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BS-001

ISOLATION OF HEAT SHOCK PROTEINS GENE (HSPs-GENE) IN THE SILKWORM, *Bombyx mori* (C301)

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ABSTRACT

Isolation of Genes Encoding Heat Shock Proteins (HSPs) in the Silkworm (*Bombyx mori* C301) performed by RT-PCR method. Primer on the design of a gene bank based on data HSPs genes in the gene bank. HSPs gene isolation begins with the isolation of total RNA derived from silkworm larvae head of the instar V. The results showed that HSPs gene fragment with a length of 750 kb. Based alignment HSP gene fragments with gene banks homolog to *Bombyx mori* of heat shock proteins gene 25.4 with E value of 0.0 (max identity 99%).

Keywords: *Bombyx mori, HSPs, gene isolation, sequencing*

INTRODUCTION

Silkworms are insects are poikilothermic (Sam-Eun, 1998). Silkworms are very easily influenced by environmental conditions. Temperatures vary widely in Indonesia (BPS, 1995). Medan temperature can reach 38^0C, well above the normal temperature growth of silkworm is 20^0C-28^0C (Sam-Eun, 1998). Growth silkworm requires optimum temperature (Singh *et al*., 1997; Brasla and Matei, 1998). Therefore, the high temperature is one of the environmental factors that can cause stress on the silkworms imported. Higher consumtions dry matter intake, reduced feed conversion efficiency silkworms reared at 28°C (Muniraju *et al*., 2004).

The phenomenon that often occurs as a result of the import activity is the interaction between genetic and environmental. Noor (1996), stated that the interaction of genetic and environmental impacts are less favorable for tropical regions, especially programs export-import of eggs. Inclusion policy of quality eggs from a cold or temperate regions to the tropics is not an alternative that is most appropriate. In silkworms, the phenomenon of the interaction between genetic and environmental very influence of the weight, survival and hatchability of eggs (Jakaria et al., 2001). Noor (1996), that one of the approaches that can be done to address the interaction between genetics and environment that is by performing are genetically unable to adapt to the environment without changing its form. Individuals who are able to adapt to changing environmental conditions or capable of displaying more than one form of

morphological, physiological and behavioral status, then it is said to have phenotypic flexibility that allegedly controlled by genes. Phenotypic flexibility phenomenon has been reported to exist in plants, insects, amphibians, fish (Noor, 1996), ducks (Dewantari, 1998) and mice (Mus musculus) (nafiu, 1996).

The success of each strain silkworms depending on the molecular mechanisms of cell response involving heat shock proteins (HSP). This event is a fast synthesis of specific proteins that heat shock protein (Garrido *et al*, 2001; Kregel, 2002; Park *et al*., 2008). Heat shock proteins (HSPs) to act as 'molecular chaperones' to ensure better survival under stress conditions, including thermostress (Santoro, 2000; Parcellier *et al*, 2003; Mosser and Morimoto, 2004) and has been involved in the immunogenicity of cancer/infectious diseases (Srivastava, 2002)

HSP is a type of protein that allows cells to overcome the problem of proteins after stress that can recognize and bind to the affected side. Thus molecular chaperones prevent side groups bound to engage in inappropriate interactions with other cellular components, as well as stabilize the bound proteins in the unfolded state, or the companion can target proteins bound to degradation or removal from the cell. Heat shock protein heat constitutive perform this role to form a new polypeptide or protein that is expressed during normal cellular processes, whereas induced HSPs function in response to protein denaturation due to stress.

METHODOLOGY

Hatching and maintenance Silkworm. Silkworm eggs obtained from pusat persuteraan alam Cantiroto Temanggung Central Java. The eggs hatched each of 100 eggs, then do so hatching egg incubation uniform. Incubation of the eggs is done in a way; propagated in eggs hatching boxes, each box containing 100 eggs and covered with a thin white paper. Having seen the blue spots in the egg, then the egg wrapped in black carbolic to avoid light so that the expected hatching can occur simultaneously. The eggs were observed every day. Three days before the maintenance, the room and all the equipment disinfected using formalin 2-3% by spraying evenly. Disinfectant made to maintain the health of the larvae. After hatching transferred to container maintenance.

Maintenance includes maintenance silkworm I-V instar. Maintenance instar I-II was preceded by hakitate activities that newly hatched handling accompanied by first feeding. The newly hatched (first-instar) desinfected with a mixture of lime and chlorine powder (95: 5), and given that the young mulberry leaves and fresh cut into small pieces, then larvae moved to a box lined with paper or paraffin oil. Feeding is done 3 times a day. At each instar will undergo a period of rest and change of cuticle. If most of the larvae breaks (90%), feeding is stopped and

sprinkled with lime. At the end of each instar separation and capacity adapted to the development of a larvae. Cleaning a larvae, pest and disease management is done on a regular basis.

In instars I and II, respectively cleaning done one time while instar III to V performed 2 times that after the second feeding and before the turn cuticula. Larvae body desinfection carried out every time the larvae change cuticles and before the first feeding. After entering the third larvae instar V, given leaves intact leaves along the branch, regularly placed the tip and base. Silkworm eggs incubated at room temperature ± 28-30^{0}C and maintained until instar V.

Total RNA Isolation. Samples (head larvae instar V) as much as 5 g of crushed with the help of liquid nitrogen in a mortar until it becomes flour. Results scour put Eppendorf already containing 1 ml trizol then homogenized and lysis by pipetting, then vorteks and incubated at room temperature for 5 minutes. Was added 0.2 ml of chloroform, and shake by hand and then incubated for 3 minutes. The mixture was then vorteks and centrifuged at 12000rpm speed at 4°C for 15 minutes. Fluid is moved to the top of the new Eppendorf, added 500µl isoprophyl alcohol and incubated at room temperature for 10 minutes. The mixture was centrifuged at 12000rpm at 4°C for 10 minutes. Fluid discharged, total RNA precipitate was rinsed with 1 ml alcohol 75% and centrifuged at 12000rpm at a temperature of 4^{0}C for 5 minutes. Total RNA precipitate was dried by vacuum dryer and resuspended in dH_2O. The quality and quantity of RNA was determined by UV spectrometer at a wavelength of 260 nm and 280 nm. Integrity of total RNA was analyzed by electrophoresis on a 1% agarose gel in 1x TAE buffer solution. Visualization performed on total RNA UV transiluminator GelDoc after stained with EtBr (0.5 mg / mL) for 15 minutes and rinsed with water

Total cDNA synthesis. Total cDNA synthesis performed by mixing 1 g of total RNA, 10 pmol oligo (dT), and added dH_2O to a total volume of 20 ml reaction, then incubated at 65^{0}C for 5mn and 4^{0}C for 5 minutes. Then added to a solution of 1x (RT-PCR 5xqiagen buffter, 10 mM dNTP mix, 5-10 U DNAse inhibitors, 100 U QIAGEN one step RT-PCR enzyme mix (Invitrogen).

Isolation of cDNA fragment by PCR using primers HSP (Table 1). The composition of the PCR for the amplification of cDNA HSP is 1 mL cDNA, 1xbuffer (5 x qiagen one step RT-PCR buffer), 2 ml of dNTP mix, 10 pmol forward primer, 10 pmol primer reserve, 2 mL QIAGEN one step RT-PCR enzyme mix and dH2O with reaction volume of 20 ml. Primer is designed by means of collecting data from the gene bank obtained in the given primary and 01. Primary HSP code used can be seen in Table 1.

cDNA was amplified by using a rotor-Gene 3000 real-time PCR in a way begins with denaturation at a temperature of 95^{0}C for 90 min, and 40 cycles at 95 ^{0}C for 5 minutes and 58

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^{0}C for 30 minutes. Dissociation curve produced by PCR using gene-rotor program with analysis software 6.0: 95^{0}C for 1 minute, 55^{0}C for 1 min and the temperature was increased to 95^{0}C, the data clarity at 55-95^{0}C temperature interval of 80 cycles at 0.5^{0}C/cycle.

Table 1. Primer result own design to be used

Gene	Left Primer	Right Primer	Secuensing site (bp)
HSP01 *Bombyx mori*	atgatcgccttagtgttgtgc	cacttaatwcattaattccac	-

RESULTS AND DISCUSSION

Silkworm eggs incubated for 10 days. After hatching maintained at room temperature until the first instar larvae instar V. maintained in plastic petridis paraffin coated paper with a wet tissue pads to avoid feed mulberry leaves to dry quickly. This is done until the third instar larvae. After entering instar larvae IV, larvae in place in the basket until the fifth instar larvae instar V. Prior to the isolation of total RNA in the head (Tanjung, *et al*., 2014), based on the measurement spectrophotometer with a ratio OD260/ OD280 was 1.899 and the concentration of 1656 ng/µl. Results isolation of total RNA used as a template to synthesize cDNA total. PCR with total cDNA as template and using the primers HSP 01 (own design) (Table 1) resulted in 750 bp (Figure 1).

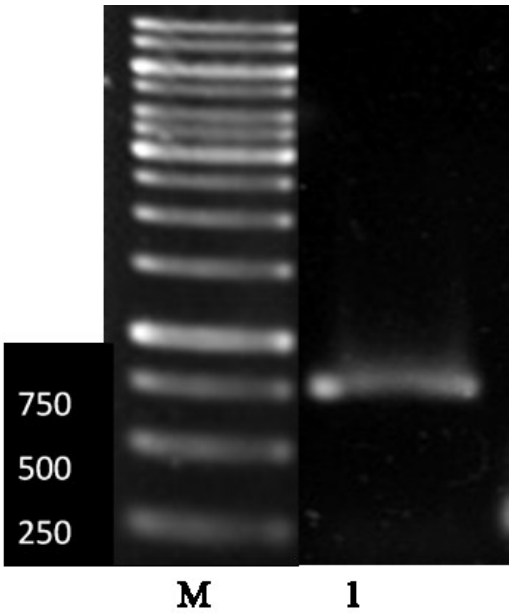

Figure 1. Results of PCR with primers HSP 01 M = marker; 1 = HSP 01 (750 bp)

After sequencing and analyzed for the primary HSP 01 can be seen in Figure 2. BlastN analysis results (www.ncbi) HSP gene larvae silk with HSP 01 primer homologous to heat shock proteins of Bombyx mori with E value 0.0 25.4 (max 99% identity).

ATGACCCGCCCTTAGTGTTGTGCGGACTGCTGGCGGCGGTCTCGGCCGCG
CCACAGTACTACCATGGCTCGTCACATTGGCCGTATCACCATTACGACCC
CTTCAGTCCTTACGTTCGGGAAAGCATGTTGGACACACATTCGCTTTGGT
CCAACCTTGCCAACGAAATGCAACACTTGGACAACATGATGAAGGAGCTG
TCGTTGAAGTTCCCCAGCATTATAAACGAAGGACGCGTGGAAGGCGACAA
GTATCAGATATCTATTCACCTGCCTGGTTACGAACAGAAAGACATCAACG
TGAAAGCGAAAAATGGAGTGCTGATGGTGCAGGCTAACAGTGCTTTTAAT
CATTACTTGAAAATACAGAACCTTCCCTGGGATGTGAATTCCGAAGGCAG
CTGGGTTTACGAGAAAGACGTGTTGAAAATCACCTTCCCGCTGAAGCAAA
AGCAGCCAGAGGATAGCAAGAGGCCAGTTGCAGAGCCCACTGAGACGACC
TCTACGAATGTAAGTCGTGAAGAGATGGAGTTCACCACCGAGAGCAACGT
GCGGGACGTTGACGTCGGCTTGGAGACAGCCCAGAAGACCAATGAGATCG
CGAAAGCTGTAGAAGCGACCACGTACGCTGTCAACATCAGAGACGATGCG
GAGTTCTTGCCGATTCCATATTAATTTGATCAAATATAAATGTGAGAATT
AATGTATTAAGTGGGGCCAACAACTAAGGGCGATCATAATA

Figure 2. The nucleotide sequence of HSP genes larval silk (*Bombyx mori*) with primer HSP 01

CONCLUSION

HSPs gene fragment with a length of 750 kb. Based alignment HSP gene fragments with gene banks homolog to *Bombyx mori* of heat shock proteins gene 25.4 with E value of 0.0 (max identity 99%).

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