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Molecular characterization and expression of a heat shock protein gene (HSP90) from the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval)

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Abstract

In this study, the cDNA of *Tetranychus cinnabarinus* (Boisduval) (Acarina: Tetranychidae) HSP90 (designated TcHSP90) was cloned using a combination of the homology cloning and rapid amplification of cDNA ends (RACE) approaches. The full-length cDNA of TcHSP90 is 2595 bp, including a 5'-untranslated region (UTR) of 177 bp, 3'-UTR of 249 bp, and an open reading frame (ORF) of 2169 bp. The ORF encodes a polypeptide of 722 amino acids with a predicted molecular weight of 83.45 kDa and a theoretical isoelectric point of 4.81. There is an mRNA polyadenylation signal of ATTAAA at the positions 2558-2564. In addition, the expression pattern of TcHSP90 mRNA relative to that of β -actin gene in the three strains of *T. cinnabarinus* (AbR, abamectin-resistant strain; HR, heat-resistant strain; SS, the susceptible strain) were examined by using fluorescent real time quantitative PCR after the impact of abamectin, high and low temperature, respectively. The results showed that under the normal condition, the mRNA level of TcHSP90 was 1.64 and 1.29-fold higher in the AbR and HR than in SS, respectively. After 8 h treatment with abamectin, the TcHSP90 mRNA levels of SS, AbR, and HR were 1.25, 1.87, and 2.05-fold higher than those of their untreated controls, respectively. The TcHSP90 mRNA levels of SS, AbR, and HR were also significantly increased after being induced at 40° C for 1 h, and they were 3.76, 3.42, and 3.79-fold higher than those of their untreated controls, respectively. The mRNA level of TcHSP90 was also significantly increased after being induced at 4° C for 1 h. These results suggest that TcHSP90 might be involved in the abamectin and extreme temperature resistance or tolerance.

Keywords: abamectin, comparative quantitative expression, gene cloning, temperature shock

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Introduction

Under high temperature or other adverse environments, many organisms can be induced to synthesize a new set of proteins, designated as the heat shock proteins (HSPs) (Tissieres et al. 1974). The HSPs are divided into four families according to their molecular weights (Morimoto et al. 1993), including the HSP90 family (83 ~ 95 kDa), the HSP70 family (66 ~ 78 kDa), the HSP60 family, and the smHSP family (small heat shock protein). Among these families, HSP70 and HSP90 are the most universal HSPs (Parsell and Lindquist 1994). Previous studies showed that HSPs, especially HSP70 and HSP90, possessed important functions in high-temperature resistance. Also, the acquiring rate of heat resistance showed a positive correlation with the accumulation rate of HSPs; the decrease of heat resistance and the degradation of HSPs evolved coordinately (Denlinger et al. 1998; Sørensen et al. 2001; Chen 2005). Unlike other HSPs, HSP90 accounts for 1-2% of all the cellular proteins in non-stress cells. Some studies show that HSP90 promotes the refolding of denatured proteins in heat or other stress conditions and degrades the protein ions (Caplan 1999; Pearl et al. 2000). A recent study shows that HSP90 is involved in many physiological functions (Christine et al. 2002).

It is known that HSPs can be induced not only by heat, but also by other factors, such as environmental pollution, pesticides, heavy metals, UV radiation, acids, antibiotics, and hormones (Anderson et al. 1983; Lee and Dewey 1988; Koga and Takumi 1995; Hartke et al. 1995; Patil et al. 1996). Interestingly, a moderate heat shock may confer *Sarcophaga crassipalpis* (Chen et al. 1987) and *Drosophila melanogaster* (Bueton et al. 1988)

an ability to tolerate a relatively low lethal temperature. In another study (Patil et al. 1996), the sub-lethal concentration of propoxur induced the resistance of *Anopheles stephensi* and *Aedes aegypti* to a higher temperature. On the other hand, exposure to high temperature for several hours can protect the insect from the pesticide (Patil et al. 1996). All these observations suggest that an organism can survive under the cross-protection of different stresses: one type of resistance can be induced by another type.

Due to its strong reproductive ability, short generation time, small movement area, high inbreeding rate and frequent insecticide-contacted opportunity, the problem of resistance to acaricides is more severe for *Tetranychus cinnabarinus* (Boisduval) (Acarina: Tetranychidae) than for other crop insect pests. *T. cinnabarinus* is widely distributed in China and is difficult to prevent or control, which has caused severe damage to cotton and vegetable crops. The abamectin used in production has become the leading medicament to control insect and mite pests because of its unique mechanisms and excellent control effect. However, pest resistance to abamectin has rapidly developed from its large-area and high-reliance use.

He et al. (2005) showed that the fecundity of the abamectin-susceptible strain had a higher fecundity than that of the abamectin-resistant strain within the temperature range of 20-28°C, while the amount of oospheres produced by the resistant strain was greater at 34°C than that of the sensitive strain (He et al. 2005), suggesting that the abamectin-resistant strain has a reproductive advantage and better adaptability at a higher temperature. Thus, it is necessary and important to elucidate the

mechanism of high temperature fitness generated by *T. cinnabarinus*, which achieves the resistance to insecticides prior to treatment with higher temperature. The efforts to study this intriguing phenomenon will help us to better understand the resistant fitness of mites and other insects under environmental stress. In this study, the full length cDNA of TcHSP90 was cloned and sequenced using RT-PCR and RACE techniques. The expression of the TcHSP90 gene in abamectin susceptible and resistant strains, as well as in a high-temperature tolerant strain, were also quantitatively investigated under the treatments of abamectin, cold, and heat using real-time fluorescence quantitative PCR. The objective of the study was to help to establish a theoretical basis for the mechanism of high-temperature fitness in abamectin-resistant *T. cinnabarinus*.

Methods and Materials

Mites

A colony of *T. cinnabarinus* was established from the mites collected from the field of Beibei, Chongqing, China, which were reared under pesticide-free conditions (26° C and 70% RH). About 1200 mites were collected from cowpea and were regarded as the original colony. This colony was never exposed to acaricides and considered as the SS.

Resistance selection and bioassay

The resistant strain of *T. cinnabarinus* was selected from the susceptible strain. Acaricide selection was carried out by spraying abamectin with Yangtze-08, and the selective pressure was maintained at about 70% of the population mortality rate. The surviving *T. cinnabarinus* were transferred to new cowpea seedlings 24 h after spraying, and the mortality rate was recorded at that time.

Surviving *T. cinnabarinus* were allowed to lay eggs on the new cowpea seedlings for 1-2 days. Then, the survivors were removed until the eggs of the same generation grew to adult, and abamectin was sprayed again. After each treatment, the survivors were cultured and selected again at the next generation. The bioassay procedures generally followed the recommended methods of Food and Agriculture Organization. After 48-generation continuous selection, the resistant fold was up to 11.05, and the colony was defined as the abamectin-resistant strain.

The high-temperature-resistant strain was also isolated from the susceptible strain. The mites were reared in a thermal incubator at 34° C, 75-80% RH and a photoperiod of 14:10 L:D. The incubator temperature was raised 1° C after every two generations. The final temperature was stabilized at 40° C, and this strain was defined as the high-temperature-resistant strain.

RNA extraction and cDNA synthesis

Total RNA was extracted from the adult female spider mites of *T. cinnabarinus* (\approx 20 mg) with TRIzol (Invitrogen, www.invitrogen.com) according to the manufacturer's instructions. The RNA quality was assessed by electrophoresis on 1.2% agarose gel. Total RNA was treated with RQ1 RNase-Free DNase (Promega, www.promega.com) to remove contaminating DNA from the total RNA, and cDNA was synthesized from 2 μ g total RNA by M-MLV reverse transcriptase (Promega) following the manufacturer's protocol with the oligo (dT) primer 5'-GGCCACGCGTCGACTAGTAC (T)16(A/C/G)-3'.

Cloning and sequencing of TcHSP90

Two degenerate primers S2: 5'-GTNTTYAT HATGGAYAAATG-3' and A2: 5'-ACRTAY

TCRTCDATNGGYTC-3' were designed based on the conserved sequence of known HSP90s to amplify the partial fragment of TcHSP90 gene from *T. cinnabarinus*. The PCR reaction was performed in a 25 µl reaction volume containing 2.5 µl of 10× PCR buffer, 1.6 µl of MgCl₂ (25 mM), 2 µl of dNTP (2.5 mM), 2 µl of each primer (10 mM), 13.5 µl of PCR-grade water, 0.4 µl (1 U) of Taq polymerase (Promega) and 1 µl of cDNA template. The PCR temperature profile was 94° C for 3 min followed by 34 cycles of 94° C for 1 min, 54° C for 30 s, 72° C for 30 s and a final extension step at 72° C for 10 min. The PCR products were separated on 1.2% agarose gel and purified by the PCR fragment purification kit (TaKaRa, www.takara-bio.com). The purified PCR product was ligated into the pMD18-T vector (TaKaRa) and transformed into competent *Escherichia coli* cells. The recombinants were identified through the blue-white color selection in ampicillin-containing LB plates and screened with both forward and reverse primers. Fourteen positive clones were selected for sequencing (Invitrogen), and the resulting sequences were verified and subjected to further clustering analysis.

The 5' end of TcHSP90 cDNA was obtained by the RACE technique. Two specific reverse primers, 5GSP1: 5'-GCAGCAACTTGTTTCCTTAGATTCACC-3' and 5GSP2: 5'-GCTTCC TTATCC TCGGCTACTTCTTCG-3' were designed based on the partial sequence amplified by the degenerate primers. The PCR amplification was performed with the same reaction system as described before with the adaptor primers (UPM) and the gene-specific primer (5GSP1) by the 5' RACE system (Invitrogen), and then a nested PCR was carried out with adaptor primers (NUP) and specific primers (5GSP2).

The 3' end of TcHSP90 was amplified with sense primer 3GSP 5'-GAAGTAGCCGAG GATAAGGAAGC-3' and adaptor primers 5'-CTGATCTAGAGGTACCGGATCC-3' with 1 µl of cDNA template. The full-length sequence was verified by sequencing the fragment amplified by the primers F4: 5'-TGTTTCATCTCACATTTCCACAC-3' and R4: 5'-AACTACTTTCTATCCCA TCCCTT-3' (located at 5' UTR and 3' UTR of TcHSP90).

Bioinformatic analysis of the target gene

The sequence similarity search at both nucleotide and amino acid levels were performed with the BLAST program at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>). The inferred amino acid sequence was analyzed with the Expert Protein Analysis System (<http://www.expasy.org/>). Multiple alignment of TcHSP90 was performed with the Clustal W Multiple Alignment program (<http://www.ebi.ac.uk/clustalw/>). A phylogenetic tree was constructed by CLUSTAL (version X1.83) (Thompson et al. 1997) and MEGA (version 3.1) (Kumar et al. 2004) based on the obtained TcHSP90 sequences and other known HSP90 sequences. The bootstrap analysis was used with 1000 replicates to estimate the confidence of the branches produced by the neighbor-joining analysis.

Quantitative analysis of the TcHSP90 mRNA expression

Cowpea seedlings with *T. cinnabarinus* SS strain were treated with the following method: for heat and cold shock experiments, six treatments (200 adult female *T. cinnabarinus* per treatment) were transferred to light-constant temperature incubator exposed to 4° C, 7° C, 10° C, 34° C, 37° C, and 40° C for 1 h and abamectin treatment (100,000 × 8 h),

respectively. The AbR and HR were treated with cold shock (4° C, 1 h), heat shock (40° C, 1 h), and abamectin treatment (100,000 × 8 h), respectively. After the *T. cinnabarinus* recovered for 30 min, total RNA of each treatment and the control sample (26° C) was immediately extracted from whole bodies using Trizol reagent (Invitrogen) according to the manufacturer's protocol. In all cases, total RNA was treated with DNase I (TaKaRa). Concentration of total RNA was determined by measuring ultraviolet (UV) absorbance at 260 nm. RNA purity was checked by determining the A_{260}/A_{280} ratio, and its integrity was checked by formaldehyde agarose gel electrophoresis. All RNAs were stored at -80° C until used.

Real-time quantitative PCR was performed on Mx3000P Florescent Real-time Quantitative PCR (Stratagene) with β -actin (GenBank accession number: ABV82698) gene from *T. cinnabarinus* as a reference. Expression of β -actin showed no response to temperature ($n = 3$ individuals) in preliminary experiment (data not shown). The efficiency of PCR amplification for gene specific primers was analyzed by one cDNA sample with five serial dilutions and three technical replications. The PCR mixture contained 10 μ l, 2 × SYBR Green Mix, 0.8 μ l primers, respectively, 2.0 μ l cDNA and 6.4 μ l ddH₂O. The primers used for TcHSP90 were 5'-TCCACAACGTCA TTCCTCTCGCAT-3' and 5'-TCCAGAG GAGGCATTTCAGCT TCA-3' with a product of 117 bp; and the primers for β -actin were: 5'-CAGCCATGTATGTTGCCATC-3' and 5'-AAATCACGACCAGCCAAATC-3' with a product of 166 bp. The PCR condition was performed as follows: 95° C for 3 min, followed by 40 cycles at 95° C for 30 s, 60° C for 30 s and 68° C for 30 s. A melting curve program was run immediately after the PCR program, and the data were analyzed with

automatic software. The threshold cycle (Ct) values were used to quantify the target gene expression for each sample. The amplification folds of TcHSP90 in SS, AbR and HR were calculated with the $2^{-\Delta\Delta C_t}$ method (Livak et al. 2001). The real-time PCR analysis was independently repeated three times for each sample.

Results

Cloning and sequencing of the TcHSP90 gene

The PCR product amplified by the degenerate primers was 485 bp long, and its nucleotide sequence was homogeneous to other known HSP90s. TcHSP90-specific primers R2, R3, and F2 were designed based on the above sequences, and used for the full-length cDNA cloning. With RACE and nested PCR approaches, two fragments corresponding to the 5' and 3' end of the TcHSP90 cDNA were amplified. Finally, a nucleotide sequence of 2595 bp representing the complete cDNA sequence of TcHSP90 was obtained by assembling the above fragments.

Characterization of TcHSP90

The cDNA sequence of TcHSP90 was deposited in GenBank (accession no. EU851046). The full-length cDNA of TcHSP90 is 2595 bp long, including a 5'-untranslated region (UTR) of 177 bp, a 3'-UTR of 249 bp, a canonical polyadenylation signal sequence ATTAAA, a poly (A) tail, and an open reading frame (ORF) of 2169 bp. The ORF encodes a polypeptide of 722 amino acids, whose predicted molecular weight is 83.45 kDa and whose theoretical isoelectric point is 4.81. The protein contains the five amino acid blocks defining the HSP90 protein family (NKEIFLRELISN[S/A]SDALDKIR, LGTIA[K/R]SGT, IGQFGVGFYSA[Y/F]LVA[E/D], IKLYVRRVFI, GVVDS[E/D]

DLPL N[I/V]SRE) as well as a consensus sequence MEEVD at the C-terminus that are highly conserved in the TcHSP90 sequence (Figure 1). The SMART program analysis revealed a typical histidine kinase-like ATPases domain at the positions 34-188, which is ubiquitous in all HSP90 family members.

Homology analysis of TcHSP90

Multiple alignments of TcHSP90 with that of other species showed high conservation (Figure 2). The BLAST results of the deduced amino acid sequence of TcHSP90 showed that the sequence obtained was closer to arthropods than to other organisms. The five HSP90 signature sequences (Gupta 1995), were observed in each of the HSP90 proteins compared (Figure 2, bold). Relative to the canonical signature sequences, somewhat fewer substitutions were observed in the signature sequences of the arthropods. Among the HSP90 proteins compared, the highest percent identity of *T. cinnabarinus* HSP90 was with HSP90 from *Bombyx mori* (79.97% identical). The lowest percent identity and similarity was with HSP90s from *D. melanogaster* (77.09% identity). Identity of HSP90 was high within the arthropods, especially in the signature regions of the HSP90 family.

Based on the sequences of HSP90s, a phylogenetic tree was constructed with CLUSTAL X1.83 (Thompson 1997) and MEGA 3.1. As shown in Figure 3, plant and animal HSP90s are separated and form two distinct branches in the tree. In the branch of animals, vertebrates and arthropods are separated and form two distinct branches in the tree. All insects are clustered together and form a sister group to the branch of mites. The relationships displayed in the phylogenetic tree are in concurrence with traditional taxonomy.

Quantitative analysis of TcHSP90 gene expression

Fluorescent real-time quantitative PCR was used to measure the mRNA expression level of TcHSP90 in *T. cinnabarinus* with the treatments of abamectin, high temperature, and low temperature. When Ct values were used to generate a log-linear regression plot, the standard curve for the house keeping gene β -actin showed a strong relationship ($r^2 = 0.9991$; PCR efficiency = 99.5%). The efficiency of PCR amplification for each target gene of TcHSP90 tested showed that the amplification efficiencies were 96.2% for TcHSP90 primers. The amplification efficiencies experiments indicated that each pair of primer of β -actin and TcHSP90 used for real-time PCR did not violate assumptions of the two ddCt method. Melting curve analysis and gel electrophoresis showed only target gene was synthesized.

The mRNA expression level of TcHSP90 in SS showed the tendency of rising after heat and cold shock, but the expression level after heat shock was higher than that after cold shock (Figure 4). Under the normal condition, the mRNA expression levels of TcHSP90 in AbR and HR were 1.64-fold and 1.29-fold higher, respectively, than that in SS. Compared with the corresponding control groups, the TcHSP90 mRNA level was increased at 8 h after the abamectin treatment, which was 1.25-fold higher in SS, 1.87-fold higher in AbR, and 2.05-fold higher in HR (Figure 5). After being induced by high and low temperature, the TcHSP90 mRNA level also increased. Specifically, after being induced at 40°C for 1 h, the mRNA levels of SS, AbR, and HR were 3.76, 3.42 and 3.79-fold higher than their corresponding control groups, respectively, all of which were significant ($p < 0.05$); after being induced at

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GGGAGTTTCATTAAATCTAACCAAGCGCATTGTGAATAAGCCATTGTTTCATCTCACATTTCCACACCTTACCAACAATTTCTGCAATTGCTTTAA 100
ATTGTTATTATTCAATGTTCTCAATTAATTTTATTGTTGATTACTTGTCTCTCATTACCCGGTATCTATCAAAATGCCGAAGAACTATGAGTGT 200
                                     M P E E T M S V 8
CGAGGAACCCGAAACCTTCTCTTTCCAAGCTGAAATCGCCAGTTGATGACTTTGATCATCAACACATTCTACTCAAAACAAAGAAATTTCTCTCGTGAA 300
E E P E T F L F Q A E I A Q L M T L I I N T F Y S N K E I F L R E 41
TTGATCTCCAACCTCTCCGATGCTCTTGATAAGATTGATATGAATCTCTTACTGATCCATCTAAATGGATAGTGGTAAAGAACTTTACATCAAGATTG 400
L I S N S S D A L D K I R Y E S L T D P S K L D S G K E L Y I K I V 75
TCCCCAACCAAGATGAACGCACCTTACCATCATCGATACTGGTATTGGTATGACTAAGGCTGACCTCATCAACAACCTGGGTACCATTGCTAAATCTGG 500
P N Q D E R T L T I I D T G I G M T K A D L I N N L G T I A K S G 108
TACTAGAGCTTTCATGGAAGCCCTTCAAGCTGGCGCTGATATTCTATGATTGGTCAATTCGGTGTGGTTTCTACTCAGCTTACTAGTTGCCGATCGG 600
T R A F M E A L Q A G A D I S M I G Q F G V G F Y S A Y L V A D R 141
GTTGTTGTTACCTCTAAGCACAATGATGATGATTGTTATACTTGGGAGTCAGCTGCTGGTGGTTCATTCACTATTAAGAAATCTGTTGACCCAGAGCTCA 700
V V V T S K H N D D D C Y T W E S A A G G S F T I K K S V D P E L T 175
CTCGTGGTACCAAGATTGTTTGTCTCTCAAGAAGATCAATCTGACTATCTCGCTGAGAAAAAGATCAAAGAAATGTGAAGAAACACAGTCAATTTAT 800
R G T K I V L F L K E D Q S D Y L A E K K I K E I V K K H S Q F I 208
CGGATATCTATCAAGCTTGTGTTGTTAGAAAGAGCGAGAAAGAGAGTTTCTGATGATGAAGCTGAAGATGAAAAGAAAGAGACGAAGAAGAGAAAAA 900
G Y P I K L V V Q K E R E K E V S D D E A E D E K K E D E E E K K 241
GAAGAGGAGAAAAAGATGATGAAGAACCCAAAGTAGAAGACGTGAAGATGAAGACAAAAAGACAAGAAAAAGAAAAAGAGTTACTGAAAGATACA 1000
E E E K K D D E E P K V E D V E D E D K K D K K K K K V T E K Y I 275
TTGAAGATGAAGAACTTAACCGAACTAAACCCATTGGATGCGAAACCTGATGACATCTCACAACCTGAATATGGTGAATCTACAAGAGTCTTACCAA 1100
E D E E L N R T K P I W M R N P D D I S Q T E Y G E F Y K S L T N 308
TGATTGGGAAGAACATCTCGCCGTGAACATTCTCAGTCGAAGGTCAACTCGAATTCGGAGCTTTACTCTTGTCTCCTAAGAGAGCTCCTTTTGACCTT 1200
D W E E H L A V K H F S V E G Q L E F R A L L F V P K R A P F D L 341
                                     S2
TTCGAAAAAGAGAAACAAAGAATAACATTAATTTGATGTACGAAGAGTATTATTATGGACAATTGCGAAGACTTAATCCAGAATACCTCAACTTTA 1300
F E N R K Q K N N I K L Y V R R V F I M D N C E D L I P E Y L N F I 375
TCAAGGGTGTGGTTGACTCTGAAGATTACCTTTGAACATTCTCGTGAAATGCTCCAACAAAAAAGATTTTACAAGTTATTAGAAAAGATTTAGTTAA 1400
K G V V D S E D L P L N I S R E M L Q Q N K I L Q V I R K N L V K 408
5GSP2 3GSP
GAAATGCTTGAATTTATTCGAAGAAGTAGCCGAGGATAAGGAAGCTTACAAGAAGTTCTACGAGCAATTACGAAGAATCATCAAGCTTGGTATTATGAA 1500
K C L E L F E E V A E D K E A Y K K F Y E Q F S K N I K L G I H E 441
GACACTCAAAACCGAAGAACTTGGTGATCTCCTTCGTTATTATACTTCTGCCTCTGGAGATGAAGTATGCTCTCTTAAGAATATGATATCCGAATGA 1600
D T Q N R K K L G D L L R Y Y T S A S G D E V C S L K E Y V S R M K 475
5GSP1
AAGAAACCAAAATCAATCTATTTTCATCACTGGTGAATCTAAGGAACAAGTTGCTGCTTCTGCATTGTTGAACGTGTTCGAAGCCGTGGTTTTGAGGT 1700
E N Q K S I Y F I T G E S K E Q V A A S A F V E R V R S R G F E V 508
A2
TGTTTACATGGTTGAGCCCATCGATGAATCTGTGTTCAACAACCTTAAGAGTATGATGGTAAACCATTAGTTTCAGTTACCAAGAAGGCTCTGAACCT 1800
V Y M V E P I D E Y C V Q Q L K E Y D G K P L V S V T K E G L E L 541
CCTGAAACCGAAGAAAGAAAGAACGTGAAGATGACAGGAAAAAATTCGAGACCTTTGCAAAGTTATGAAAGATATTCTTGATAAACGAGTCGAGA 1900
P E T E E E K K K R E D D R K K F E T L C K V M K D I L D K R V E K 575
AAGTCACTATTCTAATCGTCTGGTCACTTCTCCATGTTGATTGTCACAAGTCAATATGGTTGGTCAGCCAACATGGAACGAATCATGAAAGCTCAGGC 2000
V T I S N R L V T S P C C I V T S Q Y G W S A N M E R I M K A Q A 608
TCTCCGGGATACATCCACTATGGGATACATGGCTGCTAAAAAGCATCTTGAATTAACCCAGATCATTCAATCATTGAAAGTCTCAGAATTAAGTTGAA 2100
L R D T S T M G Y M A A K K H L E I N P D H S I I E S L R I K V E 641
GCCGATAAGAATGATAAATCAGTTAAAGATTAGTCATGTTACTCTCGAACTGCCCTCTGCTCTGGTTTCTCTCTCGAAGATCCACAACGTCATT 2200
A D K N D K S V K D L V M L L F E T A L L C S G F S L E D P Q R H S 675
CCTCTCGCATCTATCGCATGATTAACCTTGGTCTCGGTATTGATGATGATGAAATTTATGTTGGAGGTGATAAAGTAGATGAAGCTGAAATGCCTCCTCT 2300
S R I Y R M I K L G L G I D D D E I Y V G G D K V D E A E M P P L 708
GGAAGGTGACGCAGAAGATGCTTACGAATGGAGGAAGTTGATTAAATCATTCATTTTCCCTAATTTTTTTGAGTCGCCCATGAAAGTAGCTTCTCAA 2400
E G D A E D A S R M E E V D * 722
TGGATAATCCTAATTAATCAACAGTTCTAAATATTGTTTTATTCTATAATTGTCTTTTGTATTGTTAACTACTTTCTATCCCATCCCTTTTTTCA 2500
ATCAAAATGTCATAAAATGTCAACCAACCACTCCCTTAATTTTGTAGTTAAACAATTAAACAAATTCATTTAACAAAAAATAAAAAA 2595

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Figure 1. Nucleotide and deduced amino acid encoding region of **TcHSP90** (including 5' and 3' UERs). Signature sequences of Hsp90 family and C-terminal five amino acids(MEEVD) are shown in shaded parts. start codon and stop codon are boxed. The tailing signals are shown in bold. Arrows above the nucleotide sequences represent the position of the different synthetic primers (degenerate primers and Specific primers for RACE experiments) used in PCR. High quality figures are available online.

<i>Bombyx mori</i>	MPEEMETQPAEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELISNSSDALDKIRYESLTDP SKL	65
<i>Bemisia tabaci</i>	---dvn..mega---v-----v-----r-	63
<i>Tetranychus cinn</i>	---tms.ve-p---l-----t-----	64
<i>Plutella xyloste</i>	---qa-sg-----c-----	65
<i>Drosophila melan</i>	m-e-a-----a-----	58
<i>Bombyx mori</i>	DSGKELYIKIIPKNNEGTLTIIDTGIGMTKADLVNN LGTTIAKSGTKAFMEALQAGADISMIGQFG	130
<i>Bemisia tabaci</i>	e-----dr-----a-----	128
<i>Tetranychus cinn</i>	-----v--qd-r-----i-----r-----	129
<i>Plutella xyloste</i>	-----a-----	130
<i>Drosophila melan</i>	-----l---ta-----s-----	123
<i>Bombyx mori</i>	VGFYSSYL VADRVTVHSHKNDDEQYVWESSAGGSFTVRPDSGEPLGRGTKIVLVHKEDLAEFMEE	195
<i>Bemisia tabaci</i>	---af---t---v-----iks-h-----im-m---mt--l--	193
<i>Tetranychus cinn</i>	---a-----v-t-----dc-t---a-----ikksvdpe-t-----fl---qsdyla-	194
<i>Plutella xyloste</i>	---c-----t-----m---a-----i-s-as-----i---t-yl--	195
<i>Drosophila melan</i>	---a---k---t---n-----a-ns-----yi---qtdyl--	188
<i>Bombyx mori</i>	HKIKEIVKKHSQFIGYPIKLMVEKEREKELSDDEAEKEE.....EDEKPKIEDVGE	249
<i>Bemisia tabaci</i>	r---d-----l---d---e-e---v---dkeekke...dkdt-----	253
<i>Tetranychus cinn</i>	k-----v-q-----v-----d-...deekkeekkd-e-v---.	256
<i>Plutella xyloste</i>	-----g.....-d-----	250
<i>Drosophila melan</i>	s-----n-----l-----v---dd-...gdekkem....tde-----	248
<i>Bombyx mori</i>	DEDEDKKDT.KKKKKTIKEKYTEDEELNKTPIWTRNADDITQDEYGDYKSLTNDWEDHLAVKH	313
<i>Bemisia tabaci</i>	-e-g-ekk..-----p---te---e-----	316
<i>Tetranychus cinn</i>	.-----k..---kvt---i-----r---m-p---s-t---e-----e-----	318
<i>Plutella xyloste</i>	---a--k.-----e-----	314
<i>Drosophila melan</i>	---a---kda-----p---s-e---e-----	313
<i>Bombyx mori</i>	FSVEGQLEFRALLFVPRRAPFDLFENKKRKN IKLYVRRVF IMDNCELDIPEYLNFI RGV DSED	378
<i>Bemisia tabaci</i>	-----k-----k-----k-----	381
<i>Tetranychus cinn</i>	-----k-----r-q-----k-----	383
<i>Plutella xyloste</i>	-----t-----k-----	379
<i>Drosophila melan</i>	-----i---t-----q-kr-----mk-----	378
<i>Bombyx mori</i>	LPLNISRE MLQQNKILKVIRKNLVKKCLELFEELAEADKENYKYYEQFSKNLKLGIHEDSQNRAK	443
<i>Bemisia tabaci</i>	-----f--f-----t---k-----	446
<i>Tetranychus cinn</i>	-----q-----v---a--f-----i-----t--k-----	448
<i>Plutella xyloste</i>	-----n-----	444
<i>Drosophila melan</i>	-----v-----tm-i---t-----f-d-----v---n-----	443
<i>Bombyx mori</i>	LSELLRYHTSASGDEACSLKEYVSRMKENQKHIYYITGENRDQVANSSFVERVKKRGYEVVYMT	508
<i>Bemisia tabaci</i>	-ad---q---t---dv--f-d-a-----sk-----f--i---	511
<i>Tetranychus cinn</i>	-gd---y-----v-----s--f---ske---a-a-----rs--f---v-----	513
<i>Plutella xyloste</i>	-ad--f-----f-----s-----	509
<i>Drosophila melan</i>	-adf--f-----df--ad-----d---v-f---sk---s--a-----a--f-----	508
<i>Bombyx mori</i>	PIDEYVVQMQREYDGKTLVSVTKEGLELPEDEEEKKKREEDKVKFEGLCCKVMKNILDNKVEKV	573
<i>Bemisia tabaci</i>	-----kd---n-----y-----t-----d---k---i---	576
<i>Tetranychus cinn</i>	-----c---lk---p-----t-----d-rk---t-----d---kr---ti-----	578
<i>Plutella xyloste</i>	-----n-----	574
<i>Drosophila melan</i>	-----i-hlk--k-q-----s-----a--s--l--s-----	573
<i>Bombyx mori</i>	SNRLVESPCCHIVTAQYGSANMERIMKAQALRDTSTMGYMAAKKHLEINPDHSIVETLRQKAEAD	638
<i>Bemisia tabaci</i>	-s-----s---t-----s-----pvm da--v---e-----	641
<i>Tetranychus cinn</i>	-----t-----s-----i-s--i-v-----	643
<i>Plutella xyloste</i>	-----v-----	639
<i>Drosophila melan</i>	-----d---s-f-----a-----g--q-----p-----d--	638
<i>Bombyx mori</i>	KNDKAVKDLVILLYETALLSSGFTLDEPQVHASRIYRMIKLGLGIDEDEPIQVEEP.ASGDVPPL	702
<i>Bemisia tabaci</i>	---s-r--m--f-----a-ed---g--h-----vm---ekpdtam-aa	706
<i>Tetranychus cinn</i>	---s---m--f---c---s-ed--r-s-----d--iyvggdkvdaem---	708
<i>Plutella xyloste</i>	-----s-----a.sa-----	703
<i>Drosophila melan</i>	-----f--s-----s--s-----mttdaqa--a-s--	703
<i>Bombyx mori</i>	EGDADDAS RME EVD	716
<i>Bemisia tabaci</i>	d---e-----	720
<i>Tetranychus cinn</i>	---e-----	722
<i>Plutella xyloste</i>	---g-----	717
<i>Drosophila melan</i>	ve-te--h----	717

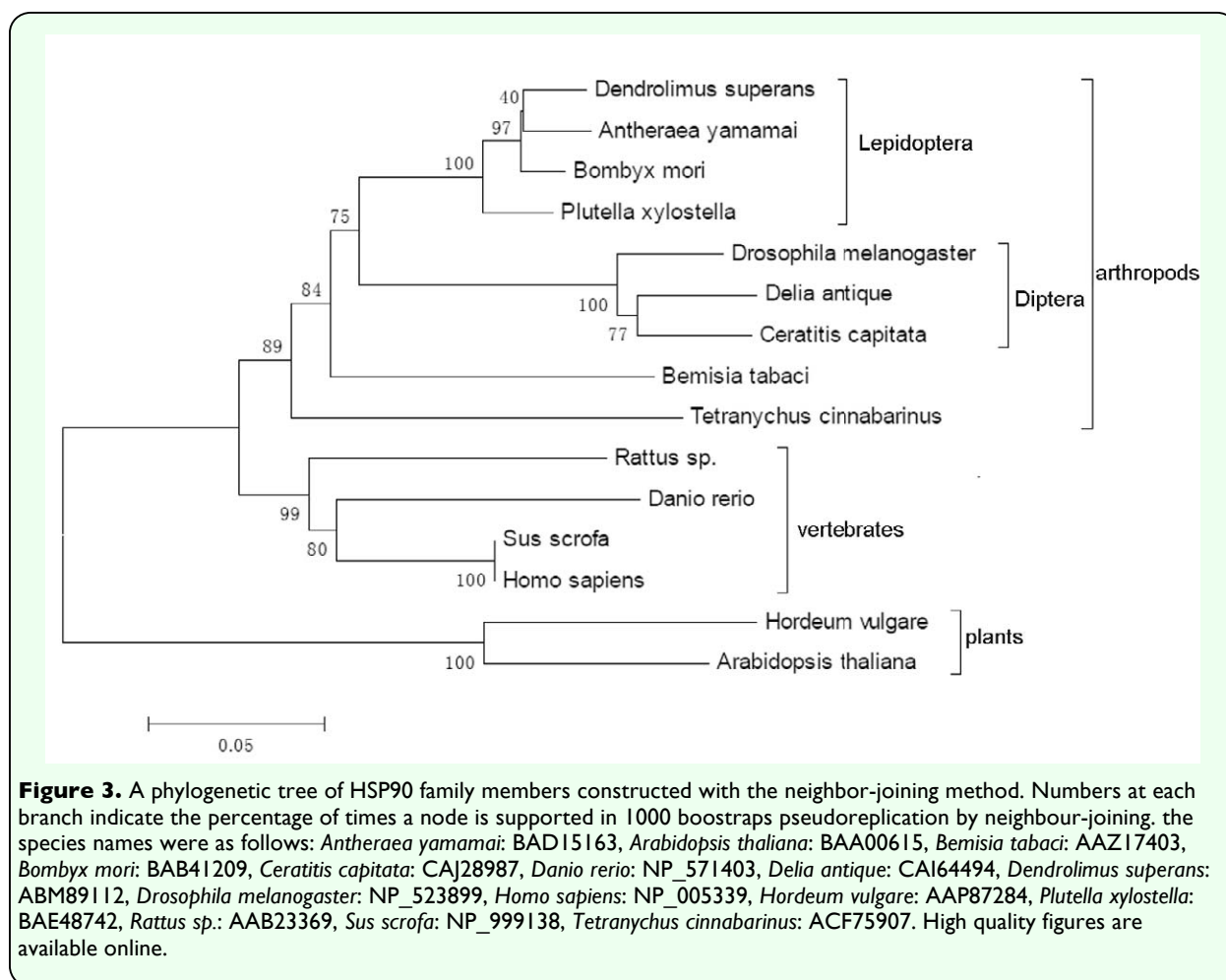
Figure 2. Comparison of the amino acid sequences of the HSP90 protein family. The GenBank accession numbers and species names were as follows: *Bemisia tabaci*: AAZ17403, *Bombyx mori*: BAB41209, *Drosophila melanogaster*: NP_523899, *Plutella xylostella*: BAE48742, *Tetranychus cinnabarinus*: ACF75907. High quality figures are available online.

4!°C for 1 h, the mRNA levels of SS, AbR, and HR were 1.38, 2.36, and 2.06-fold higher than their corresponding control groups, respectively, all of which were also significant ($p < 0.05$).

Discussion

In this study, complete cDNA sequence of HSP90 gene from *T. cinnabarinus* was reported. Conserved sequences and characteristic motifs, such as HSP90 family signatures, and histidine kinase (from 35 to 188 amino acid residues) (Gupta 1995) were found, as well as the major structural and functional domains typically in HSP90 (Buchner 1999; Caplan 1999) in the inferred TcHSP90 protein sequence. The C-terminal EEVD motif of TcHSP90 is the cytoplasmic localization signal of the heat-shock protein

family, which is recognized by the TPR domains of HOP (HSP70 and HSP90 organizing protein) (Scheufler 2000) and involved in assembling the multiple molecular chaperone. In addition, the presence of motif MEEVD at the C-terminus is a character shared among all the eukaryotic HSP90 proteins. The sequence similarity analysis revealed that the inferred amino acid sequence of TcHSP90 shared high similarities with other known HSP90s, especially with those from insects of arthropods. Based on sequence alignment, structure comparison and phylogenetic analysis, we concluded that TcHSP90 was a cytosolic member of HSP90 family. Studying the divergence of TcHSP90 from *T. cinnabarinus* has important theoretical and practical significance in terms of how to study environmental stress response.



In many species, sub-lethal temperatures can often induce the greatest amounts of HSP mRNA (Feder and Hofmann 1999). The temperatures 0!°C and 40!°C are sub-lethal for locust eggs (Jing and Kang 2003) and can induce an increase in HSPs expression (Li et al. 2000). HSPs are generally expressed at low levels under non-stress conditions, but

increase rapidly in response to a stress (Sonoda et al. 2006).

The mRNA expression of TcHSP90 in abamectin and high and low temperature stress conditions was studied based on fluorescent real time quantitative PCR. The results showed that standard expression levels

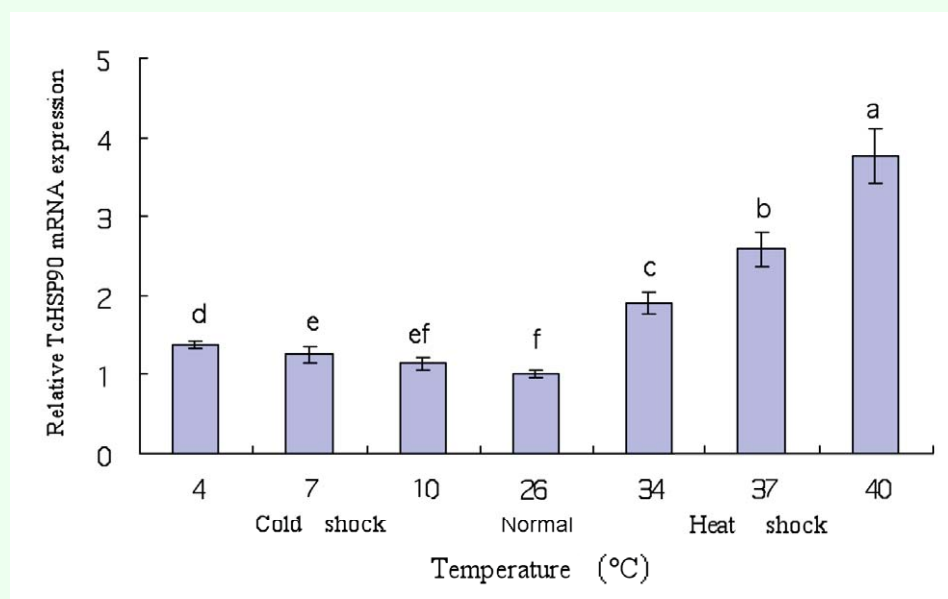


Figure 4. Comparative quantitative RT-PCR analysis of the relative expression of TcHSP90 in SS at different temperatures. Different letters above each bar indicate statistical difference by ANOVA analysis followed by the Duncan's Multiple Comparison test ($p < 0.05$). High quality figures are available online.

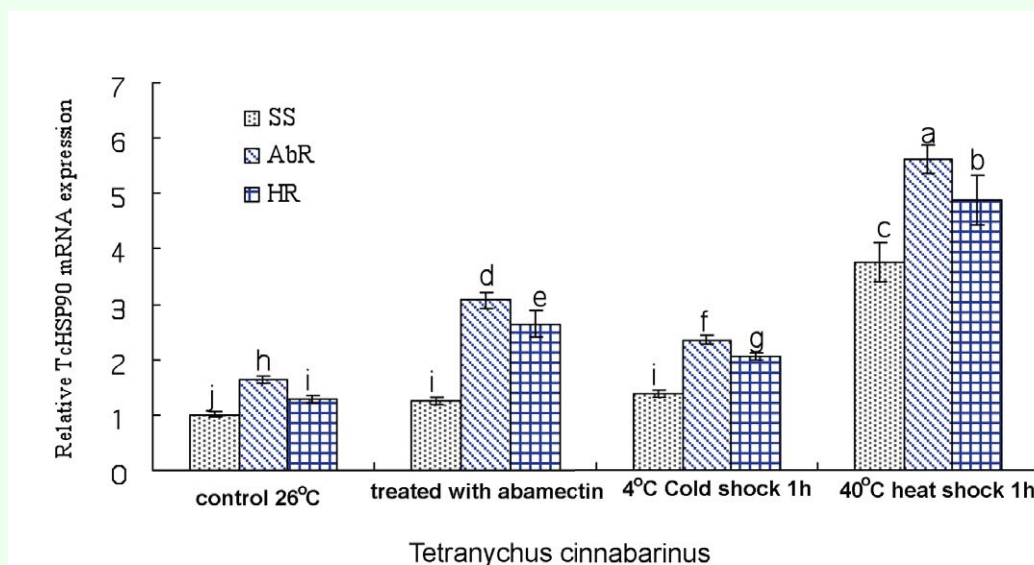


Figure 5. Comparative quantitative RT-PCR analysis of the relative expression of TcHSP90 after abamectin, cold and heat shock treatment. Different letters above each bar indicate statistical difference by ANOVA analysis followed by the Duncan's Multiple Comparison test ($p < 0.05$). High quality figures are available online.

of TcHSP90 mRNA was higher in AbR and HR than in SS, suggesting that a higher amount of HSP90 in *T. cinnabarinus* may be helpful for adaptation to temperature and abamectin stress. The TcHSP90 mRNA in AbR and HR was higher than in SS given the treatments of abamectin, high temperature, and low temperature. This observation may be ascribed to physiological functions of cross protection in *T. cinnabarinus* under different stress conditions. Greater accumulation of HSP90 in *T. cinnabarinus* induced by insecticide resistance and high temperature resistance protected the organism from stress injuries. This may provide a theoretical basis for explaining the fitness advantage of resistance of insects or mites to abamectin.

While HSP70 has been widely accepted and applied as a biomarker of unhealthy environmental factors, studies about HSP90 are rare (Arts et al. 2004; Hallare et al. 2005; Soon et al. 2006). The results of this study showed that TcHSP90 had protection functions not only in high-stress temperatures, but also under stresses from an insecticide. Therefore, HSP90 is a potential biomarker that can detect environmental factors of stress to the organism. As far as this notion is concerned, some studies have been carried out in soil and marine organisms (Köhler et al. 1998, 2001; Arts et al. 2004; Hallare et al. 2005; Soon et al. 2006). However, it should be noted that a single biomarker cannot reflect the overall physiological state of an organism. More and more attention should be paid to the comprehensive functional evaluation using various biomarkers. This study showed that TcHSP90 was susceptible to the stress responses induced by abamectin and high temperature, and further efforts are needed to explore the response to environmental effects using HSP90 and HSP70 together.

There is a significant body of evidence that shows that animal cells have evolved a variety of elaborate defense and repair mechanisms to protect them against the negative effects of natural environmental stressors (Pruski & Dixon 2007). Cloning and characterization of the TcHSP90 provides a tool for investigating stress-related responses in *T. cinnabarinus*. Furthermore, the results of our study could help determine specific techniques to manage the acarid group which includes many agricultural pests and disease vectors.

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References

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- Anderson RL, Ahier RG, Littleton JM. 1983. Observations on the cellular effects of ethanol and hyperthermia in vivo. *Radiation Research* 94: 318-325.
- Arts MJ, Schill RO, Knigge T, Eckwert H, Kammenga JE, Kohler HR. 2004. Stress protein (hsp70, hsp60) induced in isopods and nematodes by field exposure to metals in a gradient near Avonmouth, UK. *Ecotoxicology* 13: 739-755.
- Blackman RK, Meselson M. 1986. Interspecific nucleotide sequence comparisons used to identify regulatory and structural features of the *Drosophila* hsp82 gene. *Journal of Molecular Biology* 188: 499-515.
- Borkovich KA, Farrelly F, Finkelstein DB, Taulien J, Lindquist S. 1989. Hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher

temperatures. *Molecular and Cellular Biology* 9: 3919-3930.

Buchner J. 1999. Hsp90 & Co. – A holding for folding. *Trends in Biochemical Sciences* 24:136-141.

Burton V, Mitchell HK, Young P, Petersen NS. 1988. Heat shock protection against cold stress of *Drosophila melanogaster*. *Molecular and Cellular Biology* 8: 3550-3552.

Caplan AJ. 1999. Hsp90's secrets unfold: New insights from structural and functional studies. *Trends in Cell Biology* 9: 262-268.

Chen CP, Denlinger DL, Lee RE. 1987. Cold shock injury and rapid cold hardening in the flesh fly, *Sarcophaga crassipalpis*. *Physiological Zoology* 60: 297-304.

Chen B, Kang L. 2005. Insect population differentiation in response to environmental thermal stress. *Progress in Natural Science* 15: 289-296.

Christine Q, Todd AS, Susan L. 2002. Hsp90 as a capacitor of phenotypic variation. *Nature* 417: 598-599.

Dahlgaard J, Loeschcke VP, Michalak J. 1998. Induced thermotolerance and associated expression of the heat-shock protein Hsp70 in adult *Drosophila melanogaster*. *Functional Ecology* 12: 786-793.

Denlinger DL, Yocum GD. 1998. Physiology of heat sensitivity. In: Hallman GJ, Denlinger DL, editors. *Thermal Sensitivity in Insects and Application in Integrated Pest Management*. pp. 11-181. Westview.

Feder ME, Hofmann FE. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological

physiology. *Annual Review of Physiology* 61: 243-282.

Fliss AE, Benzeno S, Rao J, Caplan AJ. 2000. Control of estrogen receptor ligand binding by Hsp90. *Journal of Steroid Biochemistry and Molecular Biology* 72: 223-230.

Funes V, Alhama J, Navas JI, Lopez-Barea J, Peinado J. 2006. Ecotoxicological effects of metal pollution in two mollusc species from the Spanish South Atlantic littoral. *Environmental Pollution* 139: 214-23.

Gernhöfer M, Pawert M, Schramm M, Müller E, Triebkorn R. 2001. Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *Journal of Aquatic Ecosystem Stress and Recovery* 8: 241-260.

Gupta RS. 1995. Phylogenetic analysis of the 90 kD heat shock family of protein sequences and an examination of the relationship among animals, plants, and fungi species. *Molecular Biology and Evolution* 12: 1063-1073.

Hallare AV, Schirling M, Luckenbach T. 2005. Combined effects of temperature and cadmium on developmental parameters and biomarker responses in zebrafish (*Danio rerio*) embryos. *Journal of Thermal Biology* 30(1): 7-17.

Hartke A, Boucher S, Laplace JM, Benachoue A, Boutibonnes P, Auffray Y. 1995. UN-inducible proteins and UV-induced cross-protection against acid, H₂O₂ or heat treatments in *Lactococcus lactis* sub sp. *lactic*. *Archives of Microbiology* 163: 29-336.

He L, Zhao ZM, Cao XF, Deng XP, Wang JJ. 2005. Effect of temperature on development and fecundity of resistant *Tetranychus cinnabarinus* (Boiduval). *Acta Entomologica Sinica* 48: 203-207. (In Chinese)

- Jing XH, Kang L. 2003. Geographical variation in egg cold hardiness: A study on the adaptation strategies of the migratory locust *Locusta migratoria* L. *Ecological Entomology* 28: 151-158.
- Koban M, Yup A, Agellon L, Powers D. 1991. Molecular adaptation to environmental temperature heat shock response of the eurythermal teleost *Fundulus heteroclitus*. *Molecular Marine Biology and Biotechnology* 1: 1-17.
- Koga T, Takumi K. 1995. Comparison of cross-protection against some environmental stresses between cadmium-adapted and heat-adapted cells of *Vibrio parahaemolyticus*. *The Journal of General and Applied Microbiology* 41: 263-268.
- Köhler HR, Bartussek C, Eckwert H, Farian K, Gränzer S, Knigge T, Kunz N. 2001. The hepatic stress protein (hsp70) response to interacting abiotic parameters in fish exposed to various levels of pollution. *Journal of Aquatic Ecosystem Stress and Recovery* 8: 261-279.
- Köhler HR, Belitz B, Eckwert H, Adam R, Rahman B, Tronteli P. 1998. Validation of hsp70 stress gene expression as a marker of metal effects in *Deroceras reticulatum* (Pulmonata): Correlation with demographic parameters. *Environmental Toxicology and Chemistry* 17: 2246-2253.
- Kumar S, Tamura K, Nei M. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* 5: 150-163.
- Lee SM, Lee SB, Park CH, Choi J. 2006. Expression of heat shock protein and hemoglobin genes in *Chironomus tentans* (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. *Chemosphere* 65: 1074-1081.
- Lee YJ, Dewey WC. 1988. Thermotolerance induced by heat, sodium arsenate, or puromycin: Its inhibition and differences between 43 !C and 45 !C. *Journal of Cellular Physiology* 135: 397-406.
- Li B, Cai H, Chen Y. 2000. HSP70KD related and temperature acclimated proteins synthesis in the migratory locust, *Locusta migratoria* (L.). In: Li DM, Editor. *Chinese Entomology Towards the 21st Century*. pp. 296-302. Chinese Science and Technology Press.
- Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25(4): 402-408.
- Mayer MP, Bukau B. 1999. Molecular chaperones: The busy life of Hsp90. *Current Biology* 9: 322-325.
- Morimoto RI. 1993. Cell in stress: Transcriptional activation of heat shock genes. *Science* 259: 1409-1410.
- Parsell DA, Lindquist S. 1994. Heat shock proteins and stress tolerance. In: Morimoto RI, Tissieres A, Georgopoulous C, editors. *The Biology of Heat Shock Proteins and Molecular Chaperones*. pp. 457-494. Cold Spring Harbor Laboratory Press.
- Patil NS, Lole KS, Deobagkar DN. 1996. Adaptive larval thermotolerance and induced cross-tolerance to propoxur insecticide in mosquitoes *Anopheles stephensi* and *Aedes aegypti*. *Medical and Veterinary Entomology* 10: 277-282.

- Pearl LH, Prodromou C. 2000. Structure and in vivo function of Hsp90. *Current Opinion in Structural Biology* 10: 46-51.
- Pruski AM, Dixon DR. 2007. Heat shock protein expression pattern (HSP70) in the hydrothermal vent mussel *Bathymodiolus azoricus*. *Marine Environmental Research* 64: 209-224.
- Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H. 2000. Structure of TPR domain-peptide complexes: Critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell* 101: 199-210.
- Singer C, Zimmermann S, Sures B. 2005. Induction of heat shock proteins (hsp70) in the zebra mussel (*Dreissena polymorpha*) following exposure to platinum group metals (platinum, palladium and rhodium): Comparison with lead and cadmium exposures. *Aquatic Toxicology* 75: 65-75.
- Sonoda S, Ashfaq M, Tsumuki H. 2006. Cloning and nucleotide sequencing of three heat protein genes (Hsp90, Hsc70, and hsp19.5) from the diamondback moth, *Plutella xylostella* (L.) and their expression in relation to developmental stage and temperature. *Archives of Insect Biochemistry and Physiology* 62: 80-90.
- Soon ML, Se BL, Chul HP, Jinhee C. 2006. Expression of heat shock protein and hemoglobin genes in *Chironomus tentans* (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. *Chemosphere* 65: 1074-1081.
- Sörarensen JG, Dahlgaard J, Loeschcke V. 2001. Genetic variation in thermal tolerance among natural populations of *Drosophila buzzatii*: Down regulation of Hsp70 expression and variation in heat stress resistance traits. *Functional Ecology* 15: 289-296.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTALX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.
- Tissieres A, Mitchell HK, Tracey UM. 1974. Protein synthesis in salivary glands of *Drosophila melanogaster*, relation to chromosome puffs. *Journal of Molecular Biology* 84: 389-398.