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Transcriptome analysis reveals heat tolerance of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) adults

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ABSTRACT

Tribolium castaneum is one of the most destructive insects infesting cereals worldwide. Heat treatment, as one of the promising alternatives to chemical control, has been widely applied to disinfest empty grain storage spaces and food processing facilities. The influence of acclimation to sublethal high temperatures (36 and 42 °C for 10 h) on the heat tolerance of *T. castaneum* adults was investigated, and the transcriptome of *T. castaneum* adults was sequenced using Illumina sequencing technology for exploring the heat tolerance mechanism of *T. castaneum* adults. Acclimation to sublethal high temperatures significantly enhanced heat tolerance of *T. castaneum* adults. A total of 12,596 unigene sequences were annotated with *T. castaneum* reference genome. A total of 280, 1134, and 948 DEGs were respectively identified in the 28A-36A, 28A-42A, and 36A-42A. Seventy-three commonly up-regulated genes and 36 commonly down-regulated genes were confirmed in response to heat tolerance from 28A to 36A, and 28A-42A. The results of RT-qPCR analysis were high significantly consistent with the RNA-seq analysis. These results offer a valuable information for future improving the effectiveness of heat treatment and exploring the evolution mechanism of the heat tolerance of *T. castaneum* adults.

1. Introduction

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a worldwide stored products pest that is often found in storage spaces and food-processing facilities. *T. castaneum* infestation often results in significant damage to stored cereals (Hodges et al., 1996; Padın et al., 2002; Shi et al., 2012; Sharon et al., 2014). Due to the increasingly serious challenges to conventional chemical control, such as insect resistance (Liu and Ho, 1999; Jovanović et al., 2007), insecticide residues in food and environment (Popp et al., 2013; Kim et al., 2015), and phase-out of methyl bromide (Fields and White, 2002), the sustainable and environmentally friendly methods are urgently needed for effectively controlling stored products insects.

Therefore, heat treatment, as an environment-friendly and safe physical control method, is one of the promising alternatives to conventional chemical control, which has been widely applied to disinfest empty grain storage spaces and food processing facilities (Fields, 1992; Fields and White, 2002; Roesli et al., 2003; Mahroof et al., 2005; Opit et al., 2011). Usually, the target facility is gradually heated from ambient temperature to 50–60 °C and then maintained the temperature for 24–36 h during the heat treatment (Fields, 1992; Dowdy, 1999; Mahroof et al., 2005; Tilley et al., 2007). The stored products insects naturally experience acclimation to sublethal high temperatures during the heat treatment, which significantly affects insect heat tolerance (Neven, 2000; Johnson et al., 2004; Lachenicht et al., 2010; Lü and Zhang, 2016; Lü and Liu, 2017). Understanding the effect of acclimation to sublethal high temperatures on heat tolerance of *T. castaneum* and its molecular mechanism is in favor of successfully controlling *T. castaneum*, for example, guiding to reasonably design and implement heat treatment strategies in practice.

With the rapid development of the high-throughput next generation sequencing technology in recent years, transcriptomic analysis has been increasingly applied to explore the adaptive molecular mechanism of various insects to the environment (Schuster, 2007; Blow, 2009; Wang et al., 2009; Wang et al., 2011). Particularly, the determination of genome sequence has made the *T. castaneum* become an important model insect for researching molecular mechanism of insects to adapt to ambient environment (Richards et al., 2008; Altincicek et al., 2008). To date, little is

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known about the heat tolerance molecular mechanism of *T. castaneum* with acclimation to sublethal high temperatures. Thus, the purpose of the study was to reveal the heat tolerance molecular mechanism of *T. castaneum* by transcriptome analysis in combination with Real-time PCR (RT-qPCR) in order to determine the effect of acclimation to sublethal high temperatures and its thermotolerance mechanism.

2. Materials and methods

2.1. Insect

The *T. castaneum* adults used in this study were obtained from the Institute of Stored Product Insects of Henan University of Technology, Zhengzhou, China. The adults were maintained at 28 ± 2 °C and 75 ± 5% r.h., and a 0:24 light: dark photoperiod in 10 cm × 10 cm × 20 cm glass bottle filled with sterilized diet (wheatfeed/yeast, 9:1, w/w). All experiments were conducted on unsexed *T. castaneum* adults (14-d-old).

2.2. Experimental protocol of heat stress treatment on T. castaneum

A group of thirty adults were randomly selected and put into one empty vial (2 ml) with a few of small holes for heat air distribution, and respectively exposed to 28 (control), and 36 or 42 °C in a temperature chamber (Thermocenter TN/GDW-010B, Tainuo Experiment Instrument Factory, Wuxi, Jiangsu, China) for 10 h, and the adults were respectively referred to as 28A (control), 36A, and 42A in the paper. Subsequently, 28A, 36A, and 42A were exposed to 50 °C for 25 min, then maintained in a controlled temperature and humidity chamber at 27 ± 2 °C, and 75 ± 5% r.h. Mortality of these adults were determined after 48 h.

2.3. RNA sequencing and data analysis

The 28A, 36A, and 42A were flash-frozen in liquid N_2 and stored at −80 °C. Total RNA was isolated from each sample using Trizol Reagent (Invierogen, USA), and purified with absolute alcohol and treated with DNase (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's instructions. The integrity/quality of the mRNA was evaluated using an Agilent Bioanalyzer 2100 (Agilent Technologies). A total amount of 1 μg RNA per sample was used for library construction and sequencing. mRNAs were enriched by oligo (dT) magnetic beads, and fragmented into short pieces in fragmentation buffer, then using random hexamer primers to synthesize the first and second-strand cDNA. HiSeq 2500 (Illumina) by Beijing Biomarker Technologies CO., LTD (Beijing, China) was applied to perform sequencing analysis.

As a fundamental quality control measurement, Quality Score was used to evaluate base quality of the raw data. Clean reads were obtained by filtering the containing adapter, and low quality reads in which ploy-N accounted for more than 10% and bases (Q scores ≤ 10) were more than 50%. The *T. castaneum* genome sequence was obtained from NCBI (https://www.ncbi.nlm.nih.gov/genome/?term=Tribolium+castaneum). Clean reads were mapped to the *T. castaneum* reference using TopHat 2.0 software (Kim et al., 2013). Gene expression levels were measured using the fragments per kilobase of transcript per million mapped fragments (FKPM) method (Florea et al., 2013). EBSeq was used for differentially expression genes analysis (Leng et al., 2013). The Benjamini-Hochberg's method was used to adjust the resulting p value for controlling the false discovery rate. Gene with a checked p value < 0.05 authenticated by EBSeq were clustered as differentially expressed genes (DEGs). To reveal the molecular mechanism behind the differentially expressed genes, Blast2GO software was used to perform GO annotation (Delanghe, 2005). The WEGO software was used to perform functional classification for all of unigenes. The KOBAS software was used to perform KEGG enrichment analysis of differentially expressed genes (Xie et al., 2011).

2.4. RT-qPCR analysis

To confirm the reliability of the RNA-seq data, four up-regulated genes, including *hsp68a*, *hsp68*b, *LOC663293*, and LOC662168 of *T. castaneum* adults, were selected for RT-qPCR. Two biological replicates and three technical replicates were conducted. Four pairs of specific primers were designed using Primer Premier 5.0 software and synthesized by Sangon (Shanghai, China) (Table 1). cDNA was synthesized using the RevertAid Premium Reverse Transcriptase reagent Kit (Thermo Scientific™ EP0733) according to the manufacturer's instructions. RT-qPCR was performed on the ABI Stepone plus (Applied Biosystems) using the 2xSG Fast qPCR Master Mix (High Rox). Three independent technical replicates were

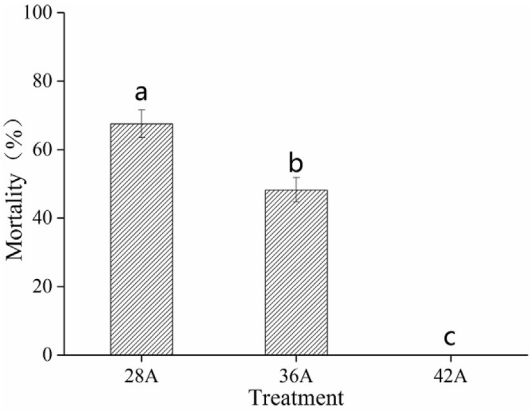

Fig. 1. Mortality of 28A, 36A, and 42A exposed to 50 °C for 25 min.

Table 1
Primer pairs for RT-qPCR.

Gene ID	Primer	ProdSize (bp)	Amplification efficiency (%)	R^2
RpS3-F	TTTGTAGTTTGTTGGCGATGG	176	102.5	0.999
RpS3-R	GAATACGACGGTTCTTCTCCC			
LOC663293-F	ACGAAAATGAAGGAAACCGC	187	96.2	0.999
LOC663293-R	TGTCAAGCCCATAAGCCAAA			
Hsp68b-F	GAAGGCTCGTTGTTTGAAGTAAG	161	101.4	0.999
Hsp68b-R	GCGGTTCTCAATCGTCGTAA			
Hsp68a-F	TTTCCGCCAAAGACACGAG	106	106.8	0.999
Hsp68a-R	TCCGCTTCTGAGACCATCCT			
LOC662168-F	AATCCTCGGTCACTTTCGG	109	98.4	0.999
LOC662168-R	ACGGTCACGGTGTTGTCTTC			