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# Expression and distribution of three heat shock protein genes under heat shock stress and under exposure to *Vibrio harveyi* in *Penaeus monodon*

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ABSTRACT

A sudden increase in temperature results in heat shock stress of the cultured shrimp. To cope with the stress, shrimp has to overcome by triggering a response known as heat shock response. To understand the heat shock response in the black tiger shrimp (*Penaeus monodon*), we examined expression patterns and distribution of three heat shock protein (*hsp*) genes in *P. monodon* juveniles. The expression levels of *hsp21*, *hsp70* and *hsp90* were determined by quantitative real-time PCR in nine tissues (gill, heart, hepatopancreas, stomach, intestine, eyestalk, pleopod, thoracic ganglia and hemocyte) under untreated and heat shock conditions. Under untreated condition, all three *hsp* genes were differentially expressed in all examined tissues where the *hsp70* transcript showed the highest basal level. Under heat shock condition, only *hsp90* was inducible in all nine tissues when comparing to its untreated level. The time-course induction experiment in gill and hepatopancreas revealed that the transcriptional levels of *hsp21*, *hsp70* and *hsp90* were inducible under the heat shock condition and in time-dependent manner. To determine the response of the *hsp* genes upon bacterial exposure, we further determined transcript levels of the *hsp* genes in gill of *P. monodon* after *Vibrio harveyi* injection. The expression levels of *hsp70* and *hsp90* were significantly increased after a 3-h exposure to *V. harveyi* where the *hsp21* transcript was induced later after a 24-h exposure. This evidence suggests for putative roles and involvement of the *hsp* genes as a part of immunity response against *V. harveyi* in *P. monodon*.



## 1. Introduction

Natural and farming environments can be variable and unpredictable. An organism needs mechanisms to adapt to a wide range of the stressful conditions. In some habitats, a stress from daily or seasonal fluctuation of environmental temperature causes an organism to respond by inducing sets of proteins including heat shock proteins (HSPs) and this process is known as a heat shock stress response (Parsell and Lindquist, 1993). The HSPs are ubiquitous, highly conserved and found in all organisms (Kregel, 2002). They play critical roles as molecular chaperones in heat tolerance by repairing and refolding denatured proteins (Morimoto, 1998; Sharma et al., 2009). In addition to heat shock stress, HSPs respond to other factors such as pathogen infection, oxidative stress, heavy metals and xenobiotics stresses (Feder and Hofmann, 1999; Moseley, 2000). The HSPs are found in various forms and categorized into different families based on their molecular weights (kDa) such as HSP110, HSP100, HSP90, HSP70, HSP60 and small HSPs (Parsell and Lindquist, 1993; Feder and Hofmann, 1999). The HSP70 family has been extensively characterized as a primary family of heat shock proteins, and the members in HSP70 family can be constitutive or inducible expressed under heat shock stress (Nover and Scharf, 1997).

The heat shock responses are well characterized in many model organisms such as *Drosophila melanogaster*, mouse and *Arabidopsis*; however, the responses can vary in different organisms (Feder and Hofmann, 1999; Cotto and Morimoto, 1999). In black tiger shrimp (*Penaeus monodon*), one of the important aquaculture species, a complete understanding of how the animal responds to heat shock stress will help improving farming condition to boost the shrimp immunity and protecting them from a risk of disease outbreak. In an attempt to understand heat shock stress response in *P. monodon*, heat shock proteins have previously been characterized by identifying full-length cDNA sequences of *hsp21*, *hsp70* and *hsp90* (Huang et al., 2008; Lo et al., 2004; Jiang et al., 2009). The *hsp21* transcript has been shown to be heat inducible in pleopods and was down-regulated during the infection by White Spot Syndrome Virus (Huang et al., 2008). The *hsp70* was inducible in hemocytes

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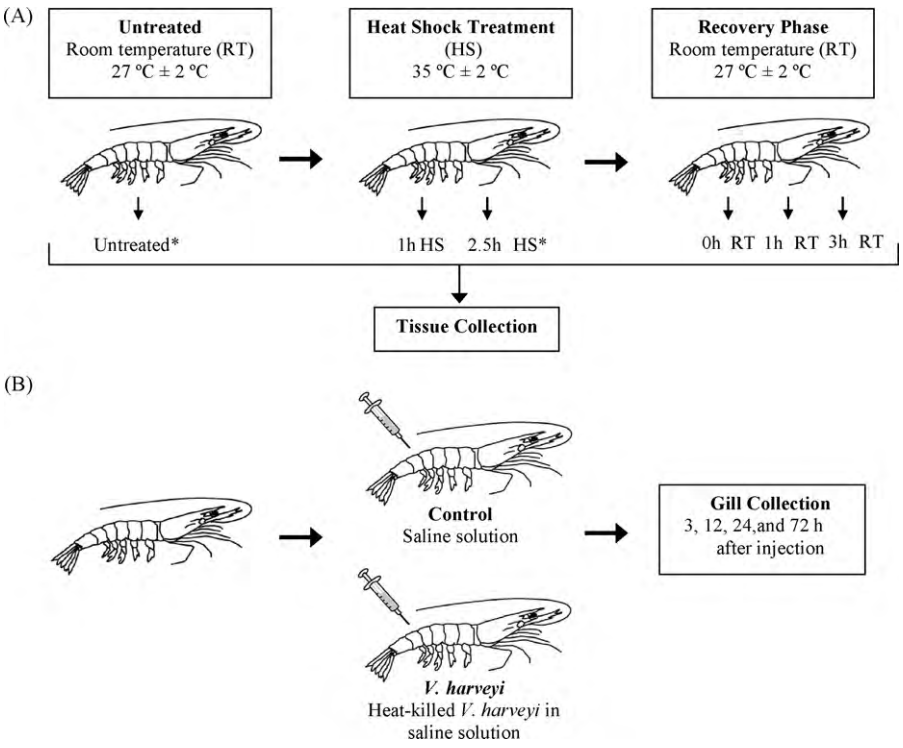


**Fig. 1.** Schematic representation of experiments performed in this study: (A) heat shock and (B) *V. harveyi* experiments. (A) In heat shock (HS) experiment, a group of 4-month old *P. monodon* juveniles was maintained at the room temperature (RT) where tissues were collected as the untreated control. The juvenile shrimp were then placed to a 35 °C seawater tank and tissues were collected at 1 and 2.5 h of exposure time (1 h HS and 2.5 h HS, respectively). For the recovery phase, the juvenile shrimp were returned to the room temperature seawater tank, and tissues were collected immediately after 0 h RT, while the remainders were allowed to recover in the seawater tank for 1 and 3 h (1 h RT and 3 h RT, respectively) before tissue collection. Gill, heart, hepatopancreas, stomach, intestine, eyestalk, pleopod, thoracic ganglia and hemocyte were collected at the sampling time with asterisk (*) ($N = 5$). Otherwise, only gill and hepatopancreas were collected ($N = 5$). (B) In *V. harveyi* experiment, a group of 4-month old juveniles was injected with 100 μl of heat-killed *V. harveyi* 1526 in the saline solution (~2.0 $OD_{600}$) at the lateral side of abdominal muscles, where a control group was injected with 100 μl of the saline solution but without bacterial cells (control). The gill tissues were dissected out from shrimp at 3, 12, 24 and 72 h after the injection ($N = 3$ for each time point).

under heat shock conditions and the increase in the *hsp70* expression was correlated with the reduction of Gill-Associated Virus (GAV) replication (Vega et al., 2006). Similarly, *hsp90* was heat inducible in brain, stomach and heart and may play a role in ovary maturation (Jiang et al., 2009). Nonetheless, the gene expression patterns for *hsp21*, *hsp70* and *hsp90* in *P. monodon* were investigated under different heat shock treatment conditions in their studies.

In this study, we aimed to characterize gene expression patterns of all three *hsp21*, *hsp70*, and *hsp90* transcripts under the same heat shock treatment conditions to give a better understanding of the response to the stress. We also investigated if *hsp* genes were inducible upon the presence of a heat-killed shrimp pathogen. The expression patterns of the three major *hsp* genes (*hsp21, hsp70* and *hsp90*) in nine tissues of *P. monodon* under both untreated and heat shock conditions were examined to validate for inducibility of *hsp* genes under the stress condition. We provided the first report on the time dependency of expression patterns of *hsp21*, *hsp70* and *hsp90* in hepatopancreas and gill tissues during and after heat shock conditions. Moreover, expression patterns of these genes were examined in *P. monodon* upon an exposure to the heat-killed shrimp pathogen, *Vibrio harveyi*, to determine the possible roles of the *hsp* genes as part of the immune response in shrimp.

## 2. Materials and methods

### 2.1. Experimental animals, heat shock treatment and Vibrio harveyi infection

The heat shock treatment was depicted in Fig. 1A. Sixty of 4-month-old *P. monodon* juveniles (13.4 ± 2.0 g) obtained from a farm in Chachoengsao province (Eastern Thailand) were transferred to seawater tanks with aeration and maintained at room temperature (27 ± 2 °C, RT) with salinity at 20 parts per thousand (ppt). In the heat shock experiment, a group of 50 juvenile shrimp was transferred from the RT seawater tank to a 35 ± 2 °C seawater tank. Tissue samples were collected from $t = 0$ (untreated at room temperature (RT), prior to heat shock treatment), heat shock (HS) = 1 and 2.5 h for heat shock treatment duration ($N = 5$ for each time point). Then, the group of remaining juveniles were returned to the RT seawater tank, the tissue samples ($N = 5$ for each time point) were collected at RT = 0 (immediate after placing back to room temperature), RT = 1 and RT = 3 h (1 and 3 h, respectively, after placing back to room temperature). The collected tissues were quickly placed in liquid nitrogen (−80 °C).

For *V. harveyi* infection (Fig. 1B), a group of 25 juvenile shrimp was injected with 100 μl of heat-killed *V. harveyi* 1526 (Rengpipat et al., 2003) (~$2 \times 10^8$ cells/ml) at the lateral side of abdominal muscles, where the control group of 25 juvenile shrimp was injected with the same diluent but without bacterial cells (100 μl of saline solution). The gill tissues were dissected out from shrimp at 3, 12, 24 and 72 h after injection ($N = 3$ for each time point).

### 2.2. RNA extraction and cDNA synthesis

Each tissue sample was ground using a mortar in liquid nitrogen and transferred to TriReagent® (Molecular Research Center). RNA extraction was performed according to supplier's instruction. The RNA pellets were resuspended in 50 μl of RNase-free water and treated with DNaseI (0.5 unit/μg, Promega) for 30 min at 37 °C to remove DNA contamination. The DNA-free RNA was purified by phenol: chloroform extraction and precipitated with 1/10 volume