Sustaining induced heat shock protein 70 confers biological thermotolerance: a case in a high-temperature adapted predatory mite

Chuanbei Tian[1], Yaying Li[2], Yixia Wu[3], Wenqiang Chu[1], and Huai Liu[2]

[1]Affiliation not available
[2]Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400715, China
[3]Key Laboratory of Entomology and Pest Control Engineering, College of Plant ProtectionSouthwest University, Chongqing 400715, China

May 22, 2020

Abstract

Tiny predators, especially like phytoseiid mites, often experience a host of threats or stresses by fluctuating environmental factors. Heat acclimation as a superior adaptation strategy critically enhances abilities for organisms to handle with changing climate, but little is known about the molecular mechanism determining tolerant plastic responses in Phytoseiid mites. The relative expression of four identified HSP70 genes in two strains of Neoseiulus barkeri increased within a short time in temperature ramping treatment; meanwhile the expression of NbHSP70-1 and NbHSP70-2 in the conventional strain (CS) sharply decreased after 4 h displaying distinct contrast with the stable expression in the high-temperature adapted strain (HTAS). Western blot analysis showed that the protein level of NbHSP70-1 in CS was dramatically elevated at 0.5 h and decreased at 6 h at 42°C. Conversely, in HTAS, NbHSP70-1 was constantly induced and peaked at 6 h changed at 42°C. Furthermore, HSP70 suppression by RNAi knockdown had a greater influence on the survival of HTAS, causing a higher mortality under high temperature than CS. The recombinant certain exogenous NbHSP70-1 protein enhanced the viability of E. coli BL21 under lethal temperature of 50°C. These results suggested that HSP70 genes were a prominent contributor promoting the thermotolerance to heat stress and plastic change of HSP70 genes conferred the thermotolerance of HTAS through long-term heat acclimation. The divergent constitutive regulation of HSP70 to thermal is conducive to the flexible adaptability of predators in higher trophic level to trade off under extremely adversity stress.

Abstract

Tiny predators, especially like phytoseiid mites, often experience a host of threats or stresses by fluctuating environmental factors. Heat acclimation as a superior adaptation strategy critically enhances abilities for organisms to handle with changing climate, but little is known about the molecular mechanism determining tolerant plastic responses in Phytoseiid mites. The relative expression of four identified HSP70 genes in two strains of *Neoseiulus barkeri*increased within a short time in temperature ramping treatment; meanwhile the expression of *NbHSP70-1* and *NbHSP70-2* in the conventional strain (CS) sharply decreased after 4 h displaying distinct contrast with the stable expression in the high-temperature adapted strain (HTAS). Western blot analysis showed that the protein level of *NbHSP70-1* in CS was dramatically elevated at 0.5 h and decreased at 6 h at 42°C. Conversely, in HTAS,*NbHSP70-1* was constantly induced and peaked at 6 h changed at 42°C. Furthermore, HSP70 suppression by RNAi knockdown had a greater influence on the survival of HTAS, causing a higher mortality under high temperature than CS. The recombinant certain exogenous *NbHSP70-1*protein enhanced the viability of *E. coli* BL21 under lethal temperature of 50°C.

These results suggested that HSP70 genes were a prominent contributor promoting the thermotolerance to heat stress and plastic change of HSP70 genes conferred the thermotolerance of HTAS through long-term heat acclimation. The divergent constitutive regulation of HSP70 to thermal is conducive to the flexible adaptability of predators in higher trophic level to trade off under extremely adversity stress.

KEY WORDS: *Neoseiulus barkeri* , heat shock protein 70, heat acclimation, thermotolerance

1 | INTRODUCTION

Changing or fluctuating environments provoke a range of stresses in arthropod which adversely affect most aspects of organismal biology and ecology. Climate change seems to have marked ecological effects on terrestrial ecosystems. Predatory phytoseiid mites are effective natural enemies and utilized as biological control agents for small insects and mite pests (McMurtry et al., 2013). However, like other living organisms, phytoseiid mites experience a variety of environmental stress stimuli under the field conditions (Ghazy et al., 2016; Schmidt-Jeffris & Cutulle, 2019) and seldom live in their optimal living environment, resulting in suppressed potential and low control efficiency on mite pests. Temperature is one of the most critical abiotic determinants affecting the behavior and physiology of living organisms. High temperatures directly bring out a myriad of organismal function shifts in terms of their metabolic balance and damage respiration patterns by inducing protein denaturation, membrane instability and cell disruption (Angilletta, 2009). Arthropod pests together with their natural enemies, as ectotherms, normally live and grow in only a limited range of temperatures, and face up to significant mortality at temperatures of 40-50°C. When the temperature was raised over 40°C, two predatory mites *Phytoseiulus macropilis* and*Phytoseiulus persimilis* ceased walking and entered heat coma (Coombs & Bale, 2013). Furthermore, changes in thermoregulatory phenotypic plasticity generally tend to vary with different trophic levels and sensitivity increases significantly with increasing trophic level (Voigt et al., 2003). High temperatures favor mite pests rather than phytoseiid mites, resulting in the reduced efficiency of predators (Stavrinides et al., 2010; Stavrinides & Mills, 2011).

Challenging physical environments can select for tolerant phenotypes of a species (Chen et al., 2018). Feasible biological control strategies should be utilized to enhance the adaptability of predatory mites in field conditions under global warming and climate change (Vangansbeke et al., 2020). In our previous study, a high-temperature adapted strain (HTAS) of the predatory mite*Neoseiulus barkeri* (Hughes) was screened from the conventional strain (CS) by long-term thermal acclimation (35°C) and regular thermal hardenings (45°C) (exposed for 2 h every fourth week for many generations). Surprisingly, long-term heat acclimation confers HTAS *N. barkeri* the thermotolerance a hefty boost. When heat stress of 45°C appeared, the half lethal time (LT_{50}) for HTAS *N. barkeri* was 15 h, which was much longer than that in CS *N. barkeri* . This better tolerance is owing to the extended pre-oviposition period, lowered fecundity and longevity under long-term heat acclimation (Zhang et al., 2018). *N. barkeri* , as the only domestic phytoseiid mite that has been commercially produced to control spider mites and thrips in China (Fan & Petitt, 1994; Wu et al., 2014), is superior to other biocontrol agents in that they are not only widely distributed, polyphagous and easy mass rear, but also have short life cycle and high fecundity (Li et al., 2017; Niu et al., 2014). These characteristics contribute to HTAS *N. barkeri* being an extremely promising agent for biological control. Heat acclimation as a superior adaptation strategy for organisms greatly enhances the ability of living organisms to handle with the changing climate. However, little is known about the molecular mechanisms determining plastic of heat tolerant response in phytoseiid mites.

Hardening to high temperature activates the heat shock or a stress response, leading to the defensive protection of producing molecular chaperones (heat shock proteins, HSPs) (Cuenca Cambronero et al., 2018; Feder & Hofmann, 1999). HSPs, as one of the most highly conserved molecular chaperone proteins, perform many functions including mediating the folding and assembly of proteins, maintaining protein homeostasis and modulating protein localization and degradation (King & MacRae, 2015). According to the sequence homology and molecular masses, HSPs are traditionally classified into five major protein families: HSP100, HSP90, HSP70, HSP60 and a small HSP family (Joly et al., 2010). Among them, the HSP70 family possesses highly conserved structures and most amount in cells suffering from stress stimuli. The rapid activation of HSPs was evidently consistent with enhanced tolerance under heat stress in invertebrate (Gu et al., 2019).

Induction of HSP70 produced abundantly has been proven to play a crucial role in the achieving the thermotolerance at extreme temperatures (Bahrndorff et al., 2010; Kostal & Tollarova-Borovanska, 2009). Heat shock at 40°C led to a higher expression level of HSP70 in mirid bug, *Apolygus lucorum* (Sun et al., 2016). The expression of HSP70 in the predatory mite*Neoseiulus cucumeris* and wheat blossom midge *Sitodiplosis mosellana* (Chen et al., 2015; Cheng et al., 2016) increased dramatically after heat shock. The HSP70 expression in *Bactrocera dorsalis* and *Bactrocera correcta* larvae of fruit flies diaplayed a significant increase responding to heat hardening at extreme temperature 39°C, which showed a remarkably consistent trend of heat stress response with their survival rates under heat shock (Hu et al., 2014).

To understand mechanisms for resisting the thermal acclimation in phytoseiid mites and the differences of defensive reaction between two strains of *N. barkeri* , the molecular mechanism of the HSP70 family in the physiological responses was investigated in this study. The full-length cDNA of four *NbHSP70s* from *N. barkeri* was cloned and identified. The different expression levels between CS and HTAS *N. barkeri* strains at transcriptional and translational levels under extremely high temperature were detected to determine the candidate *NbHSP70*participating in establishing heat resistance. To further verify the link between *NbHSP70* expression and thermotolerance, RNAi was conducted to knockdown*NbHSP70*mRNA expression in two strains. Finally, overexpression of*NbHSP70* in *E. coli* was performed to confirm its influence on the heat tolerance of *E. coli in vivo* .

2 | MATERIALS AND METHODS

2.1 |

Mite strains

The CS strain of the predatory mite*N. barkeri* was kept at a constant temperature of 25 ± 1°C, and HTAS was maintained at a heat acclimation condition of 35 ± 1°C from 2012 in this study. The HTAS of *N. barkeri* were built and reared by methods described by Zhang et al., 2018.

2.2 | Cloning of the full-length HSP70 cDNAs

Primer Premier 5 software was used to design the two pairs of gene-specific primers needed for cloning the full-length of HSP70 genes, according to the transcriptome of *N. barkeri* (Tian et al., 2020) (SRR8061289 in National Center for Biotechnology Information, NCBI) . (Table S1). Total RNA was extracted from female adult mites using TRIZol reagent (Invitrogen, Carlsbad, CA, USA) and the first-strand cDNA was synthesized using the PrimeScript RT reagent Kit (TaKaRa, Dalian, China) in accordance with the manufacturer's protocols. The PCR product was confirmed by gel electrophoresis, and cloned into the pGEM-T Easy vector (Promega, Fitchburg, MA, USA) after extraction from gel using the kit (TaKaRa). The positive clone was validated by specific primers of plasmid and then sequenced by BGI (Shenzhen, China).

2.3 | Bioinformatic analysis

Homologous domains in four HSP70 genes were determined using basic local alignment search tool at the NCBI website, whereas structural analysis of the deduced protein was performed online (http://www.expasy.org/tools/). Alignment of multiple sequences with homologs of other species was performed using DNAMAN v.7.0.2 (Lynnon Biosoft, San Ramon, CA, USA). Phylogenetic evolutionary analysis was generated using Molecular Evolutionary Genetics Analysis (MEGA v.6.0) with the neighbor-joining algorithm. The reliability of branching was tested using bootstrap method (1,000 replications) and gaps/missing data treated as complete deletions. Bootstrap values less than 50 were ignored.

2.4 | Transcript expression of HSP70 genes under 42°C exposure

For heat stress, over 300 female adults (4–5 days old) were transferred into a 1.5 mL centrifuge tube, and then exposed to 42°C for 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h in an incubator. After exposure treatment, the surviving females were collected for RNA isolation. The housekeeping gene *EΦ1a* was used as a reference gene for normalization purposes (Wang *et al.,* 2018). The primers used for qPCR were designed from the conserved regions of the genes (Table S1). Quantitative real time polymerase chain reaction (qRT-PCR)

was performed as described in our previous study (Tian et al., 2019). Each treatment was prepared with three biological replicates. The relative expression levels were calculated using the $2^{-\Delta\Delta^{\hat{}}\tau}$ method (Livak & Schmittgen, 2001).

2.5 | Preparation of antibodies and Western blot

A peptide corresponding to amino acids 360–610 of*NbHSP70-1*was designed and synthesized by Wuhan Baiqi Biotechnology Co., Ltd. (Wuhan, China) for preparation of a specific antibody. Before protein extraction, over 600 of CS and HTAS female adults were exposed to 42°C for 0.5, 1, 2, 3, 4 and 6 h with the method described above. The total proteins were ground immediately and extracted using a radioimmunoprecipitation assay (RIPA) containing protease inhibitors (Beyotime Biotechnology, China) on ice for 30 min. Then, the sample lysates were centrifuged at 15,000 g for 5 min at 4°C, and the protein concentrations of the supernatant solutions were determined using the bicinchoninic acid (BCA) method (Beyotime) according to the manufacturer's protocol. Western blot was tested by a standard procedure of Stain-Free technology. The normalized amounts of total protein from each sample were electrophoresed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred onto polyvinylidene fluoride (PVDF) membranes in 20 min, 15 V using the Trans-Blot SD Semi-Dry Transfer Cell (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% fat-free milk in TBST (blotting in TBS with 0.1% Tween-20) for 1 h at room temperature (RT) and subsequently incubated with specified primary polyclonal rabbit antibody against*NbHSP70-1*(1: 1000) for 1 h at RT. After washing three times with TBST, it was incubated for 1 h at RT with the secondary anti-body HRP-conjugated goat anti-rabbit IgG antibody (1: 1000, Cell Signaling Technology, Danvers, MA, USA). Finally, they were tested by enhanced chemiluminescence (ECL) Western blot detection reagents (Bio-Rad, Hercules, CA, USA). The photographs were scanned and analyzed using Quantity One (Bio-Rad) to quantify band densities. In total, three biological replicates for each sample were used for Western blot analysis.

2.6 | RNA interference (RNAi) andbioassay

A specific 536 bp fragment from the *NbHSP70-1* ORF was selected and amplified. The primers of *NbHSP70-1* were as follows: forward (5'-3') *TAATACGACTCACTATAGGG* ACGACTCCAGCATTCAAG, reverse (5'-3')*TAATACGACTCACTATAGGG* CTTGGAGATGTCTTTGCG. The fragment exists a short overlap with the sequence for qRT-PCR. The PCR product was used as template for dsRNA synthesis using the Transcript Aid T7 High Yield Transcription Kit (Thermo Scientific, Wilmington, DE, USA). Forty age-matched F40A female adults were beforehand starved for 24 h and then placed on a Petri dish (50 mm in diameter). In each dish, a plastic flake arena (30 mm diameter) was placed upside down on water-saturated black cloth (35 mm diameter) and round sponge (45 mm diameter). A mixture of 50 μL solution containing 500 ng dsRNA/μL in 20% sucrose (Sigma) with 3% red food dye (AmeriColor, CA, USA) was dropped on the Petri dish. Every eight Petri dishes were placed in a big preservation box (285 × 213 × 50 mm) with wet sponge at the bottom for moisturizing. F40A females were allowed to feed for 24, 48 or 72 h, respectively. Females with red dye observed in their gastric caecum were used for further experiments. Females fed on dsGFP were used as a negative control. After ingestion of dsRNA solution, all adult female mites fed on *Tetranychus urticae* for another 24 h. RNA from pooled samples (˜40 adult female mites) of*N. barkeri* was extracted using the RNeasy Plus Micro Kit (Qiagen, Frankfurt, Germany) according to the manufacturer's instructions. cDNA was synthesized and qRT-PCR was performed to determine the RNAi efficiencies with three biological replicates.

According to the highest RNAi efficiencies determined above, the oral delivery durations of dsRNA were 48 h for CS and 24 h for HTAS before bioassay. After the treatment of RNA interference, each five females were placed individually in arenas as the methods by Zhang et al., 2015. To assess their heat shock survival response, arenas with two strain female adults were exposed to 42°C with the duration of 3, 4 and 5 h in a temperature controller. Mites maintained under a normal environment (25°C for CS and 35°C for HTAS) were regarded as the control. The mites after heat shock treatment were immediately moved to normal environment until 24 h, followed by checking the mortality using a stereomicroscope. When touched with brush, the tested mites were considered dead if they could not move a distance of 1 mm. Ten arenas were

treated as one treatment and each treatment was replicated for least five times.

2.7 | Heterologous expression ofrecombinant*NbHSP70-1* and assayin*E. coli*

The ORF of *NbHSP70-1* with restriction sites was amplified by PCR with specific primers (Table S1). Then PCR products were inserted into the expression vector pET28a (Novagen, Madison, WI, USA) between NheI and BamHI sites to yield pET28a-NbHSP70-1 construct using a DNA ligation kit (Thermo Scientific, Shanghai, China). The obtained recombinant plasmid pET28a-NbHSP70-1 was transformed into *E. coli* strain BL21 (DE3) (Dingguo, Beijing, China), and protein expression was carried out according to the standard method. The 5 mL of overnight culture was transferred to 200 mL Luria-Bertani (LB) medium containing 50 mg/mL kanamycin growing at 37°C. When OD600 was reached to 0.6-0.8, IPTG was added to induce the gene expression at a final concentration of 0.3 mM for 3 h, and then further incubated at 16°C for 12 h. The cells from 200 mL culture were harvested by centrifugation (20 min, 4000 g, 4°C) and the supernatant was assessed by 12% SDS-PAGE. Western blot was tested as described above and the membrane was incubated with monoclonal mouse antibody against His-tag (1:1000, Beyotime) for 1 h at RT and subsequently with the secondary anti-body HRP-conjugated goat anti-mouse IgG antibody (1:1000, Beyotime) for 1 h at RT.

A total of 5 mL of overnight recombinant bacteria was sub-cultured into 200 mL of LB medium. The OD600 was measured every hour to evaluate bacterial density (diluted 10-folds) for a period of 12 h for *E. coli* strain BL21 (DE3) using a Multiscan Spectrum (SpectraMax iD5, Molecular Devices, USA). There were three treatments: firstly, IPTG was added to a final concentration of 0.3 mM after the recombinant cells growing at 37°C for 3 h.; second, IPTG was added and then after growing for another 2 h the transformed cells were exposed at 50°C for 1 h; thirdly, treatment was lacked of IPTG induction and heat shock. The growth curve was drawn according to OD600 measured for 12 h.

E. coli containing pET28a-NbHSP70-1 or pET28a plasmids were separately cultured as above. After the OD600 was 0.6-0.8 and grown for another 2 h at 37°C, all cells suffered from a heat shock at 50°C for 0, 30, 60, 90, 120 and 180 min. All cells were inoculated at an appropriate dilution multiple (diluted 10000-folds) in agar LB medium and cultivated overnight at 37°C. The next day, the survival rate was determined according to the number of colonies of heat-treated cells and 37 treated cells. Each treatment was performed in three replications.

2.8 | Statistical analysis

The mortality rate of female adults, transcript expression of*NbHSP70-1*and growth curve and survival rate of *E. coli* were all calculated by one-way ANOVA and significant differences between means were tested with Tukey's honestly significant difference (HSD) test using SPSS v.16.0. A two-way ANOVA was performed to analyze the effects of strains and duration exposed to high temperature on the transcript expression of*NbHSP70-1* (P =0.05). The mean differences between CS and HTAS were compared using Student's t-tests (P =0.05).

3 | RESULTS

3.1 |Characterization and phylogenetic analysis

Four HSP70 genes (*NbHSP70)* were cloned and characterized from *N. barkeri* . The sequence analysis showed that*NbHSP70-1* ,*NbHSP70-2* , *NbHSP70-3* and *NbHSP70-4* (Gene Accession No. MN399167, MN556970, MN556969 and MN556968) contained 1941 bp, 1647 bp, 1929 bp and 1899 bp of ORF length, encoding 646, 548, 642 and 632 amino acids with a calculated molecular mass of 71.33, 60.04, 70.03 and 69.04 kDa and a theoretical isoelectric point of 5.61, 5.48, 5.10 and 5.83, respectively. Three classical HSP70 protein signatures, including IGIDLGTTY(A) (7-15 in *NbHSP70-1* , 6-14 in *NbHSP70-2* , 5-13 in *NbHSP70-3* and 6-14 in *NbHSP70-4*), IFDLGGGTFDVSIL(I) (199-212 in *NbHSP70-1* , 199-212 in *NbHSP70-2* , 195-208 in*NbHSP70-3* and 198-211 in *NbHSP70-4*) and RIPR(K)I(V)QKLL (344-352 in *NbHSP70-1* , 344-352 in *NbHSP70-2* , 341-349 in*NbHSP70-3* and 343-351 in *NbHSP70-4*), existed in the amino acid sequences of *NbHSP70s* . Additionally, the ATP/GTP-binding site T(I)AEAYLGQ(S, G)P(T) (130-138 in *NbHSP70-1*

and 129-137 in*NbHSP70-2* , 128-136 in *NbHSP70-3* and 129-137 in*NbHSP70-4*) and the deduced bipartite nuclear localization signal (NLS) were found in all deduced proteins. The conserved motifs of HSP70s, EEVD, were found in C-terminal of*NbHSP70-1* , *NbHSP70-3* and *NbHSP70-4* (Fig.1A).

Pairwise comparisons between the*NbHSP70s* sequences (Table S2) displayed that *NbHSP70-1* and*NbHSP70-4* possessed the highest similarity (81%), but*NbHSP70-2* was the least similar (<67%) with other*NbHSP70s* sequences. Multiple sequence alignment revealed that the deduced amino acid sequence of*NbHSP70s*were highly homologous with the corresponding sequences in other species. Particularly, *NbHSP70-1* showed 95% similarity with HSP70s from*Gallendomus occidentalis* and *N. cucumeris* (Fig.1A). Phylogenetic tree of HSP70s was divided into two main branches: HSP70 in Arachnida and HSP70 in Insecta (Fig.1B). All Four *NbHSP70s* were all clustered into the Arachnida group. In the Arachnida group, *NbHSP70-1* ,*NbHSP70-3* and *NbHSP70-4* were first clustered with their homologs in Phytoseiidae (*G. occidentalis* and *N. cucumeris*). The *NbHSP70-2* homolog was more conserved in*Aleuroglyphus ovatus* .

3.2 | NbHSP70 expression profiles exposed to high temperature

The mRNA expression of HSP70 genes of *N. barkeri* were significantly influenced by strains, duration, and their interaction, whereas the expression of*NbHSP70-3* gene showed no significant difference between two strains ($F_{1,36}$= 3.390; P =0.074). In the control situation, HTAS *N. barkeri* showed higher transcript expression of HSP70 genes compared with CS *N. barkeri.*At 42degC, four HSP70 genes in two strains had increased expression within a short time (Fig.2). Especially, the expression levels of*NbHSP70-1*and *NbHSP70-2* in CS *N. barkeri* showed a striking upregulation over 100-fold compared with the control. *NbHSP70-3*and *NbHSP70-4* in two strains were significantly induced by high temperature during the 4 h exposure. However, the expression of*NbHSP70-1* and *NbHSP70-2* in CS sharply decreased at 4 h, and showed contrasted distinctly with that in HTAS *N. barkeri.*

In the Western blot analysis, the *NbHSP70-1* was significantly induced in HTAS by heat stress and increased with exposure time (Fig.3). The expression levels of CS *N. barkeri* were dramatically elevated at 0.5 h and down-regulated at 6 h, while in HTAS*N. barkeri* , *NbHSP70-1* protein was increased and peaked at 6 h.

3.3 | Gene knockdown onhigh temperature susceptibility

N. barkeri females fed with dsRNA were clearly observed to have ingested red dye after only 24 h feeding (Fig.4A). CS *N. barkeri*females that ingested ds*NbHSP70-1*for 24, 48 and 72 h but fed with no prey, displayed no obvious changes in transcriptional level (Fig.4B). However, when provided with Tetranychus mites after ingestion with dsRNA, the expression of *NbHSP70-1* in females had a significant decrease by 65% and 70% at 24 and 48 h respectively. The RNAi efficiency of*NbHSP70-1*in HTAS*N. barkeri* decreased over the time with oral delivery of dsRNA. The expression of *NbHSP70-1* was reduced by 66%, 22% and 11% in females that were fed with dsRNA for 24, 48 and 72 h respectively, and reduced by 66%, 37% and 17% after feeding with spider mite prey for another 1 day, respectively.

Furthermore, a significant change occurred in the mortality of females ingestion with ds*NbHSP70-1* compared with dsGFP-fed females suffering from 42degC for 4 and 5 h (Fig.4C). The mortality of CS females increased 1.91-fold and 1.85-fold compared with dsGFP control for 4 h and 5 h, respectively. The HTAS *N. barkeri* females showed more remarkable response to heat shock in mortality with a 2.7-fold reduction for 4 h and 2.9-fold reduction for 5 h compared with dsGFP control.

3.4 | Heterologous expression of recombinant*NbHSP70-1* and its role in thermotolerance of the *E. coli*host

The molecular mass of the fusion protein was estimated by comparison with the marker, and it was approximately 70 kDa for*NbHSP70-1*(Fig.5A). The Western blot analysis showed *NbHSP70-1* protein expressed in *E. coli* was in agreement with the predicted molecular weight (71.3 kDa) deduced from the amino acid sequence (Fig.5B). This confirmed the successful translation of the recombinant construct pET28a-NbHSP70-1 to the production of his+NbHSP70-1 protein in the *E. coli* cells.

The growth rate of the transformed *E. coli* strain expressing*NbHSP70-1* without the induction with IPTG displayed no significant difference compared with the control strain (Fig.6A). Once the cells were induced by IPTG, slightly adverse effects on the OD600 were observed in *NbHSP70-1* cells, with control cells exhibiting a slightly lower OD600 and the expression of*NbHSP70-1* promoted the growth of *E. coli* cell at a greater degree than in control cells. Once treated with heat shock at 50degC, the growth of control cells and*NbHSP70-1* cells showed a slowly growing period as determined *via* the reduction in OD600. However, the *NbHSP70-1* cells still kept increased growth at the later stage at 10 h while the growth of control strain was arrested.

The cell viability treated by the high temperature of 50degC was determined through counting number of colonies forming units after heat shock (Fig.6B). After 30 min, the survival rate of *E. coli* with empty pET28a vectors decreased to 20%, approximately 43% lower than that containing *NbHSP70-1.* The viability of *NbHSP70-1*cells was reduced to 18% while control cells were nearly unable to provide any protection with 5% viability for 90 min. These results indicated the capability of *NbHSP70-1* expression in providing thermotolerance capability of cells *in vivo* .

4 | DISCUSSION

In the evolution of arthropods, many practical strategies have been utilized to handle with a serious of environmental stress stimuli, including high temperature, the main environmental factor affecting the performance of predatory mites in agro-ecosystems. For example, high temperature favors *Tetranychus*mite populations rather than Phytoseiid mites, which could reduce the effectiveness of predatory mites and cause disruption or failure of direct biological control (Stavrinides & Mills, 2011). In a previous study, we confirmed that heat acclimation dramatically promoted the thermotolerance of*N. barkeri* , and HTAS *N. barkeri* could withstand more intense heat stress (Zhang et al., 2018). HSPs are important and well-known stress proteins serving as molecular chaperones to protect stressed cells against unspecific denaturation of unfolding proteins (Feder & Hofmann, 1999). Our previous experiments have proven that HSP genes in *N. barkeri* were remarkably induced under extreme temperature conditions (Xu et al., 2018). In this study, the different response strategies of HSP70 genes in *N. barkeri*confronting thermal stresses between two strains at the molecular level was explored. The full-length cDNA sequences of four HSP70 genes were cloned from *N. barkeri* , sharing high sequence identities with other Arachnoidea HSP70 genes. Many structural features, such as the highly conserved N-terminal ATP/GTP-binding site, deduced bipartite NLS and three HSP70 family conserved signatures existed in the*NbHSP70s* . The typical C-terminal motif 'EEVD' for chaperone-binding activity was found in *NbHSP70-1* ,*NbHSP70-3* and *NbHSP70-4* , indicating the genes were cytosolic HSP70.

Under the normal environment, the four HSP70 genes showed higher expression in HTAS *N. barkeri* than in CS *N. barkeri* , which may indicate a certain degree of dehydration caused by long-term acclimation. A boost in HSP70 expression was reported to occur in response to dehydration in*Sarcophaga crassipalpis* (Hayward et al., 2004) and *Belgica Antarctica* (Lopez-Martinez et al., 2009). In comparison with expression at 25 degC,*NbHSP70-1*and *NbHSP70-2* expression were significantly induced as high as 191- and 159-folds after 0.5 h treatment in CS*N. barkeri* , whereas in HTAS*N. barkeri* it was as high as 68- and 69-folds after 0.5 h and reached a 200-folds peak after 1 h. This phenomenon proved that HSP70 could be induced by heat stress in both strains and specifically much earlier in CS*N. barkeri* . The expression of three HSP70 genes in CS *N. barkeri* showed a significant decrease at the following treatment, but it showed a high expression in HTAS *N. barkeri* , indicating that long-term heat acclimation led to considerable modification in the transcriptional regulation (Colinet et al., 2013). The heat shock-induced striking boost and steady high expression in*NbHSP70s* may confer the thermotolerance capacity of HTAS *N. barkeri* . To mitigate damage from lethal thermal of organisms, HSP proteins are overexpressed and accumulated as demonstrated by the Western blot analysis. To explore the exact difference between two strains, treatment with exposure for 6 h was added and the expected finding showed that the level of HSP70 protein in CS *N. barkeri* decreased compared with 4 h while the level of HTAS*N. barkeri* maintained high expression. These results suggested that the instant response of *NbHSP70s* led to sufficient accumulation of HSP70 protein to protect HTAS *N. barkeri*resulting in thermotolerance against heat stress.

dsRNA-mediated silencing or suppression of critical genes could disrupt the physiology of arthropods, which

generally leads to a reduction in their survivability. This valuable reverse genetic tool for molecular acarology research has been applied in two phytoseiidae mites, including *G. occidentalis* (Pomerantz & Hoy, 2015; Wu & Hoy, 2014, 2015) and *Phytoseiulus persimilis* (Bi et al., 2019; Ozawa et al., 2012). In this study, we first used the RNAi trial to explore the thermotolerance in *N. barkeri* by oral delivery with dsRNA. A study on *G. occidentalis* showed that a sucrose-only diet was unable to elicit RNAi and *Tetranychus* mite prey needed to be supplied for activating gene knockdown mediated after oral delivery of dsRNA (Wu & Hoy, 2014). Similarly, effective RNAi occurred after *Tetranychus* mite prey supplied to CS *N. barkeri.* Interestingly, in HTAS*N. barkeri* , the effective gene knockdown was not necessarily required of Tetranychus mite prey for activating the RNAi regulation, though supplementary prey improved higher RNAi efficiency. Furthermore, gene knockdown in HTAS*N. barkeri* could be activated more quickly after just 24 h of feeding with dsRNA compared with CS*N. barkeri* for 48 h of oral delivery of dsRNA. These results may suggest that long-term heat acclimation could change the water and osmotic homeostasis (Benoit, 2010; Danks, 2000), leading to a stronger desire for external water to compensate for the water loss to heat stress in HTAS *N. barkeri* . It is speculated that more intake of dsRNA and faster metabolism endowed HTAS*N. barkeri* with effective gene knockdown at an earlier time. In the loss of function experiment, HSP70 RNAi knockdown in*N. barkeri* significantly reduced the survival rate under high temperature condition and suppressed heat tolerance induction, suggesting that HSP70 is essential to inducing the heat tolerance in *N. barkeri* . Similar results have been observed in *Plodia interpunctella* (Kim et al., 2017) and *Nilaparvata lugens* (Lu et al., 2017), in which RNAi of HSP70 significantly impaired the heat tolerance induction. Furthermore, RNAi knockdown of HSP70 caused a greater influence on the survival of HTAS*N. barkeri* . In *N. lugens* , decreased survival rates were also found by injecting with HSP70 dsRNA combined with temperature stress (Lu et al., 2016). We therefore concluded that more accumulation of HSP70 protein contributed a powerful protective capability in HTAS*N. barkeri* thermotolerance.

Cytoprotective function and response of overexpression HSP70 proteins in the transformed model bacterium *E. coli* endows resistance to thermal stress. For the first time, we were able to express a phytoseiid mite HSP70 gene in the *E. coli* expression system. The recombinant HSP70 was successfully overexpressed *in vivo* to clarify the function of the HSP70 gene expressed in*N. barkeri* in heat tolerance. In *Litopenaeus vannamei* , recombinant*LvHSP70* in*E. coli* could enhance the capability in bacterial defense by inducing the immune system (Junprung et al., 2019). Significantly, the recombinant expression of N-terminal domain of hyperthermophilic L-asparaginase in HSPs from *Pyrococcus furiosus* guaranteed *E. coli* to maintain typical growth behavior at 52degC and even withstand an extreme heat shock up to 62degC (Jena et al., 2018). Overexpression of*HSP17.8* from *Rosa chinensis* enhanced the*E. coli* transformant cell viability under thermal stress (Jiang et al., 2009). The overexpression of *HSP40* from *Cydia pomonella* in *E. coli* was proven to confer *E. coli* higher tolerance to thermal stress (Yang et al., 2016). Our studies experimentally verified that overexpression of certain exogenous HSP70 gene significantly enhanced the cell viability under a lethal temperature of 50degC. In addition, overexpression of *NbHSP70* in*E. coli* could greatly promote the growth of *E. coli* , implying a protective effect of HSP70 in cells.

In conclusion, our current study provides insights about the role of HSP70 genes in the thermotolerance with long-term heat acclimation in*N. barkeri* by means of RNA interference, Western blot analysis and heterologous expression. The RNAi of *NbHSP70-1* gene expression increased the susceptibility of *N. barkeri* against high temperature. Heterologous expression can be utilized for *in vivo*functional verification, which should be expanded to gene functional study in other phytoseiid mites. Different expression patterns of the HSP70 genes between two strains revealed that the sustained accumulation of HSP70 genes conferred the thermotolerance of HTAS*N. barkeri* through long-term heat acclimation. Overall, the results in present study provide the molecular basis for future research on determining the correlation between the HSP70 genes and the resistance of HTAS *N. barkeri* to high temperature; meanwhile the divergent constitutive regulation of HSP70 to thermal conducive to flexible adaptability of high trophic organisms to conserve life under extremely hot conditions. Further functional analysis with more*NbHSP70s* and their interactions are warranted to facilitate a better understanding of the adaptive mechanisms against high temperature in *N. barkeri* .

ACKNOWLEDGEMENTS

This work was funded by the National Key R&D Program of China (2017YFD0201000), the National Natural Science Foundation of China (31772223, 31901944). We wish to thank Dr. Dong Wei (College of Plant Protection, Southwest University, China) and Dr. Tianbo Ding (College of Plant Health and Medicine, Qingdao Agricultural University, China) for assistance with improving the manuscript.

DATA ACCESSIBILITY

Gene sequences with GenBank accession number: *NbHSP70-1* (MN399167), *NbHSP70-2* (MN556970), *NbHSP70-3* (MN556969) and *NbHSP70-4* (MN556968).

Transcriptome of *N. barkeri* : No: SRR8061289 in NCBI.

All additional information is given as supporting information.

AUTHOR CONTRIBUTIONS

C.B.T., Y.Y.L. and H.L. conceived and designed the experiments. C.B.T., Y.Y.L. and Y.X.W. performed the experiments, analyzed the data, and drafted the manuscript. W.Q.C and H.L. participated in manuscript drafted and modification. All authors read and approved the final manuscript.

REFERENCES

Angilletta, M.J. (2009). *Thermal adaptation: a theoretical and empirical synthesis* . 198 Madison Avenue, New York, Ny 10016 USA: Oxford Univ Press.

Bahrndorff, S., Marien, J., Loeschcke, V., & Ellers, J. (2010). Genetic variation in heat resistance and HSP70 expression in inbred isofemale lines of the springtail *Orchesella cincta* . *Climate Research* , *43* , 41-47. https://doi.org/10.3354/cr00896

Benoit, J.B. (2010). Water Management by Dormant Insects: Comparisons Between Dehydration resistance during summer aestivation and winter diapause. In: *Aestivation: molecular and physiological aspects* (eds. Navas CA, Carvalho JE). (pp. 209-229). Berlin, Heidelberger Platz 3, D-14197 Berlin, Germany: Springer-Verlag.

Bi, S., Lv, J., Xu, J., Shi, D., Wang, E., Li, G., & Xu, X. (2019). RNAi mediated knockdown of RpL11, RpS2, and tra-2 led to reduced reproduction of *Phytoseiulus persimilis* . *Experimental and Applied Acarology* , *78* , 505-520. https://doi.org/10.1007/s10493-019-00403-2

Chen, W., Li, D., Zhang, M., Zhao, Y., Wu, W., & Zhang, G. (2015) Cloning and differential expression of five heat shock protein genes associated with thermal stress and development in the polyphagous predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae). *Experimental and Applied Acarology* , *67* , 65-85. https://doi.org/10.1007/s10493-015-9933-0

Chen, Z., Farrell, A.P., Matala, A., & Narum, S.R. (2018) Mechanisms of thermal adaptation and evolutionary potential of conspecific populations to changing environments. *Molecular Ecology* , *27* , 659-674. https://doi.org/10.1111/mec.14475

Cheng, W., Li, D., Wang, Y., Liu, Y., & Zhu-Salzman, K. (2016) Cloning of heat shock protein genes (hsp70, hsc70 and hsp90) and their expression in response to larval diapause and thermal stress in the wheat blossom midge, *Sitodiplosis mosellana* . *Journal of Insect Physiology* , *95* , 66-77. https://doi.org/10.1016/j.jinsphys.2016.09.005

Colinet, H., Overgaard, J., Com, E., & Sorensen, J.G. (2013) Proteomic profiling of thermal acclimation in *Drosophila melanogaster* . *Insect Biochemistry and Molecular Biology* , *43* , 352-365. https://doi.org/10.1016/j.ibmb.2013.01.006

Coombs, MR., & Bale, J.S. (2013) Comparison of thermal activity thresholds of the spider mite predators*Phytoseiulus macropilis* and *Phytoseiulus persimilis*(Acari: Phytoseiidae). *Experimental and Applied Acarology* ,*59* , 435-445. https://doi.org/10.1007/s10493-012-9619-9

Cuenca Cambronero, M., Beasley, J., Kissane, S., & Orsini, L. (2018) Evolution of thermal tolerance in multifarious environments.*Molecular Ecology* , *27* , 4529-4541. https://doi.org/10.1111/mec.14890

Danks, H.V. (2000) Dehydration in dormant insects. *Journal of Insect Physiology* , *46* , 837-852. https://doi.org/10.1016/s0022-1910(99)00204-8

Fan, Y., & Petitt, F.L. (1994) Functional response of *Neoseiulus barkeri* Hughes on two-spotted spider mite (Acari: Tetranychidae). *Experimental and Applied Acarology* , *18* , 613-621.

Feder, M.E., & Hofmann, G.E. (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology* , *61* , 243-282. https://doi.org/10.1146/annurev.physiol.61.1.243

Ghazy, N.A., Osakabe, M., Negm, M.W., Schausberger, P., Gotoh, T., & Amano, H. (2016) Phytoseiid mites under environmental stress. *Biological Control* , *96* , 120-134. https://doi.org/10.1016/j.biocontrol.2016.02.017

Gu, L.L., Li, M.Z., Wang, G.R., & Liu, X.D. (2019) Multigenerational heat acclimation increases thermal tolerance and expression levels of Hsp70 and Hsp90 in the rice leaf folder larvae. *Journal of Thermal Biology* , *81* , 103-109. https://doi.org/10.1016/j.jtherbio.2019.02.024

Hayward, S.A.L., Rinehart, J.P., & Denlinger, D.L. (2004) Desiccation and rehydration elicit distinct heat shock protein transcript response in flesh fly pupae. *Journal of Experimental Biology* , *207* , 963-971.

Hu, J.T., Chen, B., & Li, Z.H. (2014) Thermal plasticity is related to the hardening response of heat shock protein expression in two Bactrocera fruit flies. *Journal of Insect Physiology* , *67* , 105-113. https://doi.org/10.1016/j.jinsphys.2014.06.009

Jena, R., Garg, D.K., Choudhury, L., Saini, A., & Kundu, B. (2018) Heterologous expression of an engineered protein domain acts as chaperone and enhances thermotolerance of*Escherichia coli* . *International Journal of Biological Macromolecules* , *107* , 2086-2093. https://doi.org/10.1016/j.ijbiomac.2017.10. 081

Jiang, C.H., Xu, J.Y., Zhang, H., Zhang, X., Shi, J.L., Li, M., & Ming, F. (2009) A cytosolic class I small heat shock protein, *RcHSP17.8* , of *Rosa chinensis*confers resistance to a variety of stresses to *Escherichia coli* , yeast and *Arabidopsis thaliana* . *Plant Cell and Environment* , *32* , 1046-1059. https://doi.org/10.1111/j.1365-3040.2009.01987.x

Joly, A.L., Wettstein, G., Mignot, G., Ghiringhelli, F., & Garrido, C. (2010) Dual role of heat shock proteins as regulators of apoptosis and innate immunity. *Journal of Innate Immunity* , *2* , 238-247. https://doi.org/10.1159/000296508

Junprung, W., Supungul, P., & Tassanakajon, A. (2019) *Litopenaeus vannamei* heat shock protein 70 (*LvHSP70*) enhances resistance to a strain of *Vibrio parahaemolyticus* , which can cause acute hepatopancreatic necrosis disease (AHPND), by activating shrimp immunity. *Developmental and Comparative Immunology* , *90* , 138-146. https://doi.org/10.1016/j.dci.2018.09.011

Kim, M., Lee, S., Chun, Y.S., Na, J., Kwon, H., Kim, W., & Kim, Y. (2017) Heat tolerance induction of the indian meal moth (Lepidoptera: Pyralidae) is accompanied by upregulation of heat shock proteins and polyols. *Environmental Entomology* ,*46* , 1005-1011. https://doi.org/10.1093/ee/nvx112

King, A.M., & MacRae, T.H. (2015) Insect heat shock proteins during stress and diapause. *Annual Review of Entomology* , *60* , 59-75. https://doi.org/10.1146/. annurev-ento-011613-162107

Kostal, V., & Tollarova-Borovanska, M. (2009) The 70 kDa heat shock protein assists during the repair of chilling injury in the insect, *Pyrrhocoris apterus* . *PLoS One* , *4* .

https://doi.org/10.1371/journal.pone.0004546

Li, Y.Y., Zhang, G.H., Tian, C.B., Liu, M.X., Liu, Y.Q., Liu, H., & Wang, J.J. (2017) Does long-term feeding on alternative prey affect the biological performance of*Neoseiulus barkeri* (Acari: Phytoseiidae) on the target spider mites? *Journal of Economic Entomology* , *110* , 915-923. https://doi.org/10.1093/jee/tox055

Livak, K.J., & Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta^{\sim}\tau}$ Method.*Methods* , *25* , 402-408.

Lopez-Martinez, G., Benoit, J.B., Rinehart, J.P., Elnitsky, M.A., Lee, R.E., & Denlinger, D.L. (2009) Dehydration, rehydration, and overhydration alter patterns of gene expression in the Antarctic midge, *Belgica antarctica* .*Journal of Cellular and Comparative Physiology* , *179* , 481-491. https://doi.org/ 10.1007/s00360-008-0334-0

Lu, K., Chen, X., Liu, W., Zhang, Z., Wang, Y., You, K., ... Zhou, Q. (2017) Characterization of heat shock protein 70 transcript from *Nilaparvata lugens* (Stal): its response to temperature and insecticide stresses. *Pesticide Biochemistry and Physiology* , *142* , 102-110. https://doi.org/10.1016/j.pestbp. 2017.01.011

Lu, K., Chen, X., Liu, W., & Zhou, Q. (2016) Characterization of heat shock cognate protein 70 gene and its differential expression in response to thermal stress between two wing morphs of *Nilaparvata lugens* (Stal). *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology* ,*199* , 47-53. https://doi.org/10.1016/j.cbpa.2016.05.009

McMurtry, J.A., De Moraes, G.J., & Sourassou, N.F. (2013) Revision of the lifestyles of Phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. *Systematic and Applied Acarology* , *18* , 297-320. https://doi.org/ 10.11158/ saa.18.4.1

Niu, J.Z., Hull-Sanders, H., Zhang ,Y.X., Lin, J.Z., Dou, W., & Wang, J.J. (2014) Biological control of arthropod pests in citrus orchards in China. *Biological Control* ,*68* , 15-22. https://doi.org/10.1016/j.biocontrol.2013.06.005

Ozawa, R., Nishimura, O., Yazawa, S., Muroi, A., Takabayashi, J., & Arimura, G. (2012) Temperature-dependent, behavioural, and transcriptional variability of a tritrophic interaction consisting of bean, herbivorous mite, and predator. *Molecular Ecology* , *21* , 5624-5635. https://doi.org/10.1111/mec.12052

Pomerantz, A.F., & Hoy, M.A. (2015) RNAi-mediated knockdown of transformer-2 in the predatory mite*Metaseiulus occidentalis* via oral delivery of double-stranded RNA. *Experimental and Applied Acarology* , *65* , 17-27. https://doi.org/10.1007 /s10493-014-9852-5

Schmidt-Jeffris, R.A., & Cutulle, M.A. (2019) Non-target effects of herbicides on *Tetranychus urticae* and its predator, *Phytoseiulus persimilis* : implications for biological control. *Pest Management Science* , *75* , 3226-3234. https://doi.org/10.1002/ps.5443

Stavrinides, M.C., Lara, J.R., & Mills, N.J. (2010) Comparative influence of temperature on development and biological control of two common vineyard pests (Acari: Tetranychidae). *Biological Control* , *55* , 126-131. https://doi.org/10.1016/j.biocontrol.2010.07.006

Stavrinides, M.C., & Mills, N.J. (2011) Influence of temperature on the reproductive and demographic parameters of two spider mite pests of vineyards and their natural predator. *BioControl* , *56* , 315-325. https://doi.org/10.1007 /s10526-010-9334-6

Sun, Y., Zhao, J., Sheng, Y., Xiao, Y.F., Zhang, Y.J., Bai, L.X., ... Xu GC (2016) Identification of heat shock cognate protein 70 gene (*Alhsc70*) of *Apolygus lucorum* and its expression in response to different temperature and pesticide stresses. *Insect Science* , *23* , 37-49. https://doi.org/10.1111/1744-7917.12193

Tian, C.B., Li, Y.Y., Wang, X., Fan, W.H., Wang, G., Liang, J.Y., ... Liu, H. (2019) Effects of UV-B radiation on the survival, egg hatchability and transcript expression of antioxidant enzymes in a high-

temperature adapted strain of*Neoseiulus barkeri* . *Experimental and Applied Acarology* ,*77* , 527-543. https://doi.org/10.1007/s10493-019-00361-9

Tian, C.B., Li, Y.Y., Huang, J., Chu, W.Q., Wang, Z.Y., & Liu, H. (2020) Comparative transcriptome and proteome analysis of heat acclimation in predatory mite *Neoseiulus barkeri* . *Frontiers in Physiology* , *11* . https://doi.org/10.3389/fphys. 2020.00426

Vangansbeke, D., Duarte, M.V.A., Gobin, B., Tirry, L., Wackers, F., & De Clercq, P. (2020) Cold-born killers: exploiting temperature-size rule enhances predation capacity of a predatory mite. *Pest Management Science* , *76* , 1841-1846. https://doi.org/10.1002/ps.5713

Voigt, W., Perner, J., Davis, A.J., Eggers, T., Schumacher, J., Bahrmann, R., ... Sander ,F.W. (2003) Trophic levels are differentially sensitive to climate. *Ecology* ,*84* , 2444-2453. https://doi.org/10.1890/02-0266

Wang, C., Yang, J., Pan, Q., Yu, S., Luo, R., Liu, H., ... Ran, C. (2018) Screening of reference genes using real-time quantitative PCR for gene expression studies in*Neoseiulus barkeri* Hughes (Acari: Phytoseiidae). *Bulletin of Entomological Research* , 1-10. https://doi.org/10.1017/S000748531800072X

Wu, K., & Hoy, M.A. (2014) Oral delivery of double-stranded RNA induces prolonged and systemic gene knockdown in *Metaseiulus occidentalis* only after feeding on*Tetranychus urticae* . *Experimental and Applied Acarology* ,*63* , 171-187. https://doi.org/10.1007/s10493-014-9772-4

Wu, K., & Hoy, M.A. (2015) Cloning and functional characterization of two BTB genes in the predatory mite *Metaseiulus occidentalis* . *PLoS One* , *10* , e0144291. https://doi.org/10.1371/journal.pone.0144291

Wu, S., Gao, Y., Xu, X., Wang, E., Wang, Y., & Lei, Z. (2014) Evaluation of *Stratiolaelaos scimitus*and *Neoseiulus barkeri* for biological control of thrips on greenhouse cucumbers. *Biocontrol Science and Technology* ,*24* , 1110-1121. https://doi.org/10.1080/09583157.2014.924478

Xu, Y.J., Liu, L., Yang, Y., Wang, X., Tian, C.B., Li, Y.Y., ... Liu, H. (2018) Identification of four heat shock protein genes and their expression in response to thermal stress in two strains of *Neoseiulus barkeri* (Acari: Phytoseiidae). *Systematic and Applied Acarology* , *23* , 665. https://doi.org/ 10.11158/saa.23.4.8

Yang, X.Q., Zhang, Y.L., Wang, X.Q., Dong, H., Gao, P., & Jia, L.Y. (2016) Characterization of multiple heat-shock protein transcripts from *Cydia pomonella* : their response to extreme temperature and insecticide exposure. *Journal of Agricultural and Food Chemistry* , *64* , 4288-4298. https://doi.org/10.1021/acs.jafc.6b01914

Zhang, G.H., Li, Y.Y., Tian, C.B., Xu, Y.J., Zhou, H.W., Huang, J., ... Liu, H. (2018) Intraspecific variations on thermal susceptibility in the predatory mite*Neoseiulus barkeri* Hughes (Acari: Phytoseiidae): responding to long-term heat acclimations and frequent heat hardenings.*Biological Control* , *121* , 208-215. https://doi.org/10.1016/j.biocontrol.2018.03.004

Zhang, X., Lv, J., Hu, Y., Wang, B., Chen, X., Xu, X., & Wang, E. (2015) Prey preference and life table of *Amblyseius orientalis* on*Bemisia tabaci* and *Tetranychus cinnabarinus* . *PLoS One* , *10* , e0138820. https://doi.org/ 10.1371/journal.pone.0138820

FIGURE LEGENDS

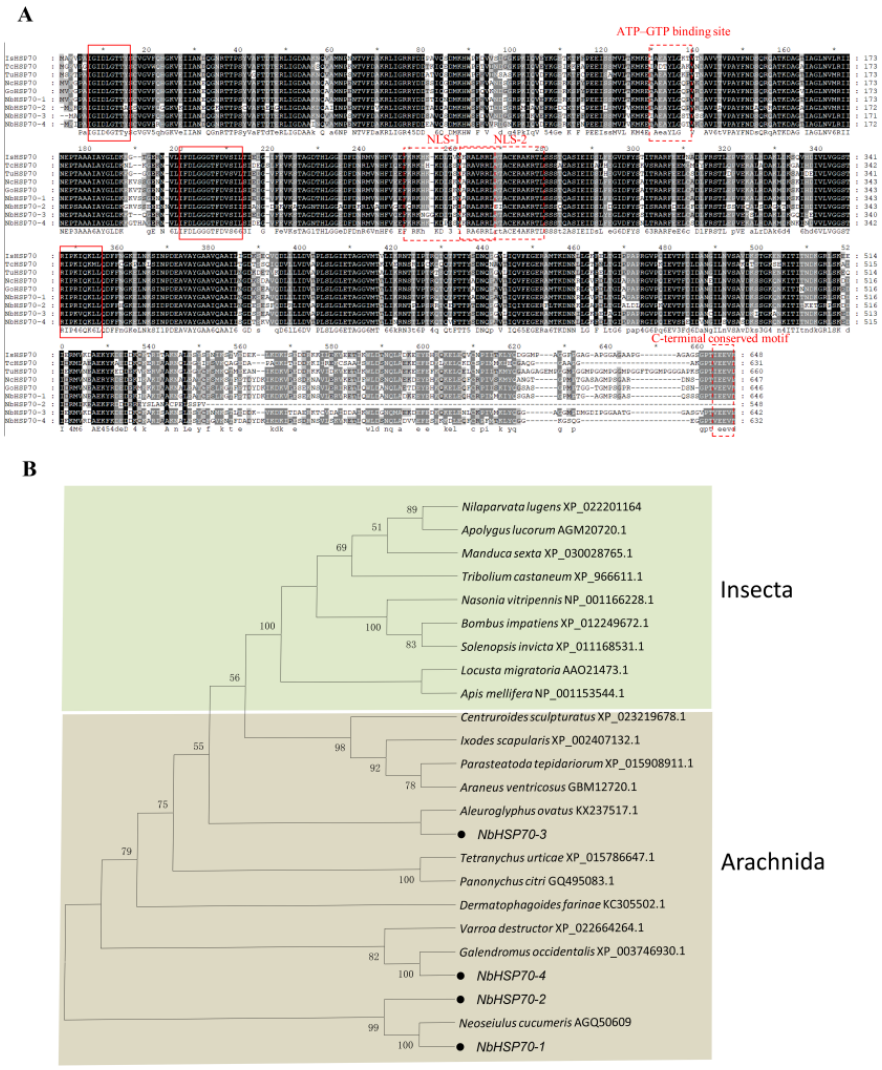


FIGURE 1. Multiple alignment and phylogenetic analysis. (A) Multiple alignment of the deduced amino acid sequence of the*NbHSP70s* with other species. Is: *Ixodes scapularis*XP_002407132.1; Tc: *Tribolium castaneum* XP_966611.1; Tu: *Tetranychus urticae* XP_015786645.1; Nc: *Neoseiulus cucumeris* AGQ50609.1; Go: *Galendromus occidentalis*XP_003738291.1. The well-conserved regions are marked in the box with solid lines. The sequence in the box with dashed lines showed the putative ATP-GTP binding site and NLS sites. (B) Phylogenetic relationships of *NbHSP70s* from different species**.** A phylogenetic analysis was conducted based on a multiple alignment of amino acid sequences deposited in the GenBank database. Numbers at the nodes are bootstrap values (1000 replications).

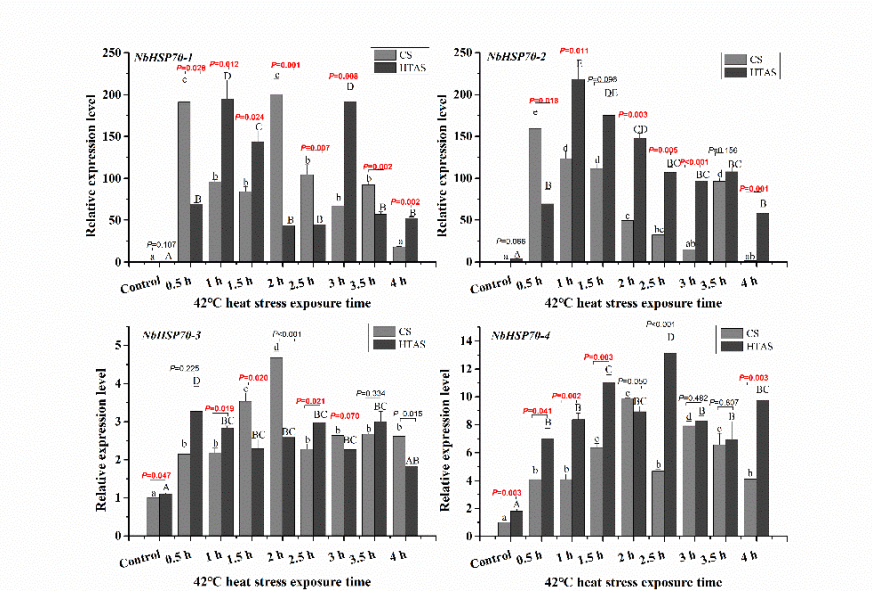


FIGURE 2. The transcript expression levels of *NbHSP70s* under 42°C heat stress. Each value represents the mean ± SE. Different lower case letters or capital letters above the bar indicated a significant difference among treatments within CS or HTAS mites, respectively, P <0.05, *Tukey's HSD* in one-way ANOVA; P value represents the significant difference of expression of *NbHSP70s* between two strains when exposed to the same treatment for a Student's t -test between CS and HTAS mites.

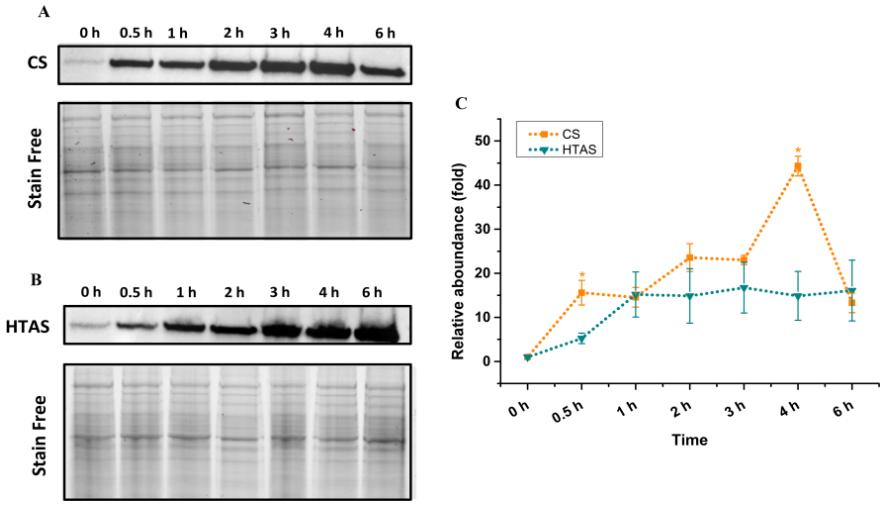


FIGURE 3. Analysis of the protein level of HSP70 in female adults of CS and HTAS strains under 42°C. (A) The protein level of HSP70 was detected in the CS strain by Western blotting. (B) The protein level of HSP70 was detected in the HTAS strain by Western blotting. (C) The graph indicates the quantitative estimation of the protein level by the standard procedure of Stain-Free technology. The relative protein levels at 0 h were assigning as 1 and the protein levels under heat stress were calculated. Error bar of each dot represents stand error of the mean from three biological replicates. The asterisk represents significant difference, while * indicates $P < 0.05$ (Student's t -test; n=3).

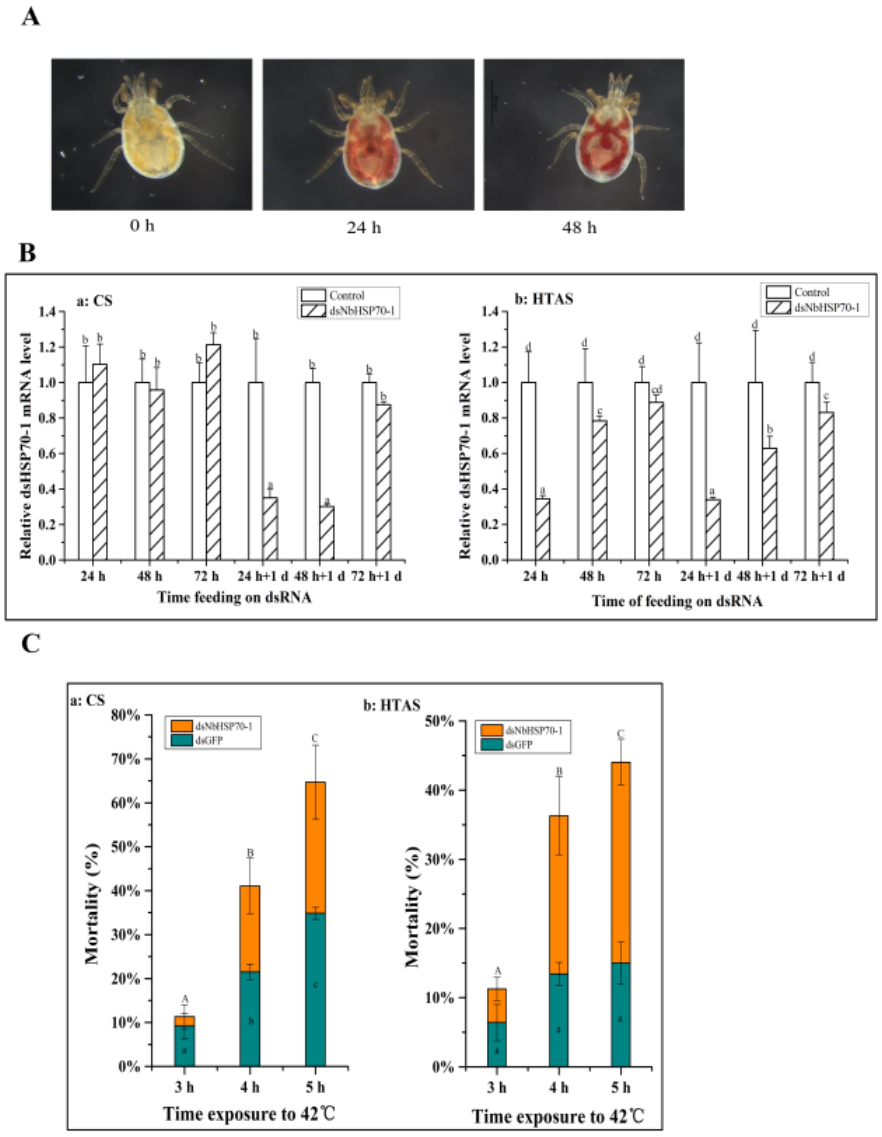


FIGURE 4. Gene knockdown of *NbHSP70-1*. (A) Female*Neoseiulus barkeri* adults consumed dsRNA with red dye for 0 h (a), 24 h (b) and 72 h (c) (Scale bar: 200 μm). (B) Effects of oral delivery of control and *NbHSP70-1* dsRNA on the expression of the*NbHSP70-1* gene assessed by qRT-PCR in *Neoseiulus barkeri*females. (a) Effect of RNAi of *NbHSP70-1* expression in the CS strain. (b) Effect of RNAi on *NbHSP70-1* expression in the HTAS strain. *NbHSP70-1* mRNA levels (open bars) in control dsGFP-treated mites were scaled to 1. *NbHSP70-1* mRNA levels in*NbHSP70-1* -dsRNA-treated females are illustrated by filled bars.*Error bars* = SEM and n = 4 for each data point. Data points labeled with different letters within the same type of dietary treatment at the same time period are significantly different (P <0.05, *Tukey's HSD* in one-way ANOVA). (C) High temperature susceptibility to 42°C of CS and HTAS strain females after RNAi. (a) The susceptibility to 42°C after RNAi of the CS strain. (b) The susceptibility to 42°C after RNAi of the HTAS strain.

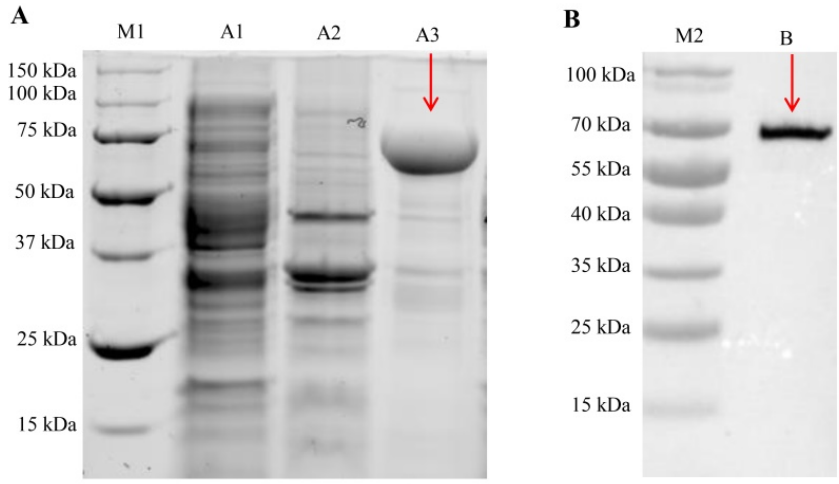


FIGURE 5. SDS-PAGE and Western blot analysis of recombinant HSP70 from *Neoseiulus barkeri*. (A) The SDS-PAGE analysis of recombinant HSP70. M1: Protein standards; A1: Control; A2: Sediment; A3: Supernatant. (B) The Western blot analysis of recombinant HSP70. M2: Protein standards; B: Recombinant protein of HSP70.

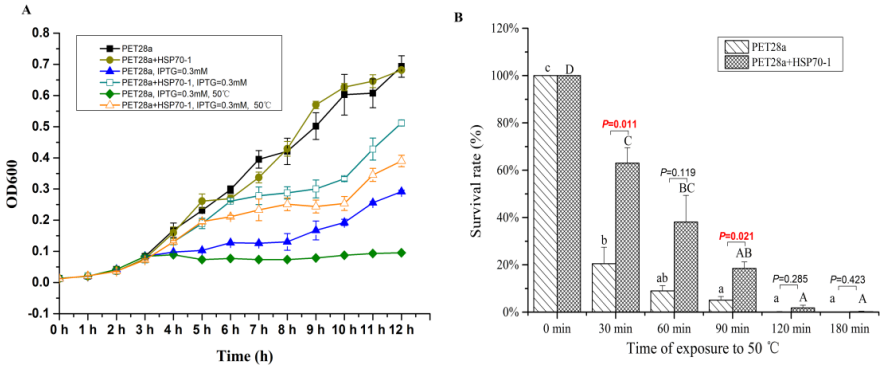


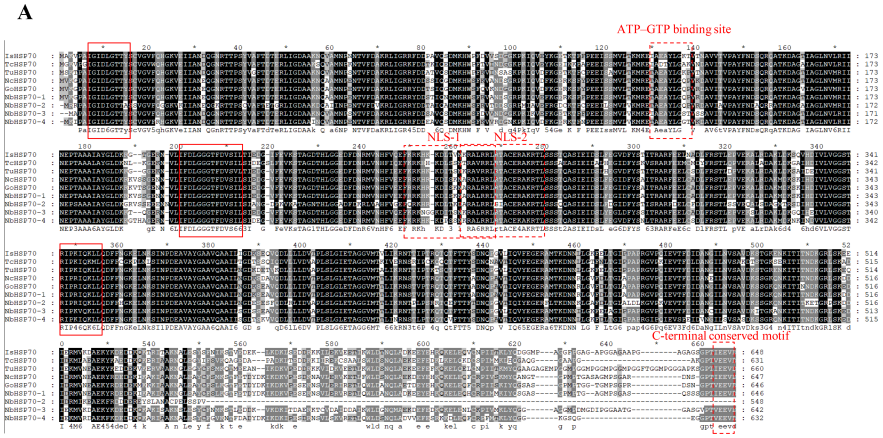
FIGURE 6. Heat tolerance assay for *E. coli*. (A) The growth curve of BL21 (PET28a-NbHSP70-1) and BL21 (PET28a). (B) The survival ratio of BL21 cells with or without over-expressing HSP70 after heat treatment at 50°C. The white bars represent the cells without over-expressing HSP70. The black bars represent the cells with over-expressing HSP70.

SUPPLEMENTARY FILES

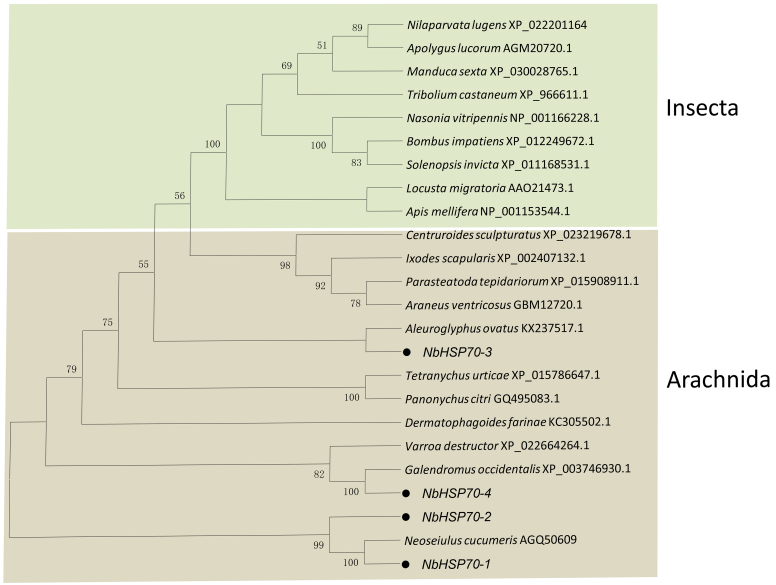
Table S1: Primers used in clone, qRT-PCR, RNAi and heterologous expression.

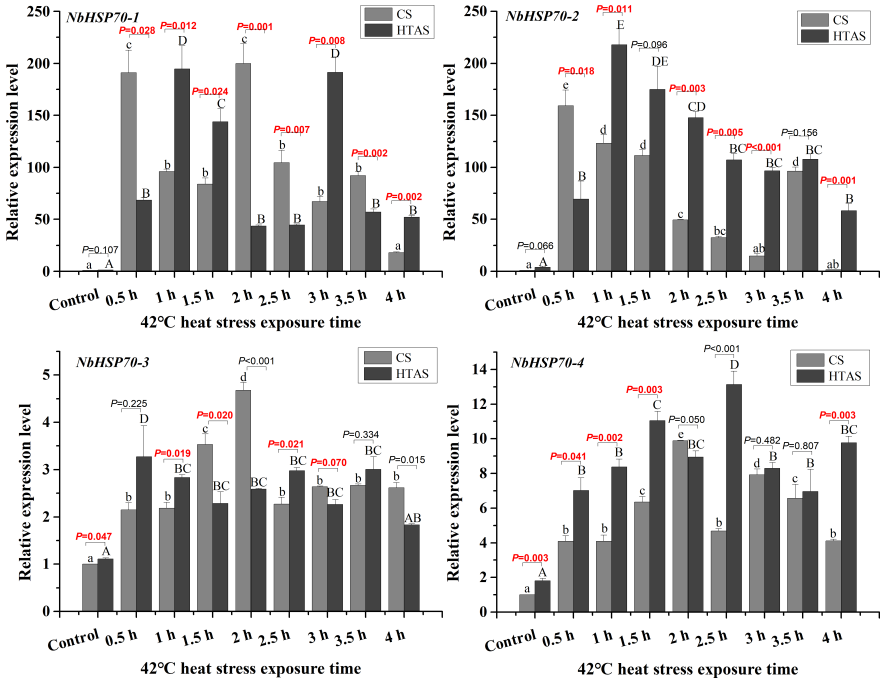
Table S2. Pairwise comparisons between the HSP70 sequences in the *Neoseiulus barkeri*.

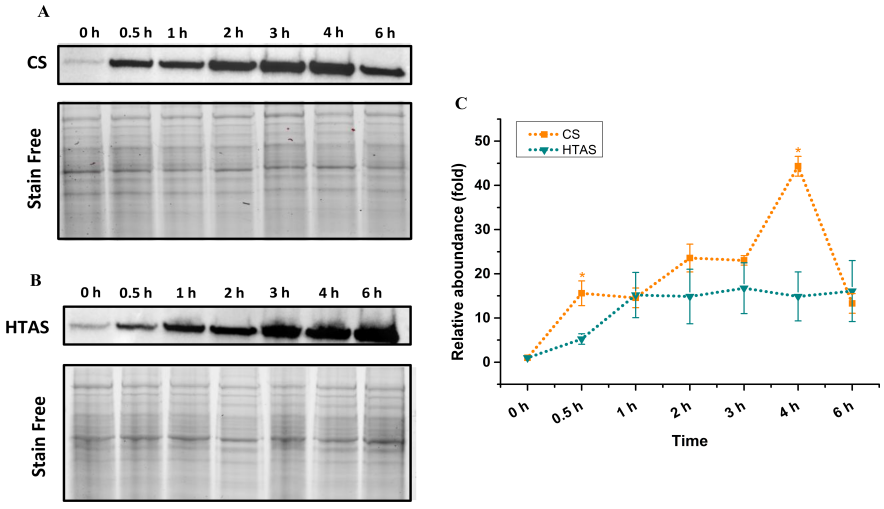
A



B

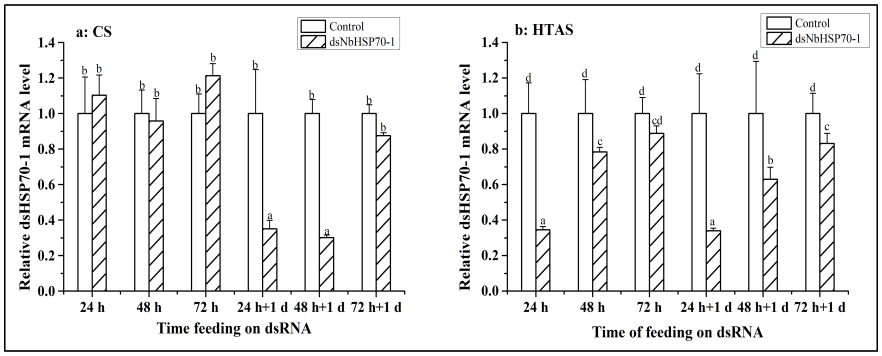






A

B



C