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The response profiles of HSPA12A and TCTP from *Mytilus galloprovincialis* to pathogen and cadmium challenge

Liping You [a,c], Xuanxuan Ning [b], Feng Liu [d], Jianmin Zhao [a,*], Qing Wang [a], Huifeng Wu [a,**]

[a] Key Laboratory of Coastal Zone Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, PR China
[b] Yantai Oceanic Environmental Monitoring Central Station, State Oceanic Administration, Yantai 264006, PR China
[c] University of Chinese Academy of Sciences, Beijing 100049, PR China
[d] China Agriculture University (Yantai), Yantai 264670, PR China

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ABSTRACT

Heat shock 70 kDa protein 12A (HSPA12A) is an atypical member of HSP70 family, and the translationally controlled tumor protein (TCTP) is a novel HSP with chaperone-like activity. They are both involved in protecting organisms against various stressors. In the present study, the cDNAs of HSPA12A and TCTP (called MgHSPA12A and MgTCTP) were identified from *Mytilus galloprovincialis* by RACE approaches. The full-length cDNA of MgHSPA12A and MgTCTP encoded a peptide of 491 and 171 amino acids, respectively. Real-time PCR was employed to analyze the tissue distribution and temporal expression of these two genes after bacterial challenge and cadmium (Cd) exposure. It was found that the transcripts of MgHSPA12A and MgTCTP were dominantly expressed in gonad and muscle, respectively. The expression level of MgTCTP at 48 h post *Vibrio anguillarum* challenge was detected to be significantly up-regulated in hepatopancreas ($P < 0.05$). As concerned to Cd exposure, 2.0-fold increase of MgHSPA12A expression compared to that of the control was observed at 48 h in 5 μg/L Cd^{2+}-treated group, while the expression levels of MgTCTP were significantly decreased after exposed to both 5 and 50 μg/L Cd^{2+} for 24 h and 96 h. These results suggested the potential involvement of MgHSPA12A and MgTCTP in the mediation of the immune responses and environmental stress in mussels.

1. Introduction

Heat shock proteins (HSPs) are a family of proteins involved in response to environmental, physical and chemical stresses [1]. According to their molecular masses and functions, HSPs were divided into several families, including HSP100, HSP90, HSP70, HSP60, and the small HSPs (sHSPs) [2]. Heat shock protein 70 (HSP70) and the translationally controlled tumor protein (TCTP) are both highly conserved protein widely existed in all eukaryotic organisms [3,4]. Heat shock 70 kDa protein 12A (HSPA12A) is a novel and atypical member of HSP70 family possibly with highly specialized function that might go beyond the HSP chaperone function [5,6]. The solution structure of TCTP from fission yeast indicated its similarity to a family of small chaperone proteins [3,7], and a more recent report confirmed that TCTP is a novel HSP with chaperone-like activity [8]. As members of HSPs family, HSPA12A and TCTP was suggested to possess cytoprotective functions in response to various stressors, especially virus or bacterial pathogen, toxic metals and heat shock [9–12]. Recently, the role of HSPs in the stresses defense system has been well documented in mollusks, such as stimulated-expression of HSP70 in *Mytilus galloprovincialis* by heat shock and *V. anguillarum* stimuli, the up-regulation of TCTP transcript in *Venerupis philippinarum* against *V. anguillarum* challenge, the over-expression of HSP70 in *Ostrea edulis* in response to heat and Cd exposures, etc. [11,13–16].

As ubiquitous, sedentary filter feeders broadly inhabiting in the coastal and estuarine areas, bivalve mollusks are easily infected by various pathogens naturally present in their habitat. For example, *Vibrio* and other pathogenic bacteria were frequently found in *M. galloprovincialis* [17,18]. Among those pathogens, *Vibrio* species have been demonstrated to be an important cause of diseases in shellfish [19], which could induce cytotoxicity and the production of reactive oxygen (ROS), etc. [20]. Furthermore, bivalve mollusks are also readily exposed to metallic xenobiotics [21,22]. As an important toxicant xenobiotic, Cd posed great threat to the marine organisms by generating oxidative stresses after penetrating into cells through calcium channels [15].

* *Corresponding author.* Tel.: +86 535 2109170; fax: +86 535 2109000.
** *Corresponding author.* Tel.: +86 535 2109190; fax: +86 535 2109000.
E-mail addresses: jmzhao@yic.ac.cn (J. Zhao), hfwu@yic.ac.cn (H. Wu).

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M. galloprovincialis is an important seafood product and a sentinel species for many countries [23]. In the present study, we aimed to clone the full-length cDNA of HSPA12A and TCTP from *M. galloprovincialis*, and also investigate the temporal expression of these two genes responded to *Vibrio anguillarum* stimulation and Cd exposure, for better understanding of the defense mechanisms of mussels responded to pathogen and toxic metal stresses.

2. Materials and methods

2.1. M. galloprovincialis and tissues collection

M. galloprovincialis (shell length 6.5–7.5 cm) were collected from a local aquafarm, and maintained at 23 °C to acclimate for 10 days before commencement of the experiment. The seawater was totally changed every day, and the mussels were fed with unicellular algal species *Chrysophyta* and *Tetraselmis chui* daily. The tissues, including hemocytes, hepatopancreas, muscle, gonad, gills, and mantle, were dissected from six individuals as parallel samples to investigate the tissue-specific expression of MgHSPA12A and MgTCTP transcripts. These tissues were subjected to total RNA extraction by using TRIzol reagent (Invitrogen) immediately.

2.2. Bacterial challenge and toxic metal exposure experiments

A total of 300 mussels were employed in *V. anguillarum* chanllenge and Cd exposure experiments, and they were classified into four groups. Mussels of the control group were cultured in the filtered seawater. For the bacterial challenge, mussels were intramuscularly injected with 10^6 CFU live *V. anguillarum* according to the method described by Cellura et al. It has been demonstrated that mechanical operations, such as handling, filing and needle insertion, exerted little effects on the variations of HSPs gene expressions [13]. During the toxic metal exposure, mussels in the other two groups were treated with environmentally relevant concentrations of Cd (prepared from $CdCl_2$) at 5 and 50 μg/L, respectively. The concentrations of Cd have been previously reported in some heavily polluted sites of the Bohai Sea [24]. The $CdCl_2$ solutions were added to the seawater when the seawater was refilled every day. After bacterial challenge and Cd exposure, the hepatopancreas of six individuals were dissected from the control (at 0 h, as the naive specimens) and treated groups at each time intervals (24 h, 48 h and 96 h), respectively, and subjected to total RNA extraction immediately.

2.3. Cloning the full-length cDNA of MgHSPA12A and MgTCTP

Partial cDNA sequences of MgHSPA12A and MgTCTP were obtained from the subtractive cDNA library constructed from the digestive gland of *M. galloprovincialis*. Nested PCR was employed to clone the full-length cDNAs of MgHSPA12A and MgTCTP. And the forward and reverse primers were listed in Table 1. The PCR amplification and sequencing were performed according to described previously [25].

2.4. Sequence analysis

The cDNA and deduced amino sequences were analyzed by using the BLAST algorithm at National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast). Multiple alignments were performed using the ClustalW Multiple Alignment Program (http://www.ebi.ac.uk/clustalw/), and the signal peptide was predicted by using the SignalP 3.0 software. Two phylogenetic trees were constructed according to the amino acid sequences of HSPA12A and TCTP by using neighbor-joining method embedded in Mega4.1 program. To test the relative support for the phylogeny analysis, bootstrap trials were replicated 1000 times.

Table 1
PCR primers used in this study.

Primers	Sequence (5′–3′)	Primer information
P1 (forward)	TTCTTTGTGATTACCGTTCC	MgHSPA12A specific primer
P2 (forward)	ATGATGTTAGCCTATGAGCC	MgHSPA12A specific primer
P3 (reverse)	CGGCTCATAGGCTAACATC	MgHSPA12A specific primer
P4 (reverse)	AGGAACGGTAATCACAAAG	MgHSPA12A specific primer
P5 (forward)	ATGGATTACAGGGAAGACGG	MgTCTP specific primer
P6 (forward)	AACTGGTGCTATGGATGCTG	MgTCTP specific primer
P7 (reverse)	CATCCATAGCACCAGTTCG	MgTCTP specific primer
P8 (reverse)	GTGTTACTCCGTCTTCCCT	MgTCTP specific primer
P9 (forward)	GTTAGCCTATGAGCCAGAAGCG	MgHSPA12A specific primer for RT-PCR
P10 (reverse)	CAGCCGTACCACCACCAAGAT	MgHSPA12A specific primer for RT-PCR
P11 (forward)	CATAATGCAATCACTACGCTCTG	MgTCTP specific primer for RT-PCR
P12 (reverse)	ATCGTCGTCGTAACAATGTCAAG	MgTCTP specific primer for RT-PCR
Actin-F (forward)	GCTATCCAGGCCGTACTCT	β-actin primer
Actin-R (reverse)	GCGGTGGTTGTGAATGAG	β-actin primer

2.5. Quantification analysis of mRNA expression

The temporal expression of MgHSPA12A and MgTCTP to *V. anguillarum* infection and Cd exposure was measured in Applied Biosystem 7500 fast Real-time PCR System. Two pairs of gene-specific primers (Table 1), were designed to amplify PCR products of 125 bp and 164 bp respectively. The products were sequenced to verify the PCR specificity. The housekeeping gene β-actin (primers information listed in Table 1) was used as the internal control to verify the successful transcription and to calibrate the cDNA template for corresponding mussel samples [26,27]. In a 96-well plate, each sample was run in triplicate along with the internal control. The hepatopancreas RNA extraction, cDNA synthesis, reaction component, thermal profile and the data analysis were conducted as previously described [28]. Dissociation curve analysis of amplification products was performed at the end of each PCR reaction to confirm that only one PCR product was amplified and detected. The $2^{-\triangle\triangle CT}$ method was used to analysis the relative expression level of the two genes [29].

2.6. Statistical analysis

One-way analysis of variance (one-way ANOVA) was performed on all data using SPSS 16.0 statistical software, and p values less than 0.05 were considered statistically significant. All data were given as means ± SE ($n = 6$).

3. Results

3.1. cDNA cloning and sequence analysis of MgHSPA12A and MgTCTP

Two nucleotide sequences of 2039 and 950 bp representing the complete cDNA sequence of MgHSPA12A and MgTCTP were

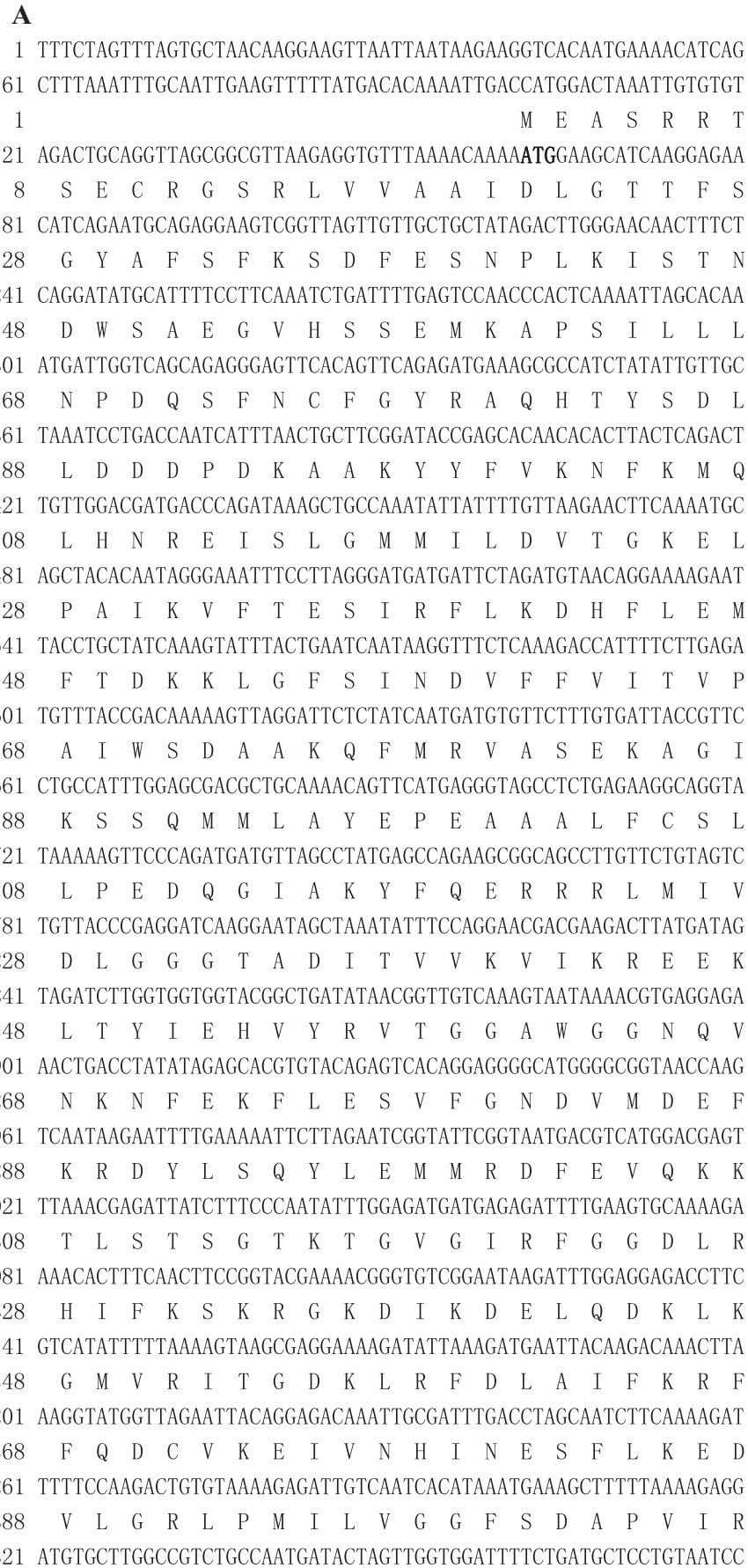

Fig. 1. The cDNA and deduced amino acid sequences of MgHSPA12A (A) and MgTCTP (B). The nucleotide and amino acid were numbered along the left margin. The start and stop codon were in bold.

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 408   E  A  I  V  D  A  F  P  V  I  K  D  V  V  S  P  V  A  T  S
1381 GGGAAGCAATTGTCGATGCTTTTCCCGTTATAAAAGATGTTGTGTCTCCCGTGGCGACTT
 428   L  A  V  L  K  G  S  V  I  F  G  H  E  P  A  I  V  T  G  R
1441 CATTGGCCGTTTTAAAAGGATCAGTTATTTTTGGTCACGAACCAGCAATAGTTACTGGCC
 448   V  C  R  E  T  L  G  L  T  L  R  R  P  Y  V  P  E  G  Q  T
1501 GAGTCTGTCGCGAAACGCTAGGCCTCACACTCCGTCGGCCATATGTGCCGGAAGGTCAAA
 468   E  K  K  T  F  K  I  D  G  Q  L  R  A  D  K  S  F  Q  K  M
1561 CTGAAAAGAAAACGTTTAAAATTGATGGACAACTTCGAGCAGATAAAAGTTTTCAAAAAA
 488   F  S  N  K  *
1621 TGTTTTCCAATAAATGAATCATTAAACTTGGACAAACCCGGTACAGTGCCAATACAGAT
1681 ATTTCATACAAAAAAAACGTTACGAAAACATCCGAAGATTATCGAGGTGTACGCTTCACA
1741 AGACGAGATTCCTGATAATATCACGGCCGCTGGTTTTTCCCTTAGAGGAAAAATCCCTGT
1801 TATTCCCCCAAGGGGAAAAGGGCCGGAAAAGGGGGAGGGTTTTTTTGTTTGGGAAACGGG
1861 GGGAACGGAAATTTTGGTCGGGTCCGGAGCCAACCATCCCGCCCAGATTATGAAGGAGGG
1921 AAAAGTTTTTATGGGATTAATTTTTTTGGAAAAAAGTTTTTTTTCCAAAAATTTGAAAAT
1981 TAAAAAGTTTTTTTTTGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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B

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   1 TTCTGACAGCCGGTGTCGCAACACTTCTGTTTATAACAGAAAATAATCTACCAACATCAG
   1      M  I  I  Y  R  C  K  I  S  G  D  E  L  F  S  D  A  F  E
  61 CCAAAATGATTATTTACAGGTGTAAAATATCAGGAGATGAATTGTTCAGTGATGCTTTTG
  20   P  M  I  V  K  E  D  F  F  Y  E  I  E  G  K  N  I  S  V  S
 121 AACCAATGATTGTGAAAGAAGACTTCTTCTATGAAATTGAGGGAAAGAATATTTCTGTCA
  40   N  K  I  D  E  S  A  I  G  A  N  A  S  A  E  E  A  A  E  G
 181 GCAATAAAATTGATGAATCTGCAATTGGAGCCAACGCTTCAGCAGAGGAGGCAGCAGAAG
  60   Q  E  D  L  V  E  T  K  I  N  V  I  Y  S  H  K  L  Q  E  T
 241 GCCAAGAAGACTTAGTAGAAACCAAAATTAATGTAATTTACAGTCACAAACTTCAAGAAA
  80   S  F  D  K  K  G  F  Q  T  F  I  K  E  Y  I  K  V  L  L  A
 301 CCAGTTTTGACAAGAAAGGTTTCCAGACATTTATCAAAGAATATATTAAAGTATTATTAG
 100   K  I  E  EE  K  G  K  E  A  A  D  E  F  K  K  R  A  A  T
 361 CTAAAATTGAAGAAGAAAAAGGCAAAGAAGCTGCAGATGAATTCAAAAAGCGAGCAGCCA
 120   G  V  K  K  V  L  E  N  F  K  N  W  Q  F  F  Q  G  E  N  M
 421 CAGGTGTAAAAAAAGTTTTAGAAAACTTCAAAAATTGGCAATTTTTCCAAGGAGAAAACA
 140   A  D  G  G  M  I  V  L  M  D  Y  R  E  D  G  V  T  P  Y  F
 481 TGGCTGATGGAGGCATGATAGTATTAATGGATTACAGGGAAGACGGAGTAACACCTTATT
 160   W  F  V  K  D  G  I  I  A  E  K  Y  *
 541 TCTGGTTTGTTAAAGATGGAATTATTGCAGAAAAATATTGAACCTCAAAACTAAGAACTG
 601 TAGTTGATATCGGCGAACTGGTGCTATGGATGCTGAACATCTCTCCTGACAATGATAGAA
 661 CAGCAGCAAATAGCACGTCTTATTTAACATTTTCATTGTGAAAATCTATACATCCTATAT
 721 CATAATGCAATCACTACGCTCTGCTTCAAAGAACCGTCATATTTAAAATTTTTAACCCAA
 781 CTATACTATGTATAATTATGGGAACTGTGTATAATTGTGGCATTGATTACAGCTGGTTTA
 841 GACCGGGCATTCTAAATATTTCTTGACATTGTTACGACGACGATGATTACATTACCTATT
 901 CTATGTCAAATAAAACATGCTATTTATTATAAAAAAAAAAAAAAAAAAA
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Fig. 1. (*continued*)

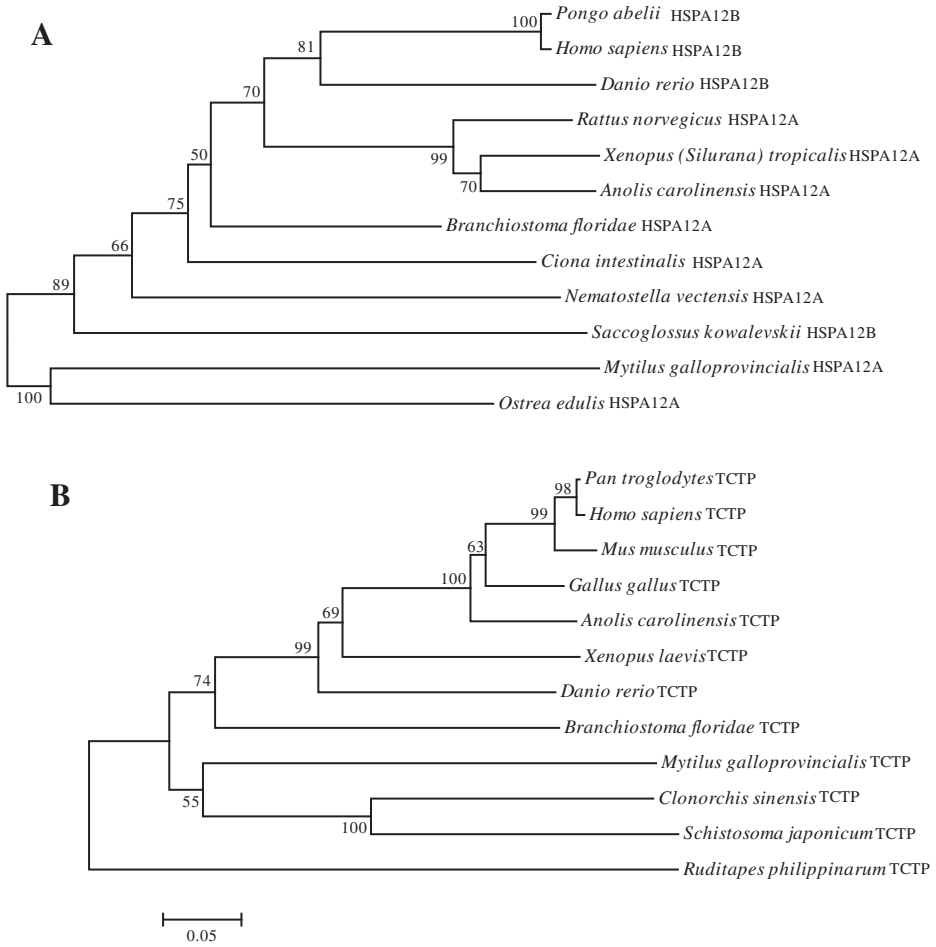

Fig. 2. Two phylogenetic trees constructed by the neighbor-joining method. (A) is for HSPA12A, and (B) is for TCTP. Bootstrap support values for the NJ tree are shown at the nodes (out of 1000 replicates). Other sequences used for phylogenetic analysis were retrieved from GenBank database as follows: *Pongo abelii* HSPA12B (XP_002830126.1), *Homo sapiens* HSPA12B (AAH90857.1), *Danio rerio* HSPA12B (NP_001036151.1), *Anolis carolinensis* HSPA12A (XP_003223545.1), *Rattus norvegicus* HSPA12A (NP_001100915.1), *Xenopus (Silurana) tropicalis* HSPA12A (XP_002941887.1), *Branchiostoma floridae* HSPA12A (XP_002601136.1), *Ciona intestinalis* HSPA12A (XP_002120765.1), *Nematostella vectensis* HSPA12A (XP_001628936.1), *Saccoglossus kowalevskii* HSPA12B (XP_002740338.1), *Ostrea edulis* HSPA12A (AFA34364.1), *Mytilus galloprovincialis* HSP90 (AM236589.2), and *Portunus trituberculatus* HSP60 (JN628037.1) for MgHSPA12A, *Pan troglodytes* TCTP (XP_001138880.1), *Homo sapiens* TCTP (AAQ01550.1), *Mus musculus* TCTP (NP_033455.1), *Gallus gallus* TCTP (NP_990729.1), *Anolis carolinensis* TCTP (XP_003226667.1), *Xenopus laevis* TCTP (NP_001080147.1), *Danio rerio* TCTP (NP_937783.1), *Branchiostoma floridae* TCTP (XP_002592847.1), *Clonorchis sinensis* TCTP (AAX84199.1), *Schistosoma japonicum* TCTP (CAX71619.1), *Ruditapes philippinarum* TCTP (ACU83235.1), *Mytilus galloprovincialis* sHSP22 (JF803804) and *Mytilus galloprovincialis* sHSP24.1 (JF803805) for MgTCTP.

obtained by RACE approach, respectively. The deduced amino acid sequences of MgHSPA12A (GenBank accession no. JN232381) and MgTCTP (GenBank accession no. JN232382) were shown with the corresponding nucleotide acid sequence in Fig. 1. The open reading frames of MgHSPA12A and MgTCTP were of 1473 and 516 bp, encoding 491 and 171 amino acids, respectively. No signal peptide was identified in both MgHSPA12A and MgTCTP by SignalP analysis, indicating that the deduced proteins should be cytosolic HSPs.

3.2. Multiple sequences alignment and phylogenetic analysis

Multiple sequence alignment of MgHSPA12A with counterparts from other organisms revealed the typical HSP70 family signatures [IV]-D-L-G-T-[ST]-*x*-[SC] and [LIVMF]-[LIVMFY]-[DN]-[LIVMFS]-G-[GSH]-[GS]-[AST]-*x*(3)-[ST]-[LIVM]-[LIVMFC] in the deduced amino acid sequence of MgHSPA12A at 20aa–27aa (IDLGTTFS) and 226aa–239aa (IVDLGGGTADITVV) (Fig. S1A), respectively. Blast analysis revealed that MgTCTP shared high identities with other registered TCTPs as well (Fig. S1B). Two potential protein kinase C phosphorylation sites [ST]-x-[RK], corresponding to the amino acid positions at 39aa–41aa (SNK) and 72aa–74aa (SHK), and one casein kinase phosphorylation site at 9aa–12aa (SGDE) were identified in MgTCTP through Prosite scan. Additionally, GANASA (48aa–53aa) in MgTCTP was predicted to be an N-myristoylation site, which was consensus to its typical sequence G-{EDRKHPFYW}-x(2)-[STAGC N]-{P}. One of the characteristic domains of the TCTP family [IFAE]-[GA]-[GAS]-N-[PAK]-S-[GTA]-E-[GDEV]- [PAGEQV]-[DEQGAV] of TC TP-1 was also found at 47aa–57aa (IGANASAEEAA).

Based on the protein sequences aligned in the multiple sequences, two phylogenetic trees were constructed by the neighbor-joining method, respectively. As revealed in Fig. 2A, MgHSPA12A formed the invertebrate group with HSPA12A counterparts from *O. edulis*, *Saccoglossus kowalevskii*, *Nematostella vectensis*, and *Ciona intestinalis*, and especially shared a common branch with *O. edulis*. Concerning to MgTCTP (Fig. 2B), it was firstly clustered into the invertebrate group with TCTP from *Ruditapes philippinarum*, *Clonorchis sinensis*, and *Schistosoma japonicum*, etc, and then formed a sister group to the vertebrate cluster. The relationships displayed in the phylogenic trees were in good agreement with traditional taxonomy.

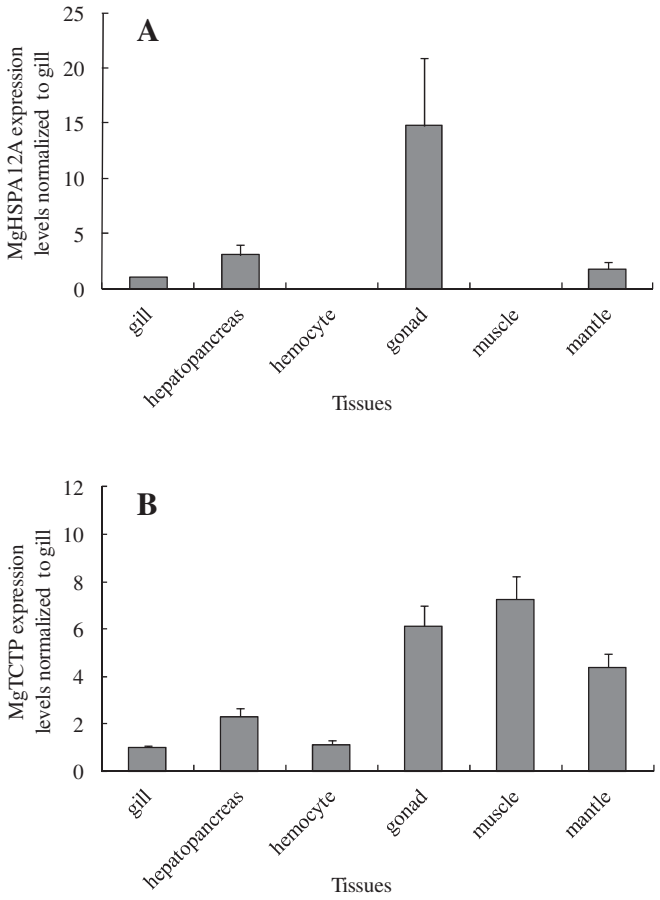

Fig. 3. Tissue distribution of the MgHSPA12A (A) and MgTCTP (B) transcript measured by SYBR Green RT-PCR. The transcript levels in hemocytes, hepatopancreas, gonad, muscle, and mantle were normalized to that in gills. Each symbol and vertical bars represented the means ± SE ($n = 6$).

3.3. Tissues distribution of MgHSPA12A and MgTCTP

The MgHSPA12A transcript was mainly detected in the tissues of gonad, hepatopancreas, mantle, and gills (Fig. 3A), while no obvious expression was observed in hemocytes and muscle. As concerned to MgTCTP, the transcript was detected in all the examined tissues, including hepatopancreas, hemocytes, gills, gonad, muscle, and mantle. It was dominantly expressed in the tissue of muscle, and moderately expressed in gonad and mantle (Fig. 3B).

3.4. The temporal profiles of MgHSPA12A and MgTCTP mRNA after V. anguillarum challenge and Cd exposure

The expression profiles of MgHSPA12A and MgTCTP genes in hepatopancreas post *V. anguillarum* infection was shown in Fig. 4. Significant increase of MgTCTP expression level (1.34-fold compared with the control, $p < 0.05$) was detected at 48 h post infection and no significant difference was observed between the control group and challenged groups for MgHSPA12A during the challenge period.

The temporal expression of MgHSPA12A and MgTCTP transcripts in hepatopancreas after cadmium exposure was shown in Fig. 5. Significant increase of MgHSPA12A expression (2.0-fold compared to the control, $p < 0.01$) was observed at 48 h in 5 μg/L Cd-treated group. However, no significant difference of MgHSPA12A expression was observed at other time points compared with the blank group in both 5 and 50 μg/L Cd exposed groups ($p < 0.05$). Concerning to MgTCTP transcript, the expression level decreased after exposed for 24 h (0.6-fold, $p < 0.05$) and 96 h (0.5-fold, $p < 0.05$) compared with that of control group at both Cd concentrations.

4. Discussion

In this study, the cDNAs encoding MgHSPA12A and MgTCTP were identified from *M. galloprovincialis*, and these two proteins were confirmed by the sequence alignments, and phylogenetic analysis. As two of three HSP70 family signatures, IDLGTTFS (20aa–27aa) and IVDLGGGTADITVV (226aa–239aa), were found in the deduced MgHSPA12A amino acid sequences, it strengthened the possibility of a functional specialization of HSPA*12A* within the group of HSP70 proteins [16]. This notion has been previously proposed by Han et al., who have reported the uniqueness of C-terminal substrate binding domain in HSPA*12A* of mice [5]. For MgTCTP, existence of the characteristic motif IGANASAEEAA (47aa–57aa) identified the homology of MgTCTP in the TCTP family [7]. Moreover, the typical sequence of an N-myristoylation site, GANASA (48aa–53aa), in MgTCTP sequence suggested the potential anti-apoptotic activity [30].

The ubiquities of HSPA12A and TCTP transcripts in different tissues have been reported in some other organisms, like mammals of human and mice for HSPA12A and mollusk of *Venerupis*

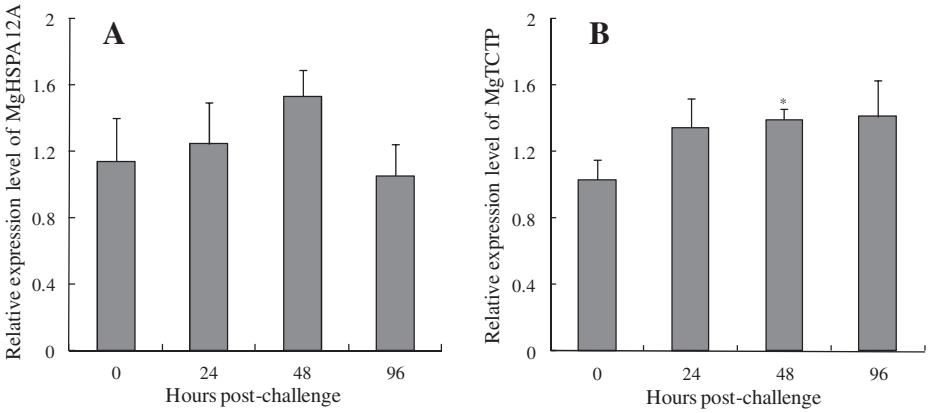

Fig. 4. The expression level of MgHSPA12A (A) and MgTCTP (B) mRNA in hepatopancreas of the mussels exposed to Cd at different concentrations (5 μg/L and 50 μg/L). The values were shown as means ± SE ($n = 6$). Significant difference between challenged and control group was indicated by an asterisk ($p < 0.05$) and extremely significant difference by double asterisk ($p < 0.01$).

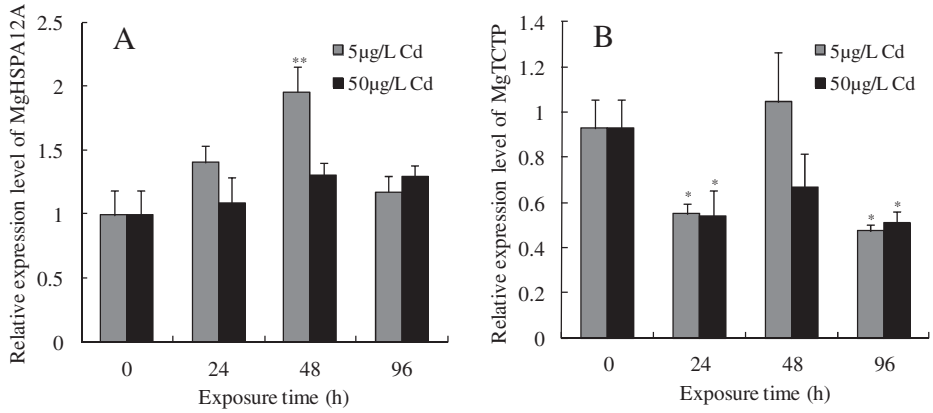

Fig. 5. The expression level of MgHSPA12A (A) and MgTCTP (B) mRNA in hepatopancreas of the mussels post *V. anguillarum* infection. Each symbol and vertical bars represent the means ± SE ($n = 6$). Significant difference between challenged and control group was indicated by an asterisk ($p < 0.05$) and extremely significant difference by double asterisk ($p < 0.01$).

philippinarum for TCTP [5,6,11]. In the present study, the expression of MgTCTP mRNA was dominantly detected in muscle, which was different from previous report on *Venerupis philippinarum* TCTP [11]. It was suggested that MgTCTP perhaps played an important role in muscle maintenance of *M. galloprovincialis*, as this tissue was frequently affected by heat, oxidative and mechanical stress [31]. Additionally, the high expression level of MgHSPA12A and MgTCTP in gonad perhaps indicated an essential role in reproductive processes, which was in agreement with Kappé et al., who found that some sHSPs were specially expressed in human testis [32].

In the aquatic environment, mollusks are frequently faced with pathophysiological and environmental stressors that can adversely affect their internal regulatory systems. It has been demonstrated that mollusk hepatopancreas represented a significant target for bacterial stimuli and toxic metal exposure [33–35]. HSP70 and TCTP, as members of HSPs family, were both implicated in the protection of cells against various abiotic stress and in the innate immune responses against bacterial challenge [3,36]. In the present study, no significant difference in the expression of MgHSPA12A was detected in hepatopancreas of mussels post *V. anguillarum* injection. This result was contrast with Cellura et al., who reported that HSP70 gene expression in *M. galloprovincialis* hemocytes was triggered by *V. anguillarum* challenge [13]. The difference perhaps further supported the notion that HSPA12 belongs to the HSP70 family but is distinct from the HSP70 family [5]. For MgTCTP, stimulated-expression in hepatopancreas was observed at 48 h post bacterial challenge. Similar phenomena were also observed in TCTP of *R. philippinarum* and sHSP22 in scallop challenged by *V. anguillarum* [11,36]. It was speculated that *V. anguillarum* was perhaps rapidly eliminated by the hemocytes [13], thus the amount of bacteria reached the hepatopancreas through the mussel circulatory system was not enough to produce significant effects. Moreover, the result might indicate that MgTCTP was more sensitive to *V. anguillarum* challenge than MgHSPA12A in hepatopancreas of *M. galloprovincialis*.

Concerning to Cd exposures, different stress responses were detected between these two genes, which indicated that they were involved in the detoxified system of mussels in different manners. The up-regulation of MgHSPA12A transcript at 48 h in 5 μg/L Cd-treated group was perhaps related to the increased production of ROS, which was in agreement with previous observations in other organisms. For example, the expression of HSP70 and HSP90 in oysters exposed to Cd showed a significant enhancement [14,37]. However, no significant difference of MgHSPA12A expression was observed when Cd concentration increased to 50 μg/L. It was deduced that strong oxidative stress caused by excess accumulation of Cd beyond a certain level in *M. galloprovincialis* drastically lowered the metabolic capacity, and then resulted in a less significant variation of MgHSPA12A expression profile in the 50 μg/L Cd-treated samples than those of by 5 μg/L Cd-treated samples. Similar result was also reported on *V. philippinarum* treated with 10, 20, and 40 μg/L Cu and Cd respectively [25]. All these might be explained by the hormesis phenomenon in toxicology characterized by low-dose stimulatory and high-dose inhibitory responses [39,40]. As concerned to TCTP, there were few reports on TCTP expression profile of shellfish exposed to toxic metals. Recently, TCTP was reported to be an anti-apoptotic protein [38]. Hence the decrease of MgTCTP mRNA was perhaps consistent with their roles as negative regulators in apoptosis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fsi.2013.04.021.

References

[1] Beere HM. The stress of dying: the role of heat shock proteins in the regulation of apoptosis. Journal of Cell Science 2004;117:2641.
[2] Trent JD. A review of acquired thermotolerance, heat-shock proteins, and molecular chaperones in archaea. FEMS Microbiology Reviews 1996;18:249–58.
[3] Bommer UA, Thiele BJ. The translationally controlled tumour protein (TCTP). The International Journal of Biochemistry & Cell Biology 2004;36:379–85.
[4] Shu Y, Du Y, Wang J. Molecular characterization and expression patterns of *Spodoptera litura* heat shock protein 70/90, and their response to zinc stress. Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology 2010.
[5] Han Z, Truong QA, Park S, Breslow JL. Two Hsp70 family members expressed in atherosclerotic lesions. Proceedings of the National Academy of Sciences 2003;100:1256.
[6] Pongrac JL, Middleton FA, Peng L, Lewis DA, Levitt P, Mirnics K. Heat shock protein 12A shows reduced expression in the prefrontal cortex of subjects with schizophrenia. Biological Psychiatry 2004;56:943–50.
[7] Thaw P, Baxter NJ, Hounslow AM, Price C, Waltho JP, Craven CJ. Structure of TCTP reveals unexpected relationship with guanine nucleotide-free chaperones. Nature Structural & Molecular Biology 2001;8:701–4.
[8] Gnanasekar M, Dakshinamoorthy G, Ramaswamy K. Translationally controlled tumor protein is a novel heat shock protein with chaperone-like activity. Biochemical & Biophysical Research Communications 2009;386:333–7.

[9] Bommer UA, Borovjagin AV, Greagg MA, Jeffrey IW, Russell P, Laing KG, et al. The mRNA of the translationally controlled tumor protein P23/TCTP is a highly structured RNA, which activates the dsRNA-dependent protein kinase PKR. RNA 2002;8:478.
[10] Gachet Y, Tournier S, Lee M, Lazaris-Karatzas A, Poulton T, Bommer UA. The growth-related, translationally controlled protein P23 has properties of a tubulin binding protein and associates transiently with microtubules during the cell cycle. Journal of Cell Science 1999;112:1257.
[11] Li C, Qiu L, Ning X, Chen A, Qin S, Wu H, et al. The first molluscan TCTP in *Venerupis philippinarum*: molecular cloning and expression analysis. Fish & Shellfish Immunology 2010;29:530–3.
[12] Margulis B, Guzhova I. Dual role of chaperones in the response of a cell and of a whole organism to stress. Tsitologiia 2009;51:219.
[13] Cellura C, Toubiana M, Parrinello N, Roch P. HSP70 gene expression in *Mytilus galloprovincialis* hemocytes is triggered by moderate heat shock and *Vibrio anguillarum*, but not by *V. splendidus* or *Micrococcus lysodeikticus*. Developmental & Comparative Immunology 2006;30:984–97.
[14] Piano A, Valbonesi P, Fabbri E. Expression of cytoprotective proteins, heat shock protein 70 and metallothioneins, in tissues of *Ostrea edulis* exposed to heat and heavy metals. Cell Stress & Chaperones 2004:134–42.
[15] Roccheri MC, Agnello M, Bonaventura R, Matranga V. Cadmium induces the expression of specific stress proteins in sea urchin embryos. Biochemical & Biophysical Research Communications 2004;321:80–7.
[16] Song L, Wu L, Ni D, Chang Y, Xu W, Xing K. The cDNA cloning and mRNA expression of heat shock protein 70 gene in the haemocytes of bay scallop (*Argopecten irradians*, Lamarck 1819) responding to bacteria challenge and naphthalin stress. Fish & Shellfish Immunology 2006;21:335–45.
[17] Araya MT, Markham F, Mateo DR, McKenna P, Johnson GR, Berthe FCJ, et al. Identification and expression of immune-related genes in hemocytes of soft-shell clams, *Mya arenaria*, challenged with *Vibrio splendidus*. Fish & Shellfish Immunology 2010;29:557–64.
[18] Ripabelli G, Sammarco ML, Grasso GM, Fanelli I, Caprioli A, Luzzi I. Occurrence of Vibrio and other pathogenic bacteria in *Mytilus galloprovincialis* (mussels) harvested from Adriatic Sea, Italy. International Journal of Food Microbiology 1999;49:43–8.
[19] Yue X, Liu B, Xiang J, Jia J. Identification and characterization of the pathogenic effect of a *Vibrio parahaemolyticus*-related bacterium isolated from clam *Meretrix meretrix* with mass mortality. Journal of Invertebrate Pathology 2010;103:109–15.
[20] Parisi MG, Li H, Jouvet LBP, Dyrynda EA, Parrinello N, Cammarata M, et al. Differential involvement of mussel hemocyte sub-populations in the clearance of bacteria. Fish & Shellfish Immunology 2008;25:834–40.
[21] Hervé-Fernández P, Houlbrèque F, Boisson F, Mulsow S, Teyssié JL, Oberhaënsli F, et al. Cadmium bioaccumulation and retention kinetics in the Chilean blue mussel *Mytilus chilensis*: seawater and food exposure pathways. Aquatic Toxicology 2010;99:448–56.
[22] Kavun VY, Shulkin V, Khristoforova N. Metal accumulation in mussels of the Kuril Islands, north-west Pacific Ocean. Marine Environmental Research 2002;53:219–26.
[23] Szefer P, Kim BS, Kim CK, Kim EH, Lee CB. Distribution and coassociations of trace elements in soft tissue and byssus of *Mytilus galloprovincialis* relative to the surrounding seawater and suspended matter of the southern part of the Korean Peninsula. Environmental Pollution 2004;129:209–28.
[24] Zhang X. Investigation of pollution of Pb, Cd, Hg, As in sea water and deposit of Bohai Sea area. Heilongjiang Environmental Journal 2001;25:87–90 [in Chinese with English abstract].
[25] Zhang L, Liu X, Chen L, You L, Pei D, Cong M, et al. Transcriptional regulation of selenium-dependent glutathione peroxidase from *Venerupis philippinarum* in response to pathogen and contaminants challenge. Fish & Shellfish Immunology 2011.
[26] Wang Q, Zhang L, Zhao J, You L, Wu H. Two goose-type lysozymes in *Mytilus galloprovincialis*: possible function diversification and adaptive evolution. PLoS ONE 2012;7:9.
[27] Zorita I, Bilbao E, Schad A, Cancio I, Soto M, Cajaraville M. Tissue- and cell-specific expression of metallothionein genes in cadmium- and copper-exposed mussels analyzed by in situ hybridization and RT-PCR. Toxicology and Applied Pharmacology 2007;220:186–96.
[28] Li C, Sun H, Chen A, Ning X, Wu H, Qin S, et al. Identification and characterization of an intracellular Cu, Zn-superoxide dismutase (icCu/Zn-SOD) gene from clam *Venerupis philippinarum*. Fish & Shellfish Immunology 2010;28:499–503.
[29] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-[Delta][Delta] CT method. Methods 2001;25:402–8.
[30] Yang Y, Yang F, Xiong Z, Yan Y, Wang X, Nishino M, et al. An N-terminal region of translationally controlled tumor protein is required for its antiapoptotic activity. Oncogene 2005;24:4778–88.
[31] Sugiyama Y, Suzuki A, Kishikawa M, Akutsu R, Hirose T, Waye MMY, et al. Muscle develops a specific form of small heat shock protein complex composed of MKBP/HSPB2 and HSPB3 during myogenic differentiation. Journal of Biological Chemistry 2000;275:1095.
[32] Kappé G, Franck E, Verschuure P, Boelens WC, Leunissen JAM, De Jong WW. The human genome encodes 10 α-crystallin-related small heat shock proteins: HspB1–10. Cell Stress & Chaperones 2003;8:53.
[33] Canesi L, Barmo C, Fabbri R, Ciacci C, Vergani L, Roch P, et al. Effects of *Vibrio* challenge on digestive gland biomarkers and antioxidant gene expression in *Mytilus galloprovincialis*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 2010;152:399–406.
[34] Company R, Serafim A, Bebianno M, Cosson R, Shillito B, Fiala-Médioni A. Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. Marine Environmental Research 2004;58:377–81.
[35] Leonard SS, Harris GK, Shi X. Metal-induced oxidative stress and signal transduction. Free Radical Biology & Medicine 2004;37:1921–42.
[36] Zhang L, Wang L, Zhao J, Qiu L, Song L, Dong C, et al. The responsive expression of heat shock protein 22 gene in zhikong scallop *Chlamys farreri* against a bacterial challenge. Aquaculture Research 2010;41:257–66.
[37] Yong KC, Pil GJ, Cheol YC. Cadmium affects the expression of heat shock protein 90 and metallothionein mRNA in the Pacific oyster, *Crassostrea gigas*. Comparative Biochemistry and Physiology, Part C 2008;147:286–92.
[38] Gnanasekar M, Ramaswamy K. Translationally controlled tumor protein of *Brugia malayi* functions as an antioxidant protein. Parasitology Research 2007;101:1533–40.
[39] Zhang J, Wang X, Guo H, Wu J, Xue Y. Effects of water-soluble fractions of diesel oil on the antioxidant defenses of the goldfish, *Carassius auratus*. Ecotoxicology and Environmental Safety 2004;58:110–6.
[40] Calabrese EJ. Hormesis: a revolution in toxicology, risk assessment and medicine. EMBO Reports 2004;5:S37–40.