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Identification of a *Bombyx mori* gene encoding small heat shock protein BmHsp27.4 expressed in response to high-temperature stress

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ABSTRACT

Elucidating the mechanisms underlying the response and resistance to high-temperature stress in the Lepidoptera is essential for understanding the effect of high-temperature on the regulation of gene expression. A tag (CATGAACGTGAAGAGATTCAG) matching the predicted gene BGIBMGA005823-TA in SilkDB identified the most significant response to high-temperature stress in a screen of the heat-treated digital gene expression library of *Bombyx mori* (*B. mori*) (Unpublished data). BLAST and RACE showed that the gene is located on chromosome 5 and has an open reading frame (ORF) of 741 bp. Phylogenetic analysis found that *B. mori* small heat shock protein 27.4 (BmHSP27.4) is in an evolutionary branch separate from other small heat shock proteins. Expression analysis showed that *BmHsp27.4* is highly expressed in brain, eyes and fat bodies in *B. mori*. Its mRNA level was elevated at high-temperature and this increase was greater in females. The ORF without the signal peptide sequence was cloned into vector pET-28a(+), transformed and over-expressed in *Escherichia coli* Rosetta (DE3). Western blotting and immunofluorescence analysis with a polyclonal antibody, confirmed that the level of protein BmHSP27.4 increased at a high-temperature, in accordance with its increased mRNA level. In this study, *BmHsp27.4* was identified as a novel *B. mori* gene with an important role in response to high-temperature stress.

1. Introduction

Heat shock proteins (Hsps) are members of a class of proteins highly expressed in organisms and cells in response to a variety of external stresses, including high-temperature, viral infection and starvation. Hsps, which are highly expressed in a range of tissues under high-temperature stress conditions, have an important role in protecting cells from damage (Cara et al., 2005; Carranco et al., 1997; Landais et al., 2001; Tammariello et al., 1998).

Bombyx mori is an economically important insect and serves as a valuable model organism. Hsps are divided according to their molecular size, structure and function into Hsp60, -70, -90 and -110 and small Hsp (sHsp), a low molecular mass protein of 15–30 kDa that represents a large class of functional proteins widespread in prokaryotes and eukaryotes (Kim et al., 1998; Norimine et al., 2004; Sugiyama et al., 2000). Sciandra and Subjeck (1983) suggested that sHsp serves as a chaperone in processes such as protein folding, aggregation, membrane transport and decomposition (Boston et al., 1996; Xu et al., 2011).

BmHsp19.9, *-21.4*, *-23.7*, *-25.4*, *-27.4* and *Hsp1* genes have been cloned and confirmed to have important functions in vivo. Their expression can be induced to various extents by exposure to high-temperature (Li et al., 2009; Sakano et al., 2006; Sheng et al., 2010). *B. mori* was treated with ecdysone and rutin and the transcriptional level of *BmHsp19.9* was changed, indicating sHsp acted as a molecular chaperone and underwent an increase in expression levels in response to heat stimulation (Xia et al., 2007). *BmHsp23.7* was highly expressed in the midgut of animals infected by cytoplasmic polyhedrosis viruses. Moreover, different types of virus, time of infection and varieties of *B. mori* tested led to differences in expression of responsive genes (Wu et al., 2011).

The *BmHsp* genes encode *B. mori* sHsps and have important roles in thermal reactions (Howrelia et al., 2011; Li et al., 2012). In this study, we isolated a new gene that encodes a 27.4 kDa *B. mori* sHsp by screening the differential gene expression library of *B. mori* at high-temperature and at a common temperature. We then cloned the gene and performed a bioinformatics and expression analysis.

Abbreviations: ACD, α-Crystallin protein; *B. mori*, *Bombyx mori*; BCA, Bicinchoninic acid; BmHsp27.4, *Bombyx mori* small heat shock protein 27.4; cAMP, Cyclic Adenosine monophosphate; cDNA, Complementary deoxyribonucleic acid; cGMP, Guanosine 3′,5′-cyclophosphate; DAPI, 4′,6-diamidino-2-phenyindole; ELISA, enzyme linked immunosorbent assay; EST, expressed sequence tag; FITC, fluorescein isothiocyanate; GRAVY, grand average hydropathicity; IPTG, isopropyl-β-d-thiogalactopyranoside; mRNA, messenger ribonucleic acid; NCBI, National Center for Biotechnology Information; ORF, open reading frame; PDB, Protein Data Bank; PVDF, polyvinylidene difluoride membrane; qRT-PCR, quantitative real-time RT-PCR; RACE, rapid amplification of cDNA ends; sHsp, small heat shock protein; SilkDB, Silkworm Genome Database; WB, Western blotting.

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2. Materials and methods

2.1. Bioinformatics web sites and software

SilkDB (silkworm.genomics.org.cn/) was used for chromosomal localization of the gene. EST and gene sequence alignment were done with BLAST on the National Center for Biotechnology Information (NCBI) website (blast.ncbi.nlm.nih.gov/), and the open reading frame (ORF) was searched using the ORF Finder at NCBI (www.ncbi.nlm.nih.gov/gorf/gorf.html). The amino acid composition, molecular mass and isoelectric point were predicted using the Protparam tool (web.expasy.org/protparam/). The transmembrane domain of the protein was predicted using DAS (www.sbc.su.se/~miklos/DAS/); the signal peptide was predicted using the SignalP 3.0 Server (www.cbs.dtu.dk/services/SignalP/); and the protein secondary structure was predicted using an online tool of SAS (www.ebi.ac.uk/thornton-srv/databases/sas/). Post-translational modification sites of the protein were predicted using Motif-scan (hits.isb-sib.ch/cgi-bin/motif_scan). Protein modeling and the prediction of its tertiary structure were done with the Swiss-model (swissmodel.expasy.org//SWISS-MODEL.html) and its subcellular distribution was predicted using PSORT Prediction (psort.hgc.jp/form2.html) and TargetP (www.cbs.dtu.dk/services/TargetP/). The amino acid accumulation map was made with Weblogo (weblogo.berkeley.edu/). The EST-based gene expression profile was obtained from the EST Profile (www.ncbi.nlm.nih.gov/unigene/).

2.2. Phylogenetic analysis

Phylogenetic analysis was performed on the multiple alignments of heat shock protein amino acid sequences. Sixteen Hsps from other species and nine BmHsps from *B. mori* were included. The analysis was performed using ClustalX and manual refinement. Phylogenetic trees were constructed using the Neighbor-Joining method, provided by the MEGA5.1 software, under the Poisson correction amino acid substitution model. Boot-strapping was performed 1000 times to obtain support values for each branch. The Pfam program was used to identify the motifs found in the Hsp proteins (Lu et al., 2012). The sequence logos for conservative analysis of α-Crystallin protein (ACD) were obtained by submitting alignment sequences to the website http://weblogo.berkeley.edu/logo.cgi (Crooks et al., 2004).

2.3. Insects and culture conditions

B. mori variety 7532 with resistance to high-temperature was used in this study. Larvae were reared on fresh mulberry leaves (*Morus* sp.) at 26–27 ºC with a photoperiod of 12 h light/12 h dark. The 5th instar larvae were selected and males and females were reared separately. The experimental larvae were exposed to high-temperature (34 ºC) for 24 h and the control group was maintained at 26 ºC. The fat body was dissected and then ground and stored in liquid nitrogen.

2.4. Cloning of a complete cDNA sequence and production of gene expression profiles

EST sequence amplification primers were used to amplify the EST sequences (Table 1). The 5′ and 3′ rapid amplification of cDNA ends was done using the 5′ and 3′ Full RACE kits (TaKaRa, Japan) with 5′ and 3′ RACE outer and inner primers. Total RNA was isolated from the fat body using Trizol® (Life Technologies, Carlsbad, CA) reagent and RNA concentrations were determined by spectrophotometry (Beckman, USA). The PCR products were examined by electrophoresis in 1% (w/v) agarose gel and stained with ethidium bromide. The selected PCR products were purified with the Agarose Gel DNA Purification Kit (TaKaRa, Japan), cloned into vector pMD19-T (TaKaRa, Japan) and sequenced by Invitrogen (Carlsbad, CA).

Table 1
Primers for preparation of cloned and prokaryotic expression.

Primer name	Sequence (5′ → 3′)
EST-S	GTCTTACTAGCCGTTGCG
EST-A	ACAAGGGCGTAGTCCAAT
3′ RACE outer	TACCGTCGTTCCACTAGTGATTT
3′ RACE GSP1	GAAGTGCGAGCGATTTACAA
3′ RACE inner	CGCGGATCCTCCACTAGTGATTTCACTATAGG
3′ RACE GSP2	AAAGCCAGAAGCCACAACAG
5′ RACE outer	CATGGCTACATGCTGACAGCCTA
5′ RACE GSP1	TCATTTGCGATTATTTGCGG
5′ RACE inner	CGCGGATCCACAGCCTACTGATGATCAGTCGATG
5′ RACE GSP2	CGACCTTGTGTCTTCTGCTCT
qPCR-S	ACCGCCACCACCAGAAAGA
qPCR-A	GTCGCCTCAGCCAAATCCA
Prokaryotic expression-S	CG GAATTC CAGAGCAGAAGACACAAGGT EcoRI
Prokaryotic expression-A	CG AAGCTT TCACTCGGAATCTGGTT HindIII
Actin 3 (A3)-S	CTGCGTCTGGACTTGGC
Actin 3 (A3)-A	CGAGGGAGCTGCTGGAT

Quantitative real-time RT-PCR (qRT-PCR) was used to analyze the levels of mRNA from the *BmHsp27.4* gene after heat treatment. A SYBR Premix Ex Taq (Perfect Real Time; TaKaRa) kit was used, in accordance with the manufacturer's instructions. The reaction volume was 20 μL, and the cycling conditions were as follows: denaturation for 1 min at 95 ºC and 45 cycles of 95 ºC for 5 s, 55 ºC for 10 s and 72 ºC for 10 s. Three replicates were tested for each sample and the data were corrected using the Sequence Detection program (v.1.3.1).

2.5. Construction and expression of the prokaryotic expression plasmid of pET28a-BmHsp27.4

The prokaryotic expression primers excluding the signal peptide sequence (amino acids 1–19) were designed (Table 1) according to the complete ORF sequence of the *BmHsp27.4* gene. The upstream and downstream primers contained EcoRI and HindIII restriction sites, respectively. Two endonucleases were used to cleave *BmHsp27.4*-T-vector and pET28a (+). The target bands were cloned into pET28a (+) via T4 DNA ligase (Fermentas, Canada) and transformed to competent Rosetta (DE3). Verified clones were selected and transferred to 500 mL of Fresh LB medium and incubated for another 3 h, then isopropyl-β-D-thiogalactopyranoside (IPTG; Sango, China) was added to a final concentration of 1 mM and incubated for 8 h at 25 °C. The fusion proteins were recovered and purified by an affinity Ni column specific to His-tailed proteins. The polyclonal antibody was recovered, purified and prepared as previously described (Yang et al., 2012).

2.6. Western blotting (WB)

Proteins were extracted from the fat body at different time points. Protein concentrations were measured using a BCA Protein Assay Kit (Beyotime, China) and a microplate reader. The extracts were subjected to SDS-PAGE (12% (w/v) polyacrylamide gel) and transferred electrophoretically to polyvinylidene difluoride membrane (PVDF). The membrane was blocked with a blocking solution (Beyotime, China), followed by incubation with the purified anti-*BmHsp27.4* antibody or the anti-β-tubulin antibody, washed and then incubated with horseradish peroxidase (HRP)-labeled anti-mouse IgG (Bioworld Technology, USA). Proteins were visualized using the EZ-ECL Chemiluminescence Detection Kit for HRP (Biological Industries, Israel).

2.7. Immunofluorescence

After treatment for 72 h, the fat body was placed onto ice and fixed in 4% (v/v) paraformaldehyde. The samples were dehydrated in an ethanol series, then embedded in paraffin and sections (8–10 μm thick) were cut with a microtome. Paraffin sections of the silk glands were

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dewaxed with xylene, rehydrated in an ethanol series then subjected to an antigen unmasking procedure by heating in sodium citrate buffer (Beyotime, China) for 15 min and cooled. Sections were incubated for 1 h with a blocking solution and then incubated overnight at 4 ºC with the anti-*BmHsp27.4* antibody or the anti-β-tubulin antibody or normal rabbit serum as a negative control. Sections were washed three times (5 min each time) with PBST (Beyotime, China) then incubated with an appropriate fluorescein isothiocyanate (FITC)-conjugated secondary antibody (CWBIO, China) for 30 min at 37 ºC. Sections were washed three times (5 min each time) with PBST then stained with 4′,6-diamidino-2-phenyindole (DAPI; Beyotime, China). Reference sections were stained with rabbit negative serum as a primary antibody. All sections were examined under an OlympusBX51 fluorescence microscope (Olympus, Japan).

3. Results

3.1. Gene information

A 709 bp target EST sequence was obtained by qRT-PCR (Fig. 1A) and the cloning strategy, which was based on the sequence BGIBMGA005823-TA, is shown in Fig. 2A. A 410 bp 3′ RACE product and a 106 bp 5′ RACE product were obtained. The gene sequence of *BmHsp27.4* is accession number KF547930 in GenBank. The full length of the gene sequence is 940 bp with a 741 bp ORF (from base pair 34-774) encoding a protein containing 246 amino acid residues (Fig. 2B). The BLASTN analysis detected no intron in the cloned gene. The gene was located at nscaf2838:1796002-1796742(+strand) on chromosome 5.

3.2. Bioinformatics analysis of the novel gene BmHsp27.4

The BmHSP27.4 protein (molecular mass 27.4173 kDa theoretical pI 5.86) contains 246 amino acids. The instability index was 45.07, which is classified as unstable. Motif-scan analysis was used to obtain 13 hits: two N-myristoylation sites; six protein kinase C phosphorylation sites; four casein kinase II phosphorylation sites; and one cAMP and cGMP-dependent protein kinase phosphorylation site (Fig. 3). The grand average hydropathicity (GRAVY) value, as predicted by ProtScale, was −0.409, which indicated that it is a hydrophilic protein. Analysis by Dense Alignment Surface showed only one transmembrane structure (6–15 amino acids) of the BmHSP27.4 protein. Using SignalP and TargetP, it had a predicted signal peptide with 20 amino acid residues in the N terminus causing them to be secreted from the cell. The score of it being located in the secretory pathway was 0.845 and it was located in the cytoplasm. Together, these results suggest that it is a secretory protein.

Analysis of the secondary structure of the BmHSP27.4 protein, by SAS, indicated that the α helix, β fold and random coil contents were 32.52%, 27.64% and 39.84%, respectively (Fig. 2B). The 3D structure of the protein was predicted by SWISS-MODEL (Fig. 2C). We found a protein homologous with alignment length 175 and corresponding PDB ID: 2ygd, which encodes the 24 meric Eye Lens Chaperone α-Crystallin protein (ACD) as an important human molecular chaperone (Braun et al., 2011). The BmHSP27.4 protein and this homolog showed 32.4% identity, with 57.7% coverage.

3.3. Homologous alignment and phylogenetic tree

Analysis of the homology of sHSP27 with other species showed that BmHsp27.4 belongs to a single branch with a large genetic distance. Furthermore, its homology with invertebrate sHSP27 differed significantly compared to vertebrate sHSP27 (Fig. 4A). Phylogenetic analysis of the cloned members of the *B. mori* sHsp family showed that *B. mori* sHsp exhibited a significant gradient branch (Fig. 4B), which might be because the *B. mori* sHsp family evolved from highly homologous proteins. sHsp contains a conserved ACD at the C terminus, which can produce a large number of oligomers, preventing damage caused by protein aggregation when serving as a chaperone (Crack et al., 2002). Our results also indicate that HSP27 of different species and the *B. mori* sHsp family both contain ACD composed of the most conserved amino acids (Figs. 4C and D). However, the base stacking diagram (amino acids 73–149) showed the ACD of HSP27 in different species (Fig. 4C) was more conservative compared to the *B. mori* sHsp family (Fig. 4D), which might be an important reason for functional differences between different sHsp species.

3.4. Gene expression profile of BmHsp27.4 and response to high-temperature stress

The UniGene ID of *BmHsp27.4* is Bmo.4295 (www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Bmo.4295), so we obtained the corresponding EST profile of the gene by the EST Database. Under normal circumstances, *BmHsp27.4* is expressed mainly before the silk spinning stage and is highly expressed in the pupal and embryo stages, but the expression level is low in the larval stage (Fig. 1C). Expression levels of the gene in different tissues in decreasing order are brain, maxilla, testis, eye and fat body (Fig. 1B). Considering most Hsps can be induced by high-temperature and the fat body is an important site

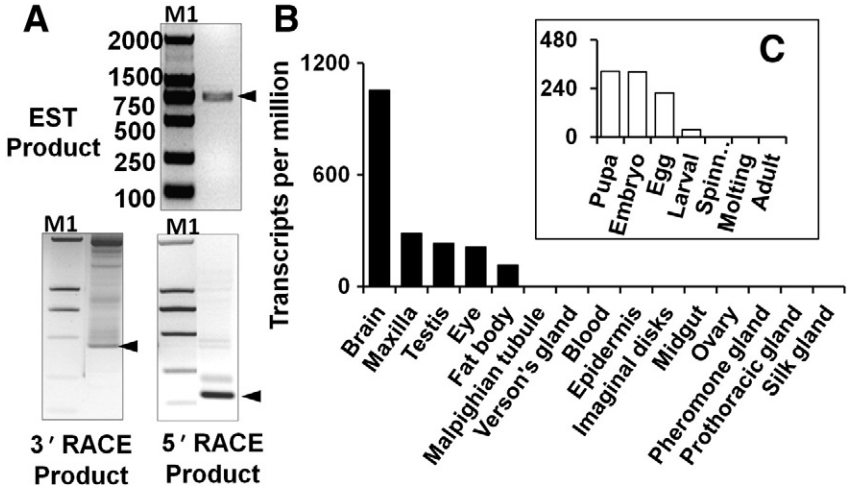

Fig. 1. Cloning method production and EST expression profiles. (A) Results of PCR amplification. (B) Digital expression profiles of *BmHsp27.4* in different tissues. (C) Expression levels of different developmental stages. The corresponding UniGene number for the *BmHsp27.4* gene was Bmo.4295.

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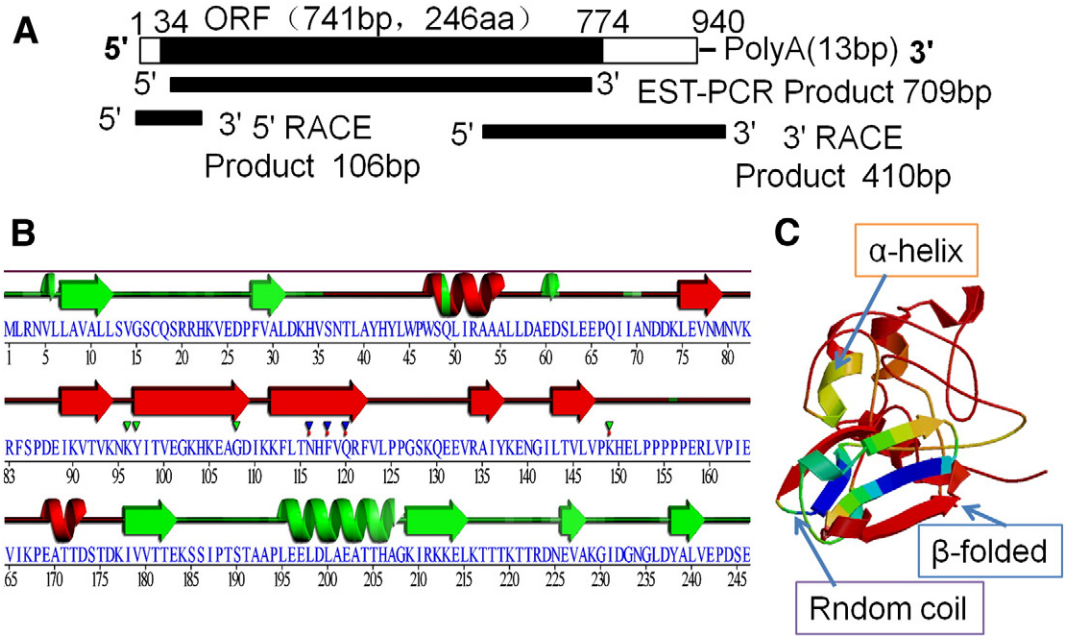

Fig. 2. Cloning strategy and protein structure analysis. (A) Cloning strategy. First, the spliced EST sequences were amplified using PCR, and the 5′ and 3′ RACE primers on both sides of the EST sequences were designed. The 5′ and 3′ RACE products were 106 bp and 410 bp in length, respectively, and were spliced with the EST sequence to obtain the gene sequence with a full length of 940 bp. The black frame is the ORF. (B) Prediction of protein secondary structure. (C) Prediction of the protein 3D structure based on 2ygd:T.

of energy metabolism in *B. mori*, we investigated gene expression in the fat body at high-temperature. The results showed that, similar to other sHsp genes, *BmHsp27.4* could be induced by high-temperature, and the expression levels were increased with treatment time at high-temperature. However, the expression level showed a downward trend after day 6. The results also showed that, during various periods of time, the gene expression levels in *B. mori* males were higher compared to females (Fig. 6A).

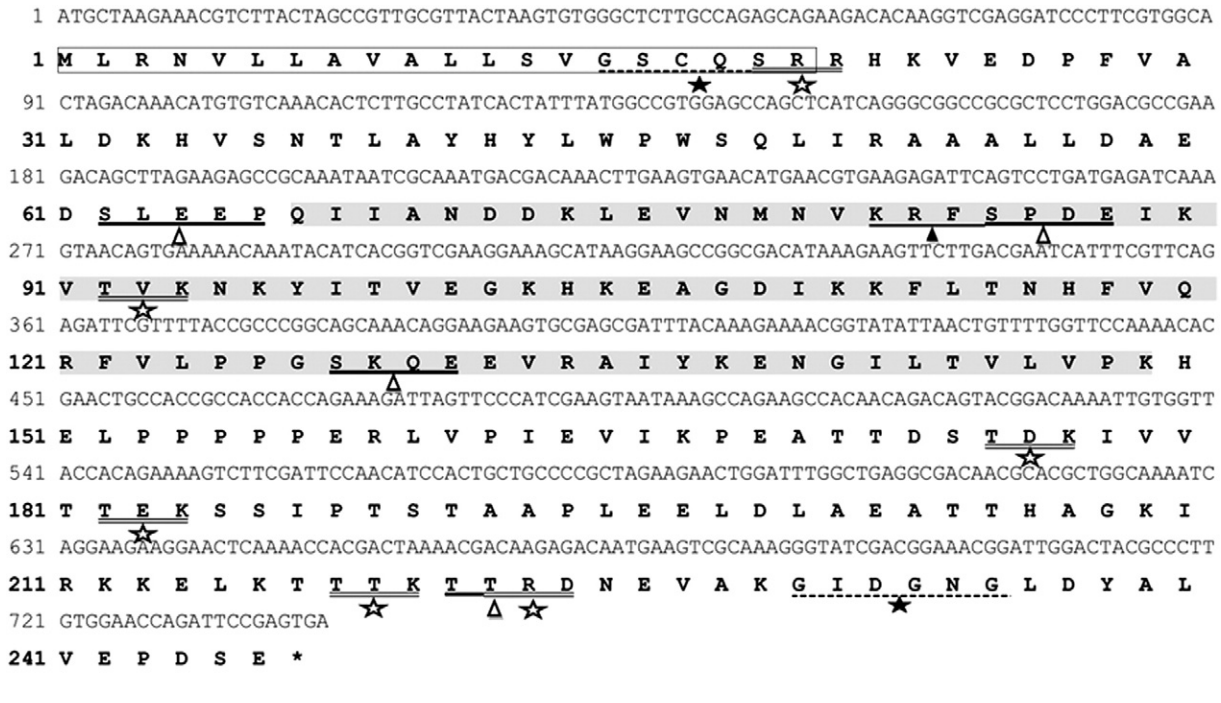

Fig. 3. Amino acid sequence analysis, Motif-scan was used to analyze the motifs and 13 hits were found.

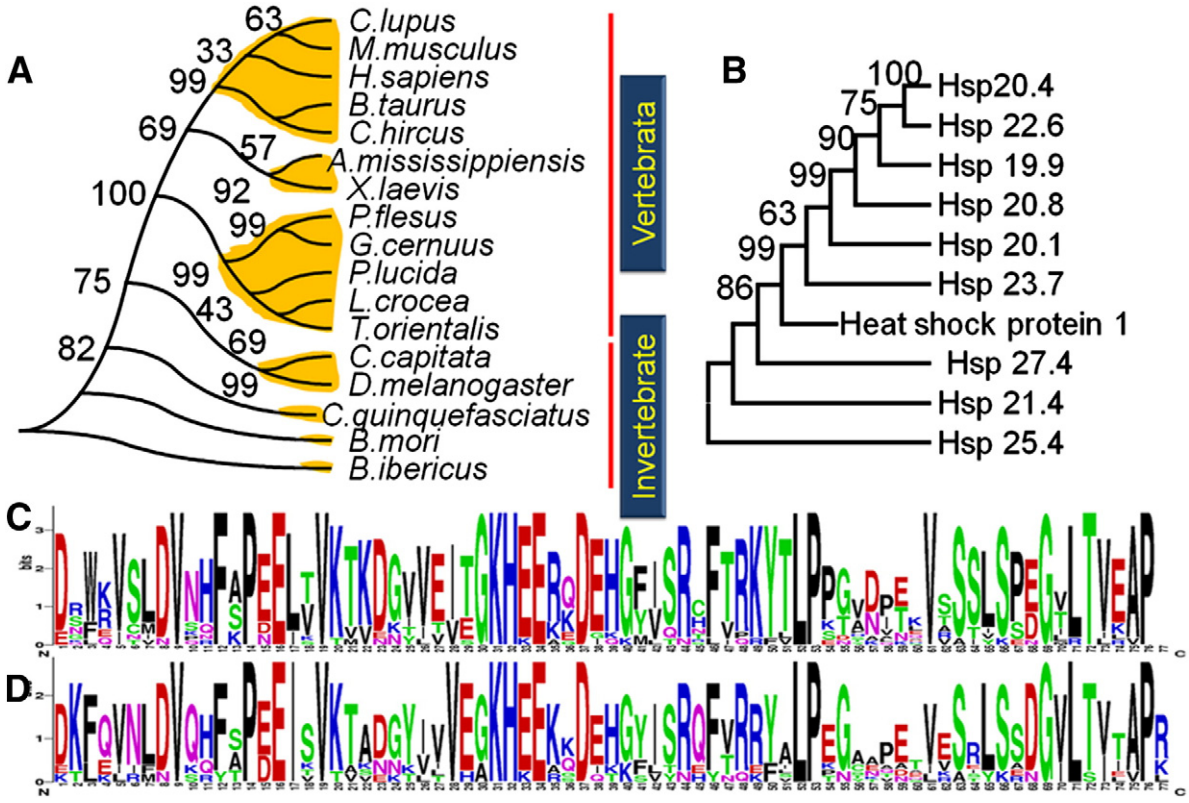

Fig. 4. Comparison of homology comparison and phylogenetic tree. (A) Phylogenetic trees of sHSP27 of different species. The phylogenetic tree was created by the neighbor joining (NJ) method, using Mega4.0. The protein sequences of BmHSP27.4 homologs were retrieved from the following species (GenBank ID), *Canis lupus* (AAA87172.1), *Mus musculus* (AAA18335.1), *Homo sapiens* (AAB51056.1), *Bos taurus* (NP 001020740.1), *Capra hircus* (AFK93550.1), *Alligator mississippiensis* (BAF94137.1), *Xenopus laevis* (ABF17872.1), *Platichthys flesus* (CCO03033.1), *Gymnocephalus cernuus* (CCO03019.1), *Poeciliopsis lucida* (AAB46593.1), *Larimichthys crocea* (ADX98507.1), *Thunnus orientalis* (BAH59273.1), *Ceratitis capitata* (ACD76913.1), *Drosophila melanogaster* (AAA28638.1), *Culex quinquefasciatus* (XP 001847191.1), *Brachionus ibericus* (ADR79277.1), (B) Homology analysis of cloned sHsp in *B. mori*. The other of *B. mori* heat shock protein sequences used were: Hsp20.8 (ACM24338.1), Hsp20.4 (AAG30945.2), Hsp19.9 (BAD74195.1), Hsp20.1 (BAD74196.1), Hsp21.4 (BAD74197.1), Hsp23.7 (BAD74198.1), Heat shock protein 1 (ABF51459.1), Hsp25.4 (ACA25336.1), Hsp22.6 (ACM24354.1). (C) Conservative analysis of ACD of sHSP27 in different species (D) Conservative analysis of ACD and sHsp cloned from *B. mori*.

3.5. Western blot and immunofluorescence analysis of protein expression

The target gene with a length of 687 bp after removal of the signal peptide was cloned and connected to the high efficiency expression vector pET28a (+). SDS-PAGE and western blot detections showed that the fusion target protein was detected only in the supernatants after centrifugation, indicating that the fusion protein was soluble

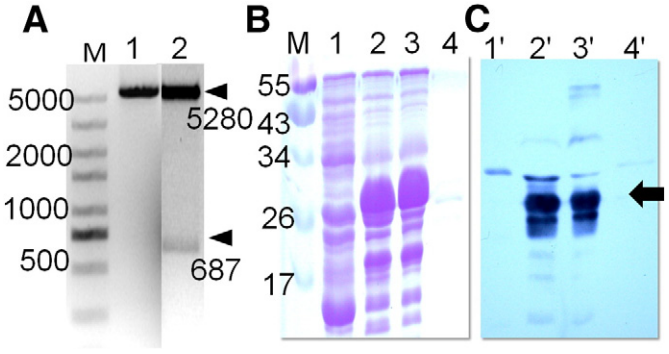

Fig. 5. Construction and expression of the prokaryotic expression plasmid, pET28a-BmHSP27.4. (A) The prokaryotic expression vector with the target gene. Lane 1, the empty vector; lane 2, the vector with the target gene. (B) SDS-PAGE of proteins after induction. Lane 1, the empty vector; lane 2, BmHSP27.4-pET28a (+) whole bacterial liquid after induction; lane 3, supernatant after sonication; and lane 4, precipitate after sonication. (C) Western blot assays of proteins after induction using the His-tag antibody as the secondary antibody for validation. Lanes 1–4 have the same material as in (B). Lanes M are molecular mass markers.

(Fig. 5A). ELISA assays showed the polyclonal antibodies had a titer of 1:51200. The western blot assays showed that the polyclonal antibodies had high specificity (Figs. 5B and C).

The results of the western blots showed that the transcriptional levels were consistent with the translational levels, reaching a peak at day 5 and then decreasing (Fig. 6B). Similarly, the protein levels in male silkworms were higher compared to females. Subsequently, the localization of BmHSP27.4 proteins in the fat body under high-temperature was done on day 5, which showed that the proteins were localized outside the nuclei (Fig. 6C) and the normal is only a low lever (figure not shown).

4. Discussion

The synthesis of Hsps in insects can be induced by low or high-temperature. The speed of acquiring heat resistance is positively correlated with the rate of Hsp accumulation, and the decrease of heat resistance is synchronized with Hsp degradation (Boston et al., 1996; Sakano et al., 2006).

BmHsp27.4 has some characteristics in common with the *B. mori* sHsps investigated earlier. First, the gene sequence contains only one exon and has no intron, which is suitable for the rapid expression of large quantities of sHsps and prevention of the impact of severe thermal shock on pre-mRNA splicing (Sheng et al., 2010). Second, the protein sequence contains a conserved 77 amino acid α-Crystallin domain (MacRae, 2000), which can prevent undesired interactions between proteins and help the refolding of denatured protein (Haslbeck, 2006). In addition, Hsps have thermal reactivity; some proteins also have

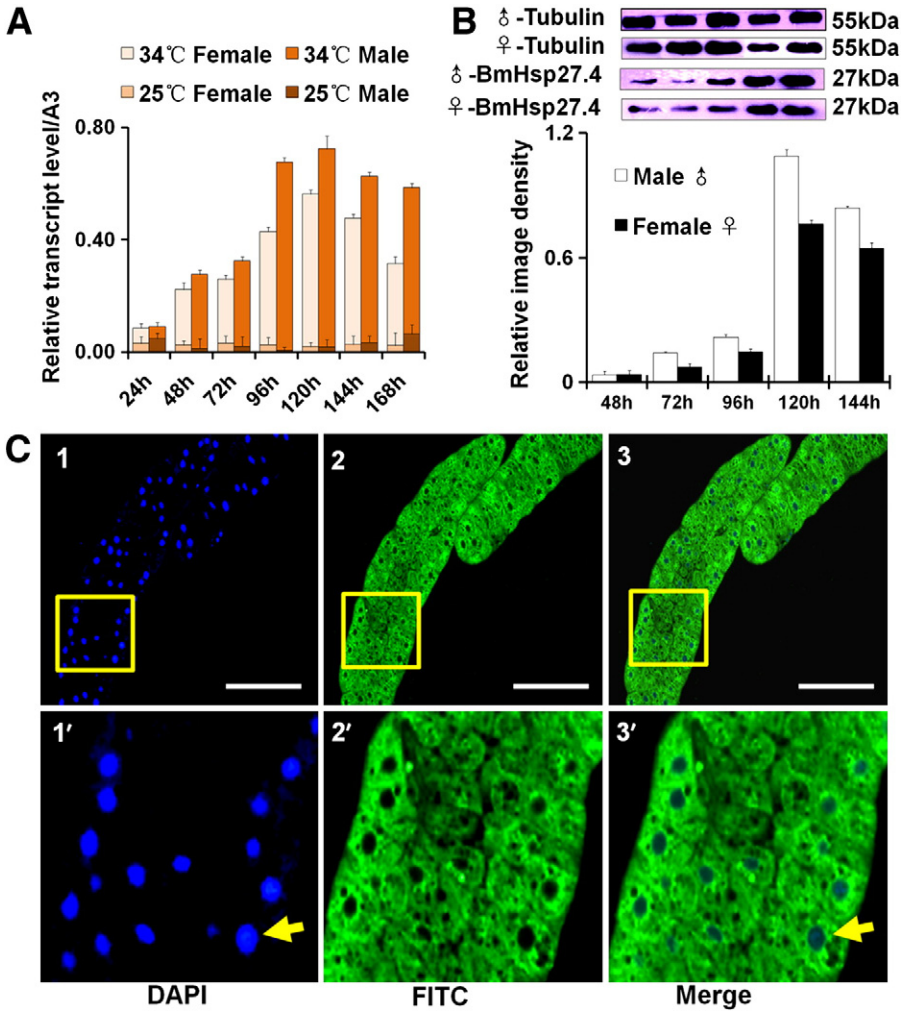

Fig. 6. Expression of gene and protein in response to high-temperature stress. (A) Expression levels of the *BmHsp27.4* gene in the fat body in a high-temperature environment. After feeding for 24 h, *B. mori* 5th instar larvae in the experimental group were transferred to 34 ºC (control group 26 ºC) and sampled once every 24 h. *B. mori* actin A3 was used as the internal reference gene. The *BmHsp27.4*/A3 ratio represented the relative gene expression level of *BmHsp27.4*. (B) BmHSP27.4 protein expression in a high-temperature environment. (C) Localization of the BmHSP27.4 protein in the fat body under high-temperature stress. Column 1, genomic DNA labeled with DAPI; column 2, BmHSP27.4 (green fluorescence) stained by the anti-BmHSP27.4 antibody and FITC; column; 3, merged images for FITC and DAPI. Diagrams 1′, 2′ and 3′ show amplified sections (yellow frames) of 1, 2 and 3. Yellow arrowheads indicate genomic DNA. The scale bars represent 50 μm. The expression data were downloaded from the EST database and the calculation was: TPM (transcript per million) expression values (pool) = Gene EST/Total EST in pool. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

important roles in other adverse environments (Sakano et al., 2006). BmHSP27.4 constitutes an independent and relatively primitive branch in the phylogenetic tree of the sHsp of *B. mori* or other species.

The prokaryotic expression of the BmHSP27.4 protein was found to be highly expressed only in the *E. coli* Rosetta (DE3) strain, but not in the normal BL21 strain (DE3). Rare codon analysis found that the translation initiation site contained a leucine residue (CTA) and could not be expressed in BL21 (DE3). However, it had a good expression in Rosetta (DE3) and could be expressed only in the viral supernatant, indicating good solubility. sHsps with Cys are prone to form interchain disulfide bonds that affect correct folding, leading to aggregation (Fu et al., 2003). Therefore, these proteins often exist in the form of inclusion bodies. The BmHSP27.4 protein sequence has only one Cys and thus cannot form a disulfide bond.

Both PCR and western blot assays showed that *BmHsp27.4* gene transcription and expression levels at high-temperature were higher in males compared to females. Sorensen et al. (2007) found a significant difference between male and female fruitflies in the adaptability of Hsps to high-temperature in both the initial and at the late stages. It is known that high-temperature resistance is significantly higher in male compared to female silkworms (Traut et al., 2007). The subcellular localization of BmHsp25.4 under normal circumstances is mainly in the cytoplasm and starts to appear in the membrane after a heat shock (39 ºC) for 3 h, suggesting that the transfer of BmHsp25.4 to the cell membrane could maintain its mobility and integrity (Sheng et al., 2010). The subcellular localization of BmHSP27.4 protein indicates that it is distributed mainly in the cytoplasm at high-temperature. However, the question of whether BmHSP27.4 has the same function as BmHsp25.4 remains to be answered.

Conflict of interest

There is no conflict of interest.

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Jiangsu Higher Education Institutions, and the Research Innovative Projects for average college graduate students of 2011 in Jiangsu Province, China.

Database:

PDB ID:2ygd (http://www.rcsb.org/pdb/explore.do?structureId=2ygd)

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