding14 molluscs, three insects, two nematodes, three crustaceans, four teleosteans, two reptiles, one bird and three mammals. The species

A)

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HsHSP90         --MPEEVHHGEEEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELISNASDALDKIRYES
MyHSP90         MPEPEGQAMEDGEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELISNCSDALDKIRYES
                   **     : ***********************************.**********

HsHSP90         LTDPSKLDSGKELKIDIIPNPQERTLTLVDTGIGMTKADLINNLGTIAKSGTKAFMEALQ
MyHSP90         LTDPSKLDSGKELEIKIVPNKDDNTLSIMDTGIGMTKADLVNNLGTIARSGTKAFMEALQ
                ************:*.*:** ::.**:::**********:*******:**********

HsHSP90         AGADISMIGQFGVGFYSAYLVAEKVVVITKHNDDEQYAWESSAGGSFTVRADHGEPIGRG
MyHSP90         AGADISMIGQFGVGFYSAYLVADRVVVETKNNDDEHYIWESSAGGSFTVKSGDGS-FSRG
                **********************::*** **:****:* **********:::.*. :.**

HsHSP90         TKVILHLKEDQTEYLEERRVKEVVKKHSQFIGYPITLYLEKEREKEISDDEAEEEKGEKE
MyHSP90         TLITLHMKEDQAEYLEEKKVKEIVKKHSQFIGYPIKLQVEKERDVEVSDDEEEEEKKEED
                * : **:****:*****::***:************.* :****: *:**** **** *::

HsHSP90         EEDKDDEEKPKIEDVGSDEEDDSGKDKKKKTKKIKEKYIDQEELNKTKPIWTRNPDDITQ
MyHSP90         KDAEKTEDEKPKVEDLDDEDDDEDKSKDKKKKKIKEKYIEDEELNKTKPLWTRNPDDITQ
                :: :. *::    :  .**:**..*.*.**.********::********:**********

HsHSP90         EEYGEFYKSLTNDWEDHLAVKHFSVEGQLEFRALLFIPRRAPFDLFENKKKKNNIKLYVR
MyHSP90         EEYGEFYKSLTNDWEDHLAVKHFSVEGQLEFRALLFVPRRAPFDLFENKKKKNNIKLYVR
                ************************************:**********************

HsHSP90         RVFIMDSCDELIPEYLNFIRGVVDSEDLPLNISREMLQQSKILKVIRKNIVKKCLELFSE
MyHSP90         RVFIMDNCDEVIPEYLNFVRGVVDSEDLPLNISREMLQQSKILKVIRKNLVKKCMELFDD
                ******.***:*******:*******************************:****:***.:

HsHSP90         LAEDKENYKKFYEAFSKNLKLGIHEDSTNRRRLSELLRYHTSQSGDEMTSLSEYVSRMKE
MyHSP90         IAEDKENYKKFYEQFAKNLKLGIHEDTTNRKKIADFLRYHTSQSGDEMTSFKEYVSRMKE
                :************ *:**********:***::::::**************:.********

HsHSP90         TQKSIYYITGESKEQVANSAFVERVRKRGFEVVYMTEPIDEYCVQQLKEFDGKSLVSVTK
MyHSP90         NQKSIYYITGESREVVQSSAFVENVKKRGIEVIYMVDPIDEYAVQQLKEYEGKALVSVTK
                .***********:* * .*****.*:***:**:**.:*****.******::**:******

HsHSP90         EGLELPEDEEEKKKMEESKAKFENLCKLMKEILDKKVEKVTISNRLVSSPCCIVTSTYGW
MyHSP90         EGLELPEDEEEKKRFEEATAEFEGLCKVVKEILDKKVEKVTVSNRLVTSPCCIVTSQYGW
                *************::**:.*:**.***::************:*****:******** ***

HsHSP90         TANMERIMKAQALRDNSTMGYMMAKKHLEINPDHPIVETLRQKAEADKNDKAVKDLVVLL
MyHSP90         SANMERIMKAQALRDSSTMGYMAAKKHLEINHEHAIIKALKEKAGLDKYDKSVKDLVLLL
                :**************.****** ******** :*.*:::*::**  ** **:*****:**

HsHSP90         FETALLSSGFSLEDPQTHSNRIYRMIKLGLGIDEDEVAAEEPNAAVPDEIPPLEGDED-A
MyHSP90         FETSMLASGFSLEEPGTHANRIHRMIKLGLGIDDDDSGAPETSDENVEEPPPLEGDEDDA
                ***::*:******:* **:***:**********:*: .* *..    :* ******** *

HsHSP90         SRMEEVD
MyHSP90         SRMEEVD
                *******
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B)

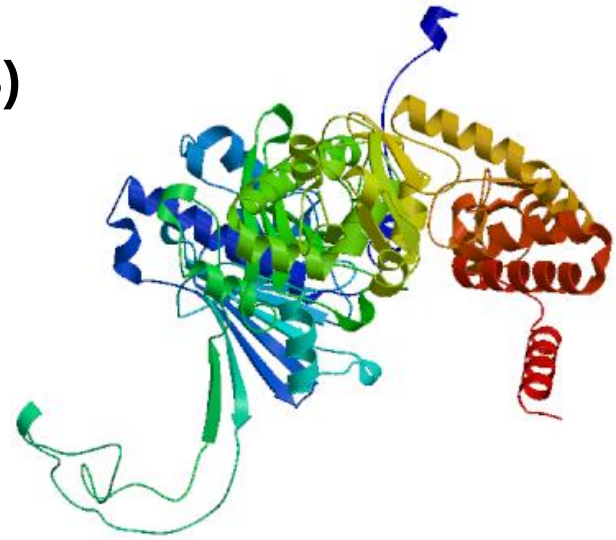

Fig. 2 (A) Alignment of the sequence of template human heat shock protein 90 (HSP) (PDB ID: 5FWP) chain A with the target protein heat shock protein 90 of *Mizuhopecten yessoensis*. The sequence identity is 77.21%. Conserved residues are represented by asterrisk (*), strong and weak conserved amino acids are denoted by (:) and (.), respectively. Gaps are represented by (-); (B) The three-dimensional (3D) structure of MyHSP90 in *M. yessoensis*, modeled using SWISS-MODEL

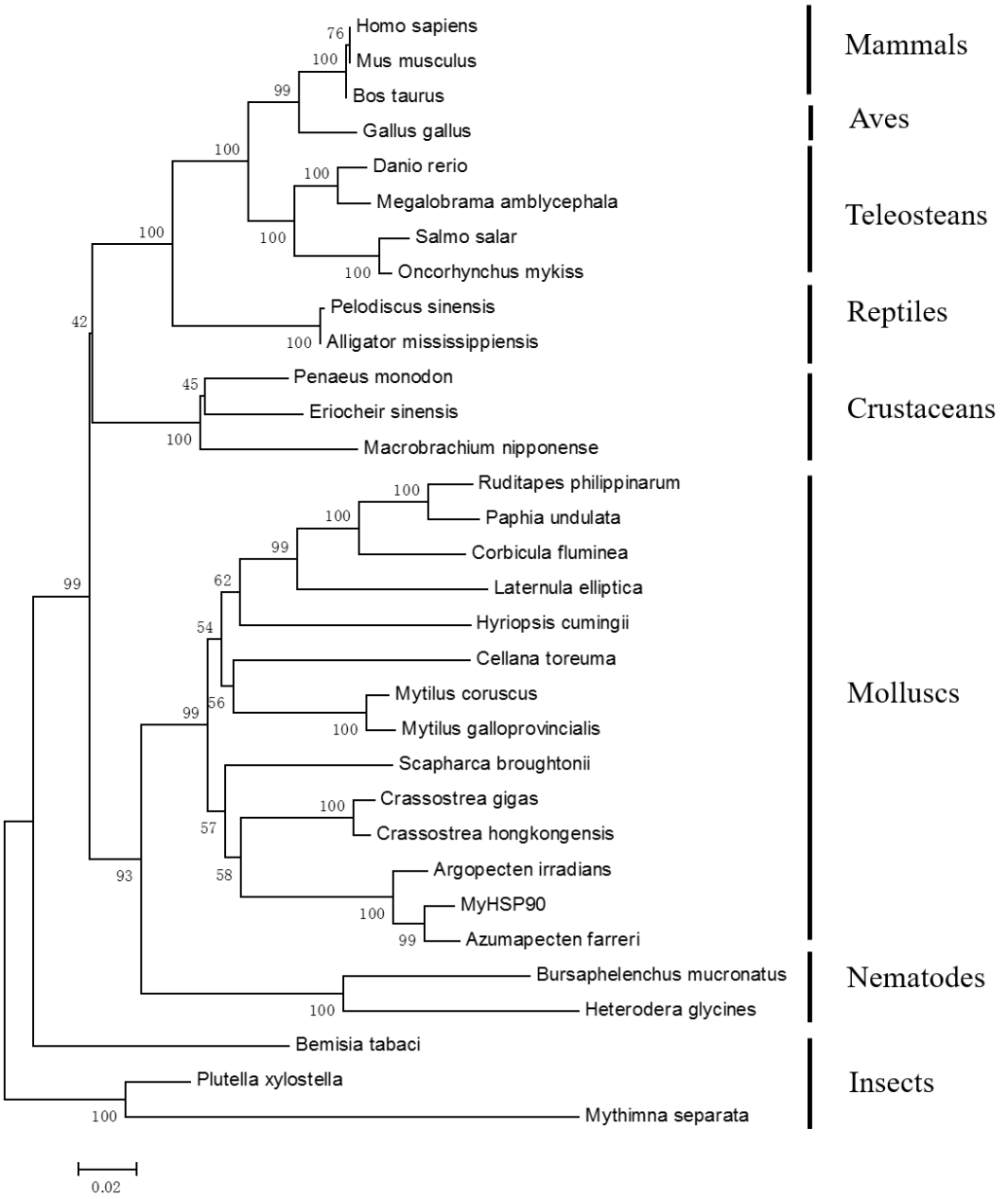

Fig. 3 Neighbor-joining phylogenetic tree of MyHSP90 amino acid sequences and other representative species. The representative species names and the GenBank accession numbers are the same as those in Table 2. Numbers on the nodes indicate the confidence level of the bootstrap analyses

name, the GenBank accession numbers, and identity are listed in Table 2. Searching for sequence similarities revealed that MyHSP90 shared more than 73 % similarity in all the matches except with *Mythimna separata*. Similarity with the HSP90s of other molluscs was over 80 %, MyHSP90 presented the highest identity with two other species of scallops, sharing 97.66 % identity with *A. farreri* (AAR11781.1) and 94.75 % with *A. irradiants* (ABS50431.1), respectively. In mollusks, MyHSP90 displayed high similarity to other species and all the levels of identity were higher than 80 %. Multiple sequence alignment of MyHSP90 with other HSP90s showed that they were highly conserved, especially in the regions of HSP90 family signatures.

To examine the relationship between various HSP90, phylogenetic trees were generated with the MEGA 6.0 NJ methods (Fig. 3). The resulting phylogenetic treeswere composed of two major large clusters, including the eight major groups: mammals, aves, reptiles, teleosteans, crustaceans, nematodes, insects, and molluscs. MyHSP90 was divided into the mollusc cluster.

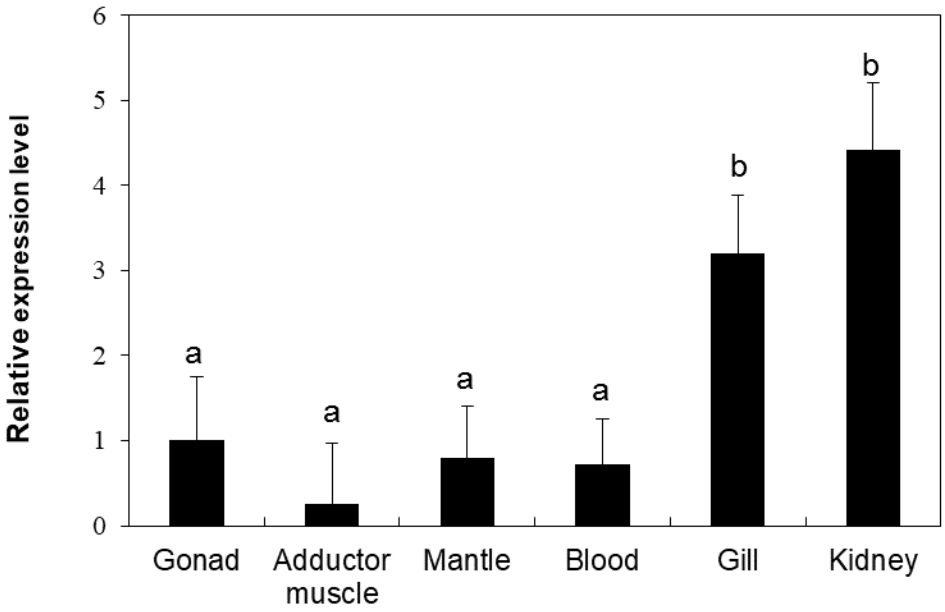

Fig. 4 Relative expression level of MyHSP90 mRNA in different tissues by quantitative real-time PCR analysis. The tissues include gonad, adductor muscle, mantle, blood, gill, and kidney. After normalization to Cytochrome b (*cytb*), relative expression of MyHSP90 mRNA were calculated with $2^{-\Delta\Delta Ct}$ method. Bars represent the mean ± SD. Different letters indicated significant differences ($p < 0.05$)

Relative expression of MyHSP90 mRNA in different tissues

MyHSP90 mRNA expressions were quantified in different tissues with qPCR with *cytb* as an internal reference gene and expressed values with the mean of the gonad set as 1 (Fig. 4). MyHSP90 mRNA was expressed in each of the six tissues examined, and the relative expression level was from high to low in kidney > gill > gonad > mantle > blood > adductor muscle. There was strong expression in both the gill and kidney, which was much higher than other tissues ($p < 0.05$), and there was no significant difference among the gonad, adductor muscle, mantle and blood ($p > 0.05$).

Expression level of MyHSP90 mRNA under temperature stress

MyHSP90 mRNA expression levels in different tissues responded differently to temperature stress (Fig. 5). MyHSP90 mRNA expression was temperature-dependent following temperature stress. In the gill, MyHSP90 expression was the highest level at 26 °C, and the maximal expression level was 130 times higher than that of the control temperature (15 °C) ($p < 0.05$). In the kidney and mantle, the maximal expression levels at 26 °C were 100 times and 70 times higher than the control. Compared to other tissues, MyHSP90 mRNA expression was lowest at 15 °C – 26 °C in the gonad, though the maximal was 10-fold that of the control group, no significant differences were found between the heat shock treatment and the control treatment ($p > 0.05$). At 24 °C, the expression level peaked in gonad and muscle, 10-fold and 40-fold of the control group, respectively.

Discussion

In the present study, we obtained and characterized the full-length cDNA sequence of the MyHSP90 gene from the gill of *M. yessoensis* using RACE methodology, and studied the expression pattern of MyHSP90 in different tissues in order to investigate the mechanism of heat shock response under temperature stress. This is the first time the expression of MyHSP90 has been investigated under temperature stress.

The full-length of MyHSP90 cDNA was 2639 bp and encoded a 726-aa protein. Unlike the HSP70 protein which contains three HSP family signature sequences, there was only one signature sequence in the HSP90 protein. Similar to other HSP90 family members, MyHSP90 contained highly conserved sequences, an ATP-binding domain in the N-terminal, a family signature sequence (MEEVD) in the C-terminal and a ribosomal protein S5 domain, as well as major structure and functional domains (Gupta,1995; Caplan, 1999), which is consistent with other studies (Prodromou *et al*., 1997; Gao *et al*., 2007, 2008).Based on the presence of the C-terminal MEEVD sequence (Ahn *et al*., 2003), the MyHSP90 was concluded to belong to the HSP90 family.

Multiple sequence alignment and homology analysis revealed that the deduced aa sequence of MyHSP90 shared high similarity with theHSP90 proteins of other eukaryotic organisms (65.27 % - 97.66 %). Gao *et al*. (2007, 2008) obtained the full-sequence of HSP90 cDNA from the Zhikong scallop *A. farreri* and bay scallop *A. irradians*. MyHSP90 shared higher identities with *A. farreri*

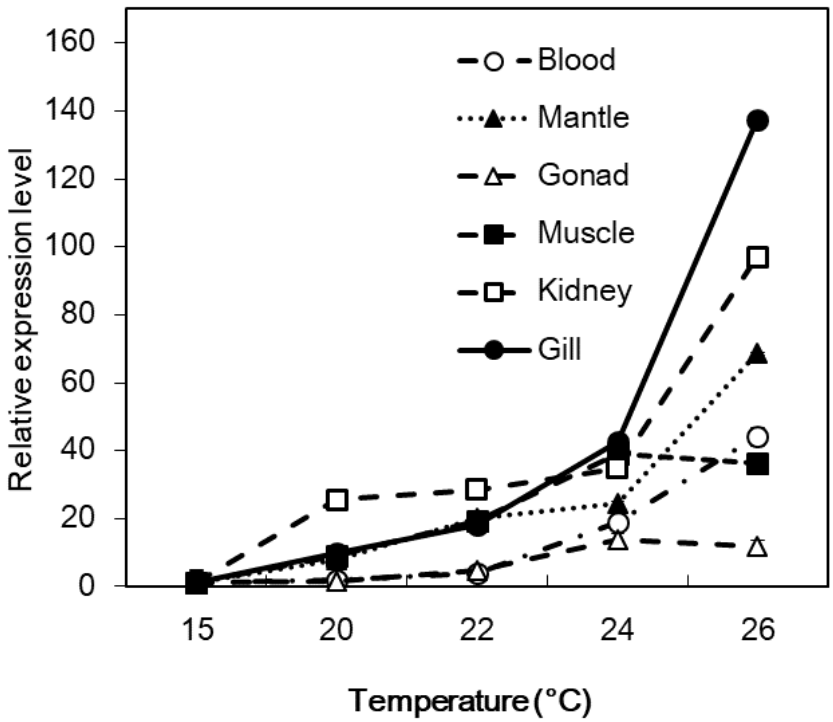

Fig. 5 MyHSP90 mRNA expression levels in different tissues under various temperatures stress. Each group of *Mizuhopecten yessoensis* was treated at 15 (as control), 20, 22, 24 and 26 °C for 7 days respectively, and each temperature was replicated three times (n = 3). After normalization to Cytochrome b (*cytb*), the relative expressions of MyHSP90 mRNA were calculated with $2^{-\Delta\Delta Ct}$ method. Vertical bars represent the mean ± SD

(97.66 %) and *A. irradians* (94.75 %). The sequence alignment and phylogenetic analysis further suggested that the MyHSP90 was a member oftheHSP90 family.

There are two different subtypes of HSP90 in vertebrates (HSP90α and HSP90β), which are different in the structure of glutamine-rich sequence (QTQDQ) at the N-terminus, a site of phosphorylation by a dsDNA-dependent kinase (Lees-Miller and Anderson, 1989; Theodoraki and Mintzas, 2006; Manchado *et al*., 2008). HSP90α plays an important role in cell growth, apoptosis, and the cell cycle. HSP90β is involved in maintaining normal physiological cell function through its role in cell differentiation and maintenance of cell structure and defense. In contrast, there is only one HSP90 gene in most invertebrates. MyHSP90 lacks the QTQDQ sequence at the N-terminus and shared higher similarity with HSP90β. These facts suggest that MyHSP90 is more closely related to the vertebrate HSP90β isoform.

There is presently no crystal structure of scallop HSP90 deposited in PDB, hence, in this study, a homology model was performed to determine the 3D structure of MyHSP90. Using BLAST search, *Homo sapiens* HSP90was selected as the template (PDB ID: 5FWP), which had an identity of 77.21 %. The cartoon representation of MyHSP90 is depicted in Fig. 2. All alpha-helices and beta-sheets, and the backbone structure resembling the same alignment that had been found in the template structure. Based on the present knowledge of the HSP90 structure in other organisms (*e.g*., human, yeast), HSP90 is an intact dimeric chaperone which forms homodimers, and each monomer has three structural domains: a C-terminal domain (CTD) responsible for dimerization; a middle domain (MD) implicated in client binding; and the N-terminal domain (NTD) that binds ATP (Meyer *et al*., 2003).

In the present study, tissue expression analysis of the MyHSP90 gene revealed ubiquitous expression in each tissue examined, indicating that MyHSP90 was synthesized under control conditions (15 °C). MyHSP90 mRNA expression was detected in all tissues tested in *M. yessoensis*, though there was no significant difference among the tissues examined in this study ($p > 0.05$). The MyHSP90 gene was expressed at a higher level in the gill and kidney, and at a lower level in the adductor muscle and blood (or hemocytes) (Fig. 4), which is consistent with previous studies in abalone *Haliotis diversicolor* HSP90 (Huang *et al*., 2014) and clam *Ruditapes philippinarum* (Liu *et al*., 2015). In contrast, the HSP90 gene expression level was highest in ovary when compared with other tissues tested (hepatopancreas, hemocytes, gill and muscle) in shrimp and crab (Jiang *et al*., 2009; Zhang *et al*., 2009; Li *et al*., 2012). MyHSP90 expressed in scallop gonad, which implied that HSP90 might have a certain relationship with genital organ development and maturation.

Heat shock stress could cause denaturation and aggregation of proteins, disrupt the integrity of essential organelles, and inhibit vital processes such as transcription and mRNA translation (Richardson *et al*., 2011). However, as a general molecular chaperone, the function of HSP90 is in promoting proper folding or refolding and preventing potentially damaging interactions or protein aggregations, and in assisting the disassembly of already formed protein aggregates (Pratt and Toft, 1997; Wang *et al*., 2008). *M. yessoensis* is a cold-water shellfish with an optimum growth temperature of 15 °C. The metabolic activity and feeding behavior of this species decreased greatly when the ambient environment temperature is above 23 °C (Chang 2007). The scallops were able to adapt to temperatures between 15 °C and 22 °C and maintain a survival rate >82 % (Hao *et al*., 2014).

After the scallop was exposed to heat shock treatment (15 °C – 26 °C) for 7 days, the expression level of MyHSP90 mRNA in all the six tissues tested except gonad was up-regulated significantly ($p < 0.05$) (Fig. 5). The different MyHSP90 mRNA expression levels in these tissues may be caused by different sensitivity to temperature stress. The expression level showed a significant increase in the gill (15 °C – 26 °C), which was 130 times higher than that at the control temperature (Fig. 5). The gill is an important organ for respiration in the scallop and presents a large surface area in direct contact with the ambient environment, which would act as a first response to seawater temperature changes (Jiang *et al*., 2016). In the gonad, there was no significant difference between the expression level under heat shock and the control. This could further imply that the MyHSP90 gene expressed stably in scallop gonad might have other functions in organ (gonad) development and maturation.

Conclusion

We identified full-length HSP90 cDNA in the scallop *M*. *yessoensis* (MyHSP90). This is the first time that the expression of MyHSP90 has been investigated under temperature stress (15 °C -26 °C).In the environment, water temperature changes had a strong effect on MyHSP90 mRNA expression level, and this gene was up-regulated significantly in gill, kidney, adductor muscle and mantle of scallop but not in the gonad. These results will be useful in understanding the mechanism of heat shock response in *M*. *yessoensis* under temperature stress and in understanding the massive summer mortalities of this species. These results are also an important reference for exploiting shellfish resources and optimizing their culture management, andMyHSP90 could be used as a potential biomarker to monitor environmental changes in future studies.

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