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Research paper

Starvation-, thermal- and heavy metal- associated expression of four small heat shock protein genes in *Musca domestica*

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ABSTRACT

In this study, starvation-, thermal- and heavy metal-associated expression of four small *Musca domestica* HSPs (abbreviated as *Mdom*HSPs and they are *Mdom*HSP10, *Mdom*HSP27, *Mdom*HSP27.1 and *Mdom*HSP27.2) were determined. The following results were found: All *Mdom*HSPs were significantly higher expressed during the active larval and adult stages than the egg and pupal stages; All *Mdom*HSPs were expressed at relatively equal levels in the head, thorax and abdomen of adults; The expression of *Mdom*HSP27 was significantly down-regulated in 4-day-old larvae that were starved for 6 h, while the other 3 *Mdom*HSPs were not significantly affected; Thermal treatment altered the expression of *Mdom*HSPs in 4-day-old larvae: *Mdom*HSP10 was significantly down-regulated in 4-day-old larvae that were maintained at 4 °C and 37 °C than in those that were maintained at 25 °C; Lead, cadmium and chromium exposure influenced larval expression of *Mdom*HSPs to varying degrees. The expression dynamic profile of *Mdom*HSPs would contribute to the understanding of their physiological role in *M. domestica*.

1. Introduction

Heat shock proteins (HSPs) are ubiquitous among various types of organisms that are connected with multiple physiological processes, which are named from their earliest thermal responses (Carper et al., 1987; Skowyra and Zylicz, 1987; Feder and Hofmann, 1999). Based on their molecular weights and phylogenetic homology, HSP family members are divided into five types: HSP100, HSP90, HSP70, HSP60, and small HSPs (sHSPs) (Parsell and Lindquist, 1993; Nguyen et al., 2016). Insect HSPs also include sHSPs, HSP60, HSP70 and Hsp90 as four major HSP families which are also associated with various environmental factors, such as heat/cold, anoxia and starvation (King and Macrae, 2014). Researches of insect small HSPs started since the first identification of sHSPs in *Drosophila melanogaster* (Tissiéres et al., 1974). Thus far, many sHSPs have been identified from many other insects, especially those insects with genome, transcriptome or other sequencing information (Gusev et al., 2011; Scott et al., 2014; Lu et al., 2015; Wang et al., 2015).

Insect sHSPs are involved in physiological activities, especially in stresses (e.g., thermal and oxidative stresses) (King and Macrae, 2014). For example, HSP27 of *D. melanogaster* was associated with starvation resistance (Hao et al., 2014) and *HSP22*, *HSP23*, *HSP26* and *HSP27* could be induced by cold (Colinet et al., 2009). The expression of *HSP27* in *Chironomus riparius* was sensitive to temperature variations (Martínez-Paz et al., 2014). In the flesh fly *Sarcophaga crassipalpis*, *HSP23*was highly up-regulated during cold induced diapause (Yocum et al., 1998). The expression of multiple sHSPs of *Plutella xylostella* responded differently to heavy metals and *HSP27* gene in *C. riparius* (Diptera) had dose effect by cadmium (Cd) (Martínez-Paz et al., 2014; Chen and Zhang, 2015). In addition to their role in stress, insect sHSPs have also been reported to be associated with developmental and reproductive processes (Michaud et al., 1997; Michaud et al., 2002; Lu et al., 2015). Some insect sHSPs presented stage- and tissue-specific expression features. For instance, *HSP23*, *HSP26* and *HSP27* were highly expressed in the nervous system and in the gonads of *D. melanogaster* (Marin et al., 1993).

Housefly *Musca domestica* (Diptera: Muscidae) is a vector and resource insect (Abbas et al., 2014; Bahrndorff et al., 2014). This insect is

Abbreviations: Cd, cadmium; Cr, chromium; EF1, elongation factor 1; H, high dose; L, low dose; MdomHSP, heat shock protein genes in *Musca domestica*; MEGA, molecular evolutionary genetics analysis; Pb, Lead; PCR, polymerase chain reaction; Rpl8, ribosomal large subunit 8; sHSP, small heat shock protein; TUB, tubulin

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distributed worldwide and highly adaptable to various environments, such as starvation, unsuitable temperature, bacterial and hazard metal challenge (Coulson and Bale, 1990; Tiwari and Mohan, 1997; Hicks et al., 2004; Wang et al., 2006; Larrain and Salas, 2008; Zhang et al., 2012; Kjærsgaard et al., 2015). For *Musca domestica* HSPs (*Mdom*HSPs), most of the work was conducted on *Mdom*HSP70 and *Mdom*HSP60. For example, *Mdom*HSP70 could be induced by insecticide dimethoate and alkilobenzene sulfonate in a dosage effect and proved to function in the immune processes during heat shock, Cd stress and bacterial challenge (Pyza et al., 1997; Tang et al., 2012). *Mdom*HSP60 contributed to the development and maturation of eggs in *M. domestica* (Sharma et al., 2007). However, few studies were known on small HSPs in *M. domestica*, which might facilitate the understanding of heat shock genes in response studies, especially in stress condition (Colinet et al., 2009).

Therefore, development, tissue specific expressions of four small HSPs in *M. domestica* (*Mdom*HSP10, *Mdom*HSP27, *Mdom*HSP27.1, *Mdom*HSP27.2) and starvation, thermal and heavy metal associated expressions of these *Mdom*HSPs were explored in this study, which would contribute to the understanding of their physiological responses in *M. domestica*.

2. Materials and methods

2.1. Insect sampling

Houseflies were obtained from the laboratory population of the housefly *Musca domestica* at the Hubei International Scientific and Technological Cooperation Base of Waste Conversion by Insects (Huazhong Agricultural University, China). The light: dark cycle was 13 h:11 h, and the temperature was 25 ± 3 °C in the housefly rearing room. Adults were kept in nylon mesh cages (40 × 30 × 35 cm) and supplied with water and sugar and the total count of adults per cage was approximately 2000 individuals. Eggs were laid on a small plastic box of moist wheat bran and then transferred to large plastic boxes with sufficient moist wheat bran. For all treatment, larva density and rearing condition were constant: every 15 g of wheat bran was homogenized with 30 mL of distilled water to make feed for 50 larvae in plastic box (10 diameter × 5 height cm) with cotton cloth cover. All samples were prepared in triplicate and the following description was for a biological repetition of sampling procedure.

2.2. Experimental treatments

2.2.1. Development

Insects were reared as introduced. For various developmental stages, twenty eggs, five 4-day-old larvae, five pupae and five 6-day-old males and five 6-day-old females were sampled and quick-froze in liquid nitrogen before stored at − 80 °C refrigerator. Males and females were further mixed as groups of adults. *Tissues*: The head, thorax and abdomen of 20 6-day-old adult houseflies were dissected and quick-froze in liquid nitrogen before stored at − 80 °C refrigerator.

2.2.2. Starvation

Twenty 4-day-old larvae were separated from wheat bran feed and placed into petri dishes (9 cm in diameter) with wet filter paper for 6 h and then ten of them were randomly sampled and quick-froze in liquid nitrogen before stored at − 80 °C refrigerator. Meanwhile, the same number of normal-feeding 4-day-old larvae were separated in the same way as the treatment and also placed in petri dishes (9 cm in diameter) with new wheat bran feed as a control group. No death occurred in the treatment and the control group.

2.2.3. Diverse temperature treatments

Three groups of twenty 4-day-old larvae were placed into petri dishes (9 cm in diameter) with moist wheat bran, transferred into a constant temperature incubator at the temperatures of 4 (Bluepard®, Yiheng science apparatus company limited, Shanghai), 25 and 37 °C (HP250GS, Ruihua science apparatus company limited, Shanghai) and then placed for 2 h. Then ten of them were sampled and quick-froze in liquid nitrogen before stored at − 80 °C refrigerator. No death occurred in this treatment.

2.2.4. Heavy metal treatments

The routine rearing one-day-old larvae were used for heavy metal treatments. Then 50 one-day-old larvae were reared with feeds with or without metals according to proportion 15 g solid per 30 mL water or metal solution. The metal solution was lead (Pb^{2+}, lead nitrate) solution, cadmium (Cd^{2+}, cadmium chloride) solution and chromium (Cr^{6+}, potassium chromate) solution. These solutions were applied to the wheat bran feed as water source and water without saline was kept as the control group for heavy metal treatments. The doses were defined as follows: low dose (1×10^{-4} mol/L, abbreviated as L) and high dose (1×10^{-3} mol/L, abbreviated as H). For individual ions, the dose was calculated as follows: low (PbL, 41.44 mg/kg dry weight) and high (PbH, 414.40 mg/kg dry weight); low (CdL, 22.48 mg/kg dry weight) and high (CdH, 224.82 mg/kg dry weight); low (CrL, 10.40 mg/kg dry weight) and high (CrH, 103.99 mg/kg dry weight). Then ten 4-day-old larvae were sampled from each group. All the sampling was performed by immediately chilled in liquid nitrogen and then transferred into the − 80 °C refrigerator before use.

2.3. Phylogenetic analysis

Phylogenetic analysis was performed with MEGA 6.0 software with Neighbor-Joining tree method (Tamura et al., 2013). Previously, four small *M. domestica* HSPs (*MdomHSPs*) were identified as small HSPs from transcriptome database which were submitted to the National Center for Biotechnology Information (NCBI) under the accession number of PRJNA268405. These genes were cloned, sequenced and then submitted to NCBI (AQY54361, AQY54362, AQY54363 and AQY54358).

2.4. RNA and cDNA preparation

RNA from samples was extracted with TRIzol® Reagent (ThermoFisher Scientific, China) according to the product manual. RNA concentration and purity were measured for quality validation by Nanodrop 2000 Spectrophotometer (ThermoFisher Scientific, USA). RNA with a value of OD260/280 ratio between 1.9 and 2.1were applied into further cDNA synthesis. Approximately 2 μg of quality total RNA was applied to produce first strand DNA with a cDNA synthesis kit (NEWBIO Tech., Canada).

2.5. Quantitative real-time polymerase chain reaction

The primers of target and reference genes are listed in Table 1. Quantitative real-time polymerase chain reaction (qPCR) was performed with (SYBR Green) Real Master Mix (NEWBIO Tech., Canada) on a Bio-Rad iQ5 Optical System (Bio-Rad, USA). The qPCR conditions were set as follows: 95 °C for 30 s; 40 cycles of 95 °C for 5 s, 60 °C for 20 s and 72 °C for 20 s; and then 72 °C for 2 min.

2.6. Statistical analysis

The relative expression of HSPs was calculated by the $2^{-\triangle\triangle CT}$ method (Schmittgen and Livak, 2008). The expression of HSPs was firstly normalized with the relative expression of a reference gene. Then relative expressions of treatments were normalized to that of control groups. Some studies had provided valuable reference genes before (Wang et al., 2006; Bahrndorff et al., 2014), however, they had not been validated in this study. Rpl8 could be used as reference gene for development samples; EF1 could be used as reference gene for tissue,