Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014; 39 (2) ; 155–161
doi: 10.5505/tjb.2014.43265

Research Article [Araştırma Makalesi]

Yayın tarihi 30 Haziran, 2014 © TurkJBiochem.com

[Published online 30 June, 2014]

A partial genomic characterization and up-regulation of HSP90 by imidazole derivative KK-42 in the shrimp *Penaeus vannumei*

[İmidazol türevi KK-42 ile karides *Penaeus vannumei*'den HSP90'ın kısmi genomik karakterizasyonu ve upregülasyonu]*

Xia Xi-chao[1,2],
Wang Wen-feng[1,3],
Yang Hong[1],
Ning Qian-ji[1]

[1]College of Life Sciences, Henan Normal University, [2]College of Life Sciences, Nanyang Medical University, [3]College of Life Sciences, Xinxiang Medical University, Xinxiang 453002, Henan, China

Yazışma Adresi
[Correspondence Address]

Ning Qian-ji

College of Life Sciences, Henan Normal University, Xinxiang 453007, Henan, China
Tel. +86 0373 3326340.
E-mail. ningqianji1964@163.com.

***Translated by [Çeviri]** Dr. Samiye Yabanoğlu

Registered: 12 March 2013; Accepted: 4 December 2013

[Kayıt Tarihi: 12 Mart 2013; Kabul Tarihi: 4 Aralık 2013]

ABSTRACT

Aim: HSP90, one of the most important and multifunctional stress proteins in cells, plays important role in cell self-protection and environmental adaptability in crustacean. Our previous study showed that application of imidazole derivative KK-42 can increase survival rate of *Penaeus vannumei*. In an attempt to research the possible molecular mechanism of KK-42 increasing survival rate in *P. vannumei*, one HSP90 cDNA sequence from the hepatopancreas was first isolated and then its spatiotemporal expressions were analyzed.

Material and Methods: The HSP90 was cloned from the hepatopancreas of *P. vannumei* using the rapid amplification of cDNAends methods and its sequence was analyzed with biological software. Expression level was determined by real-time PCR.

Results: The full-length cDNA contains a 2163 bp open reading frame encoding a predicted protein of 721 amino acids. The *P. vannumei* HSP90 protein sequence is most closely related to other HSP90 enzymes, the higher mRNA level is detected in hepatopancreas. The hepatopancreas HSP90 expression is significantly induced by KK-42 and increases more than 69.1 %, even up to 505.5 % and 481.7 % on day 2 and day 3, respectively.

Conclusion: The up-regulation of HSP90 expression induced by KK-42 is beneficial to improve adaptability that is likely one of the molecular mechanisms of KK-42 increasing survival rate in *P. vannumei*.

Key Words: HSP90, Up-regulation, KK-42, *Penaeus vannumei*

Conflict of Interest: There is no conflict of interest in respect to this manuscript.

ÖZET

Amaç: Hücrelerdeki en önemli ve çok fonksiyonlu stres proteinlerinden biri olan HSP90, kabuklularda hücrenin kendini korumasında ve çevreye adaptasyonda önemli bir rol oynamaktadır.

Daha önceki çalışmamız, imidazole türevi KK-42 uyulamasının *Penaeus vannumei*'nin hayatta kalma oranını arttırdığını göstermiştir. KK-42'nin *P. vannumei*'nin hayatta kalma oranını arttırmasının olası moleküler mekanizmasını araştırmak amacıyla, hepatopankreastan bir HSP90 cDNA sekansı izole edilmiş ve spatiotemporal ekspresyonları analiz edilmiştir.

Gereç ve Yöntem: HSP90, cDNAend yöntemlerinin hızlı amplifikasyonunu kullanarak *P. vannumei*'nin hepatopankreasından klonlanmış ve sekansı biyolojik yazılımla analiz edilmiştir. Ekspresyon seviyesi real-time PCR ile belirlenmiştir.

Bulgular: Tam uzunluktaki cDNA,721 aminoasitten oluştuğu tahmin edilen bir protein kodlayan 2163 bç'lik bir açık çerçeve içerir. *P. vannumei* HSP90 protein sekansı digger HSP90 enzimleriyle yakından ilişkilidir; hepatopankreasta yüksek mRNA seviyesi tespit edilmiştir. Hepatopankreas HSP90 ekspresyonu KK-42 ile belirgin biçimde indüklenmiştir ve % 69.1'den fazla ve hatta 2. gün ve 3. günlerde sırasıyla % 505.5 ve % 481.7 artmıştır.

Tartışma: KK-42 ile HSP90 ekspresyonunun upregülasyonu uyumluluğu arttırmak için gereklidir ki bu *P. vannumei*'de KK-42'nin hayatta kalma oranını arttıran moleküler mekanizmalardan biridir.

Anahtar Kelimeler: HSP90, Upregülasyon, KK-42, *Penaeus vannumei*

Çıkar Çatışması: Yazarlar arasında çıkar çatışması bulunmamaktadır.

Heat shock proteins (HSPs) are ubiquitously found in all organisms, and essential for the molecular defense machinery against environmental stress, pathogen infection and oxidative stress [1, 2]. HSPs can be grouped into several families according to their molecular weights [3, 4]. HSP90 is one of the most important and multifunctional stress proteins in cells that play an important role in cell self-protection and environmental adaptability in crustacean. In *Penaeus monodon*, HSP90 is involved in immunity response against *Vibrio harveyi* [5]. Up-regulation of HSP90 expression from the crab *Portunus trituberculatus* results in increase of adaptability coped with temperature, Cu^{2+} stress and salinity [6, 7]. Thus, a better understanding of the relationship between tolerance mechanisms against environmental stress and HSP90 expression may give rise to the possibility of more efficient control of mortality [6,7]. Up to the present, only little gene information regarding HSP90s has been obtained, including the marine crab *P. trituberculatus* [8], the prawns *Metapenaeus ensis* [9] and *P. monodon* [10].

The white shrimp *Penaeus vannumei*, an important economical species, is extensively reared in many countries. Our previous study showed that application of imidazole derivative KK-42 can in crease survival rate of *P. vannumei* (0.8~1.2 cm long), but the mechanism remains unclear [11, 12]. In the present study, a full-length HSP90 cDNA was first isolated from hepatopancreas of *P. vannumei* and its expression was analyzed at different time after KK-42 treatment compared the control group with treated one using real-time PCR.

Animals and experimental design

KK-42 (purity ≥ 95%) was supplied from Department of Chemistry, Yantai University. Shrimps farming (*P. vannumei*) were carried out in a 3500 m^2 pond. When their body length reached 3.5~5.0 cm, 200 shrimps were randomly collected from the pond as control group and KK-42-treated one, respectively. The shrimps were treated as previously described [11, 12] with KK-42 at a concentration of 1.95×10^{-4} mol/L or in the solution without KK-42, and then grew in the normal way (water temperature 20~33°C, feeding four times per day). The control and KK-42-treated shrimps were respectively put in two nets (100 m^2 per net), which were commonly located in the pond mentioned above. On different day after KK-42 treatment, 9 samples were randomly collected / each time point / each group, hepatopancreas and other tissues were dissected, immediately frozen in liquid nitrogen, and then stored at -80°C until use.

Total RNA isolation and synthesis of the cDNA first strand

Total RNA was extracted from the hepatopancreas, brain, muscle and eyestalk tissues using TRIzol (Invitrogen Life Technologies, USA) according to the manufacturer's protocol. The quality of RNA was monitored by 1.2 % agarose gel electrophoresis. The contaminated genomic DNA was removed by DNase I. The first-strand cDNA was synthesized by reverse transcription in a buffer containing 1 μg of total RNA, 2 μl oligo-dT_{18} primer, 0.5 μl RNase inhibitor, 2.5 μl dNTP mix and 1 unit of RTase M-MLV reverse transcriptase (Takara, Japan). The cDNA was used as the template for PCR reaction.

Gene cloning and sequencing

The HSP90 cDNA fragment was amplified using two degenerate primers HSP90 -F1 and HSP90 -R1 (Table 1), which was designed according the conserved domain of HSP90 sequences. The PCR product was subcloned into the pMDT-19 (Takara, Japan) and sequenced from both directions (Invitrogen Life Technologies, USA).

BLASTx (NCBI) search identified the partial sequence with high homology to other HSP90. Highly stringent primers designed from the partial cDNA sequences were used to characterize the 5′ and 3′ regions of the HSP90 cDNAs by RACE-PCR (Takara, Japan) according to the manufacturer's protocol. 5′ Raceouter primer and HSP90 5-1 (Table 1) were used for the first-round PCR of 5' RACE, 3′ Raceouter primer and HSP90 3-1 (Table 1) for the first-round PCR of 3' RACE, respectively. Subsequently, the first-round PCR products were used as the template to perform the nested PCR using Raceinner primers (5'iner, 3'iner) included in the kit, and gene specific primers HSP905-2, HSP903-2 (Table 1). The 5′ RACE and 3′ RACE PCR products were cloned and five clones were sequenced using the method described above.

Oligonucleotide primers used in the experiments

Primer	Sequence (5′–3′)
HSP90-F1	CATYAACACRTTCTAWAGAYACA
HSP90-R2	TRCTYCTGCTNGCYGTRCTC
HSP903-1	CTGAACAAGACGAAGCCTG
HSP903-2	CGTGAAGCACTT CAGCGTG
HSP905-1	TACGGCGAGTTCTACAAGTCG
HSP905-2	CTTCTCCTTGTCGGCGTCTTC
HSP90-F1	CAAGAACAACGACGACGAGC
HSP90-R2	GCACGACGTCGAAGAACTAG
β-F	CATCCACGAGACCACCTACAAC
β-R	GAAATACTGCCTCGCTCCCTC

Multiple sequence alignment and phylogenetic analyses

The search for nucleotide and amino acid sequence similarities was conducted with BLAST programs at the National Center for Biotechnology Information. HSP90 deduced amino acid sequence was aligned with HSP90 from other known species using the software Meg Align

(DNASTAR, USA). A phylogenetic tree was constructed using the software MEGA 4.0 (USA) based on the amino acid sequences of HSP90 and other known HSP90.

Expression study

The spatial expressions of the HSP90 were conducted by Semi-quantitative RT-PCR and the temporal expressions of were performed with real-time quantitative PCR following the manufacture instruction for SYBR *Premix Ex Taq*™ (TaKaRa, Japan). The PCR primers HSP90-F2 and HSP90-R2 (Table 1), and the internal standard *β-actin* primers *β*-F and *β*-R (Table 1) were designed based on reported sequences of the shrimp (*P. vannumei*) (GenBank accession No. HQ008268，AY486466). The real-time PCR was performed using an ABI 7500 Real-Time Detection System (Applied Biosystems) under the following conditions: initial denaturation at 95 ℃ for 30 sec and amplifying for 40 cycles (9°C, 5 sec; 60°C, 34 sec). Based on standard curve constructed, the expression level of *CYP* was calculated by $2^{-\Delta\Delta CT}$, and the data obtained were subjected to the statistical analysis followed by an unpaired sample t-test. Significant difference was accepted at $P<0.05$.

Identification and analysis of HSP90 cDNA

A full-length cDNA of 2543 bp, encoding one of HSP90, was obtained from the hepatopancreas of the shrimp *P. vannumei*. The HSP90 cDNA had a 2163 bp open reading frame encoding a predicted protein of 721 amino acids with a predicted molecular mass of 82.8 kDa (Fig. 1). A 105 bp untranslated region was preceding the open reading frame, which was followed by a 275 bp untranslated 3′ region, containing a polyadenylation signal closely followed by a poly (A) tail. Furthermore, the HSP90 protein shared common characteristics of the HSP90 protein family: NKEIFLRELISN[S/A]SDALDKIR, LGTIA[K/R]SGT, IGQFGVGFYSA[Y/F]LVA[E/D], IKLYVRRVFI, GVVDS[E/D]DLPLN[I/V]SRE and the consensus sequence MEEVD at the C-terminus (Fig. 1). The ATP binding domain as well as the major structural and functional domains typically in HSP90 was detected in the deduced HSP90 amino acid sequences.

Phylogenetic analysis of the HSP90

A comparison of *Ps*HSP90 amino acid sequence with those of other HSP90 protein indicated that it was most close to various crustaceans HSP90, especially from shrimp (Fig. 2), and then insect HSP90, while the similarity with vertebrate HSP90 was lower.

The spatial and temporal expression of the HSP90 in P. schmitti

In order to detect HSP90 expression in four tissues (hepatopancreas, muscle, eyestalk and brain), a semi-quantitative RT-PCR experiment was used. The results showed that the higher HSP90 mRNA level was detected in the hepatopancreas, then in brain and muscle, is lower in eyestalk (Fig. 3).

In control, the HSP90 transcript in hepatopancreas showed an obvious fluctuation during experiment observed, and the expression level reduced 53.3 %, 71.5 % and 56.5 % on day 1, day 3 and day 4 compared with that of day 0, respectively (Fig. 4). In comparison to the control group, treatment of KK-42 at the dose of 1.95×10^{-4} mol/L resulted in the significant up-regulation of HSP90 expression during the whole test. The mRNA level in hepatopancreas administrated by KK-42 increase to > 69.1 %, even to 505.5 % and 481.7 % on day 2 and day 3, respectively, compared to that in control group at the same time (Fig. 4). In the KK-42 treatment group, the expression of HSP90 increased 4.34 times from day 0 to day 2, then decrease 58.7 % at end of the experiment.

HSP90 is highly conserved molecular chaperones that contribute to the folding, maintenance of structural integrity and proper regulation of a subset of cytosolic proteins [13-15]. Analysis of the deduced amino acid sequence of *P. vannumei* HSP90 shows that it shares the characteristic motifs of HSP90 family. Five highly conserved motifs have been previously regarded as the HSP90 protein family signature, and they were also found in the HSP90 (Fig. 1). In addition, the presence of sequence MEEVD on its C-terminus (Fig. 1), the HSP90 is concluded to be a cytosolic HSP90 homolog, similar to HSP90 proteins in other species [16]. In order to perfectly dissect different tissues, the shrimps with body length 3.5~5.0 cm are selected for sampling. The HSP90 transcript is detected in four tissues, but relative higher in hepatopancreas which is a major organ for the digestion, absorption and secretory of compounds in crustaceans and also sensitive to environmental changes [17].

In insect, KK-42 is mostly used as a JH antagonist to regulate growth [18-20]. But, the knowledge of its functions in crustacean is poor. We first found that KK-42 can increase shrimp (*P. vannumei*) survival rate [10, 11]. The present study further demonstrates that the HSP90 expression is obviously induced by KK-42 in hepatopancreas of the shrimp *P. vannumei* (Fig. 4), suggesting the up-regulation of HSP90 expression derived from KK-42 treatment is potentially contributes to improve adaptability that is likely one of factors for shrimp survival. It has reported that stimulation of HSP90 expression associates with evaluating adaptability against the environmental stress and protecting cells from damage [17]. It is worth our attention that the HSP90 transcript is sharply induced by KK-42 treatment occurred on day 2 (Fig. 4), postulating hepatopancreas HSP90 is likely involved in other cell components such as protein kinases in signal transduction pathways, transcriptional factors and hormone receptors, except as molecular chaperone in protein folding. The relational studies of HSP90 have been reported in different field [21-24]. Of course, many factors are involved in affecting the shrimp survival, so great efforts will be required before fully elucidating the KK-42 increase-survival mechanism on *P. vannumei*. One interesting finding is that the expression of HSP90

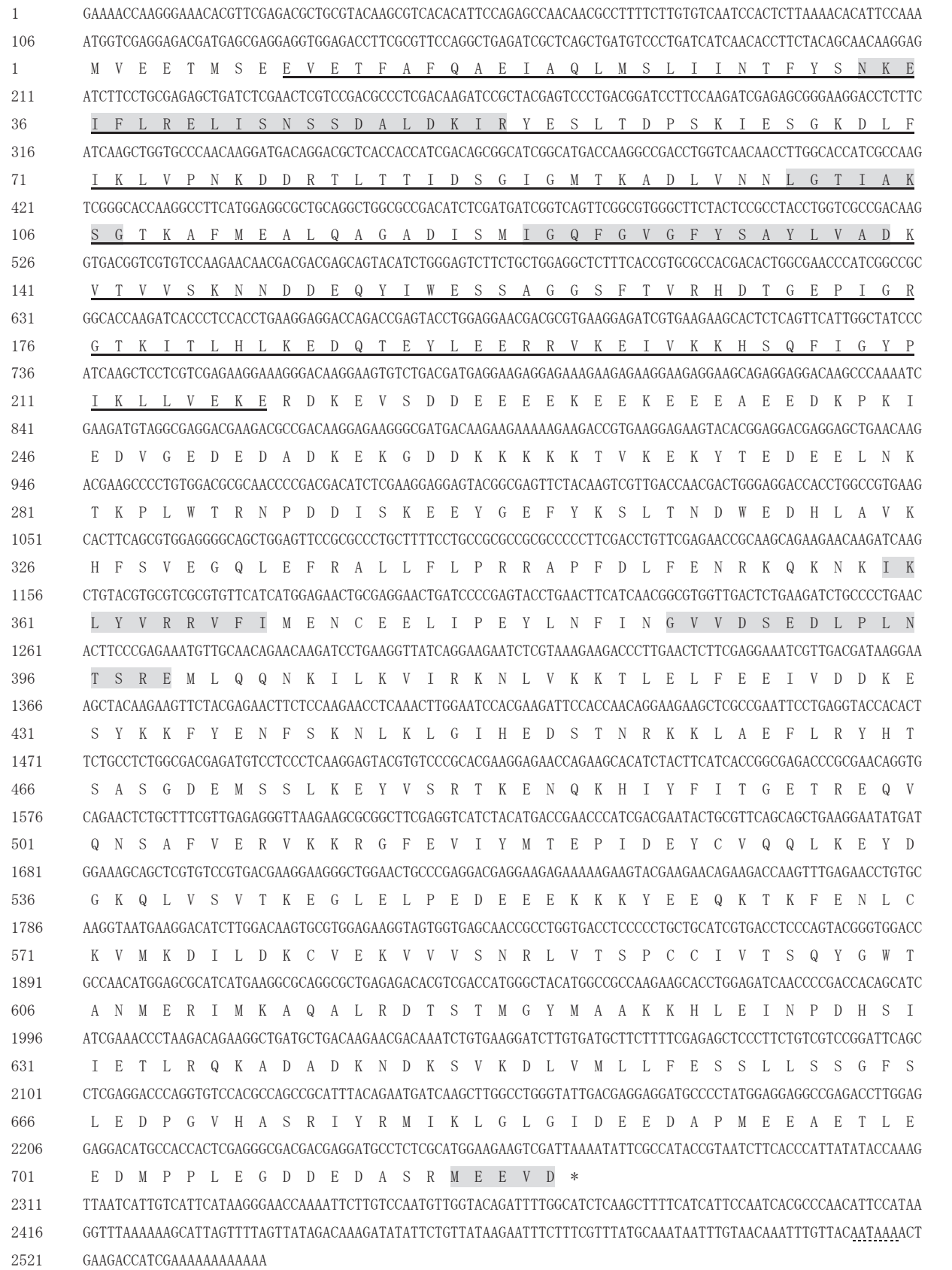

Figure 1. Nucleotide and deduced amino acid sequences of *Ps*HSP90 from *P. vannumei*. Heat shock protein 90 family signature motifs are highlighted as shaded regions. The ATPase domain of *Ps*HSP90 is underlined. The putative polyadenylation signal sequence is underlined with a dotted line. The stop codon is indicated by *.

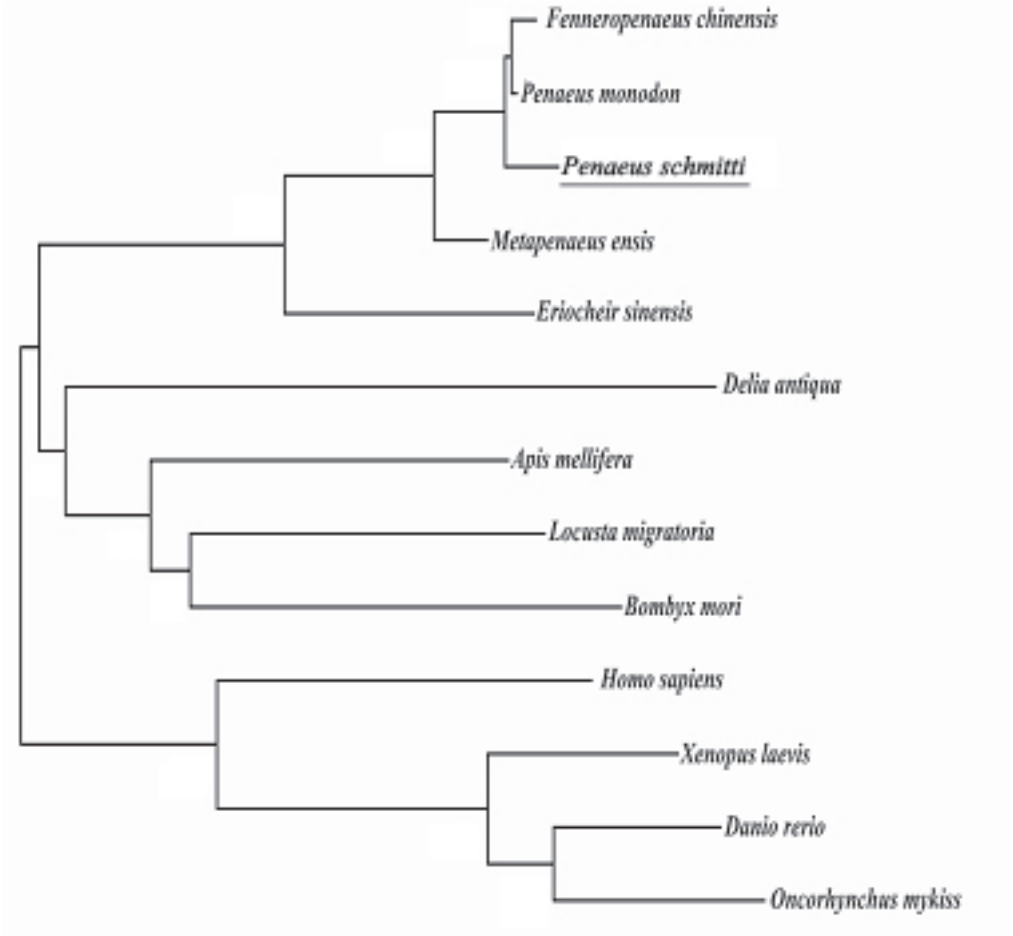

Figure 2. Phylogenetic Neighbor-Joining tree of 15 representative full-length HSP protein sequences from vertebrates, insects and crustaceans. The *P. vannumei* HSP90 sequence was underlined, bootstrap percentages from maximum parsimony (MP, 1000 replicates) analyses.

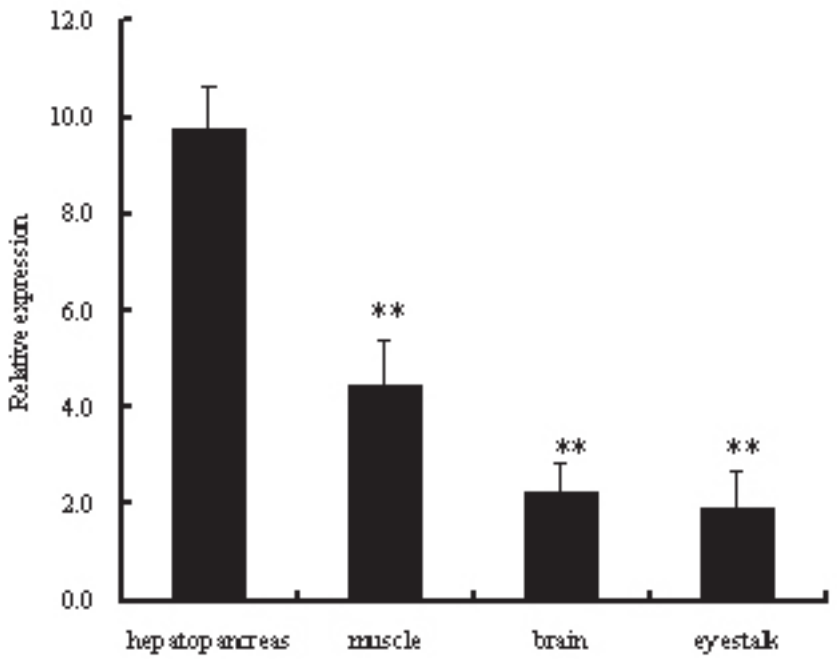

Figure 3. The analysis of HSP90 distribution in different tissues. *$P<0.05$, **$P<0.01$ vs control group at hepatopancreas result.

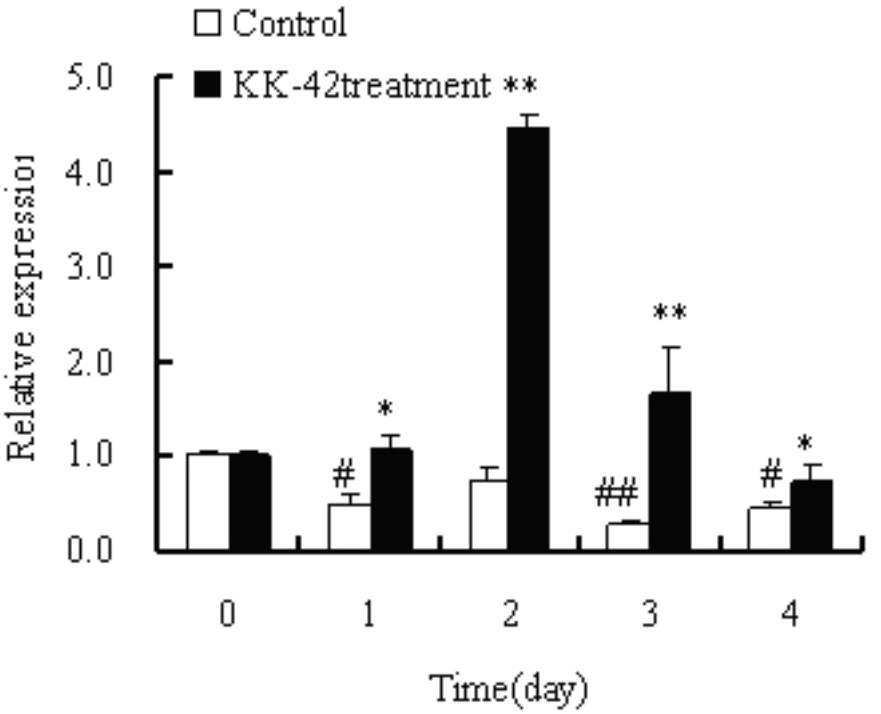

Figure 4. The up-regulation effect of KK-42 on the hepatopancreas HSP90 mRNA level in *P. vannumei*. Bars represented means ± SE; n = 9/each group/each time point. *$P<0.05$, **$P<0.01$ vs control group at the same time. #$P<0.05$, ##$P<0.01$ vs control group at day 0.

shows an obvious fluctuation in the control group from day 0 to day 4, which is likely related to the environmental conditions. The animals are cultured in the outdoor in the experiment. Temperature, dissolved oxygen in water body and food intake may impact the shrimp growth. HSP90, that might be considered a key element to adapt the environment, should be susceptible to these changes which could be helpful to enhance the animal's adaptability.

This research was funded by the National Natural Science Foundation of China (No.30940008), and Henan Province Science and Technology Innovation Talent Support Program (No.2009HASTIT022). We thank Engineer G. Jiang-tao for helping us in culture of the shrimps.

There is no conflict of interest in respect to of this manuscript.

[1] Craig EA, Weissman JS, Horwich AL. Heat shock proteins and molecular chaperones: mediators of protein conformation and turnover in the cell. Cell 1994; 78: 365–372.

[2] Ayoola O, Miodrag Belosevic. Immune evasion strategies of trypanosomes: a review. J Parasitol 2012; 98(2): 84–292.

[3] Sanders BM. Stress proteins in aquatic organisms: an environmental perspective. Critical Reviews in Toxicology 1993; 23: 49–75.

[4] Gupta RS. 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 1995; 12: 1063–1073.

[5] Wanilada R, Rungnapa L, Pikul J, Sirawut K, Nitsara K. Expression and distribution of three heat shock protein genes under heat shock stress and under exposure to *Vibrio harveyi* in *Penaeus monodon*. Developmental and Comparative Immunology 2010; 1447: 1–8.

[6] Zhang XY, Zhang MZ, Zheng CJ, Liu J, Hu HJ. Identification of two hsp90 genes from the marine crab, *Portunus trituberculatus* and their specific expression profiles under different environmental conditions. Comparative Biochemistry and Physiology C 2009; 150: 465–473.

[7] Li YH, Deng W, Yang KL, Wang WM. The expression of prophenoloxidase mRNA in red swamp cray fish, *Procambarus clarkii*, when it was challenged. Genomics 2012; 99: 355–360.

[8] Li P, J Zha, Zhang ZH, Huang H, Sun HY, Song DX, Zhou KY. Molecular cloning, mRNA expression, and characterization of HSP90 gene from Chinese mitten crab *Eriocheir japonica sinensis*. Comparative Biochemistry and Physiology B 2009; 153: 229–235.

[9] Wu LT, Chu KH. Characterization of heat shock protein 90 in the shrimp *Metapenaeus ensis*: evidence for its role in the regulation of vitellogenin synthesis. Molecular Reproduction and Development 2008; 75: 952–959.

[10] Jiang SG, Qiu LH, Zhou FL, Huang JH, Guo YH, Yang K. Molecular cloning and expression analysis of a heat shock protein (Hsp90) gene from black tiger shrimp (*Penaeus monodon*). Molecular Biology Reports 2009; 36: 127–134.

[11] Ning QJ, Fu SG, Xu XJ, He JT. A new and practical application of JH antagonist KK-42 to promoting-growth of shrimp *Penaeus schmitti*. Aquaculture 2007; 270: 422–426.

[12] Wang WF, Yang H, Liu F, Chen XL, Lv YJ. Qian-Ji NingA novel effec t of imidazole de rivative KK-42 on increasing survival of *Aeromonas hydrophila* challenged prawn *Macrobrachium nipponense* Fish & Shellfi sh Immunology 2013; 34:167–17 2.

[13] Picard D. Heat-shock protein 90, a chaperone for folding

and regulation. Cellular and Molecular Life Sciences 2002; 59: 1640–1648.

[14] Queitsch C, TA Sangster, Lindquist S. HSP90 as a capacitor of phenotypic variation. Nature 2002; 417: 618–624.

[15] Chen B, Zhong D, Monteiro A. Comparative genomics and evolution of the HSP90 family of genes a cross all kingdoms of organisms. BMC Genomics 2006; 7: 156–174.

[16] Gao Q, Song LS, Ni DJ, Wu LT, Zhang H. Chang YQ. cDNA cloning and mRNA expression of heat shock protein 90 gene in the haemocytes of Zhikong scallop *Chlamys farreri*. Comparative Biochemistry and Physiology B 2007; 147: 704–715.

[17] Bhavan PS, Geraldine P. Biochemical stress responses in tissues of the prawn *Macrobrachium malcolmsonii* on exposure to endosulfan. Pesticide Biochemistry and Physiology. 2001; 70: 27–41.

[18] Asano S. Anti-juvenile hormone activity of imidazole Compound (KK-42) and its diminution by metroprene in the 4th instar silkworm *Bomby xmori*. Applied Entomology and Zoology 198; 621: 63–69.

[19] Hirai M, Kamimura M, Kikuchi K, Yasukochi Y, Kiuchi M, Shinoda T, Shiotsuki T. cDNA cloning and characterization of *Bombyx mori* juvenile hormone esterase: an inducible gene by the imidazole insect growth regulator KK-42. Insect Biochemistry and Molecular Biology 2002; 32: 627–635.

[20] Wu JM, Wu ZD, Xu JL, Cao MX. The effect of trimolter inducer KK-42 on the endocrine system in the silkworm *Bombyx mori*. Acta Entomologica Sinica 1991; 34: 278–283.

[21] Sreedhar AS, Kalmár E, Csermely P, Shen YF. Hsp90 isoforms: functions, expression and clinical importance. FEBS Letter 2004 562: 11–15.

[22] Du HH, Hou CL, Wu XG, Xie RH, Wang YZ. Antigenic and immunogenic propert ies of truncated VP28 protein of white spot syndrome virus in *Procambaru s clarkia*. Fish & Shellfish Immunology 2013; 34: 332–338.

[23] Xu W, Mimnaugh EG, Kim JS, Trepel JB, Neckers LM. Hsp90, not Grp94, regulates the intracellular trafficking and stability of nascent ErbB2. Cell Stress Chap 2002; 7: 91–96.

[24] Dong CH, Zhang P. A putative G protein-coupled receptor involved in innate immune defense of *Procambarus clarkii* against bacterial infection. Comparative Biochemistry and Physiology Part A 2012; 161: 95 –101.