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Study of Heat Shock Protein: In Housefly.

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Abstract
Insects and other animals, including humans, produce heat-shock proteins in response to extremely high temperatures. Hsp is vital in withstanding stress conditions which insects normally thrive, the aim of our work was to study Hsps and find out the molecular weight of Hsps in *Musca domestica*. Houseflies were collected. The samples prepared were loaded on SDS gel along with protein molecular weight marker and study of Hsps was done by using silver staining technique. The test samples which was exposed to heat shock at 42ºC & 45ºC were compared with control which was prepared by growing larvae at optimum temperature i.e. 26°C-28°C. It was found that survival rate was not decrease along with increase in temperature. Thus, Hsp's play very important role when Hsp's expressed through stress conditions. Hsps was increased when cells were exposed to elevated temperature. A band of approximately 70 kDa was seen to be expressed in all the Hsps as against control sample.

Key Words
Heat-shock proteins,

Introduction

Heat shock proteins (Hsps) are a group of proteins whose expression is increased when the cells are exposed to elevated temperatures or other stress conditions **(**Tissieres A et al, 1974). Hsps are named according to their molecular weight such as Hsp60, Hsp70, Hsp90, etc. Hsps are present in circulation of normal individuals and their circulating levels decrease during aging and increase in a number of pathological conditions such as hypertension, atherosclerosis and after open-heart surgery (Lindquist et al,1988; Morimoto et al,1994).When excessive heat is applied to normal proteins, they begin to loose their shapes. When the interior of these proteins gets exposed, proteins can adhere and form globs. This can make them dysfunctional. Protein conformational defects are responsible for a number of pathologies, ranging from Alzheimer's disease and oncogenic transformation in humans to heat and drought susceptibility in plants (Sherr CJ, 1995). Chaperones protect against denaturization. Hsps bind to denatured proteins to prevent aggregation. Hence, the heat shock proteins are important.

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Experimental Methods

A. Maintenance of Culture

Houseflies were collected from the chicken shop and fish shop. Cotton bed was made & put it in the jar. Spread some milk on it & collected houseflies kept in jar & tie the musclin cloth on top of jar. After 2-3 days, 3rd instar larvae were observed & these were used for protein extract.

B. Protein Extraction

Equal quantities of larvae were taken in 3 different vials and washed them thrice with D/W. That was transfer into 3 different Petri plates for giving heat shock. Heat shock was given as follows:

The following procedure is same for all the samples:

1. These larvae were transferred into another autoclaved vials and kept it in ice and 40-50 strokes were given to these larvae with the help of homogenizer by maintaining ice condition.
2. Homogenize buffer (300µl) was added and again 40 strokes were given to larvae in ice condition.
3. Vials were spin at 10,000 rpm for 20 min at 4ºC and supernatant was transferred into another vial.
4. Heat samples in water bath at 100ºC for min and then load 25µl of sample on SDS gel.

C. SDS-page method.
D. Silver staining method.

Results and Discussion

When the housefly larvae are exposed to heat shock at 42ºC & 45ºC, bands of approximately 70kDa is seen to be expressed in all the heat shock protein sample as against control sample. The optimum temperature for the growth of insects was found to be between 26°C-28°C. Hsps expression was increased when samples were exposed to elevated temperature (42ºC & 45ºC). Their survival rate was not found to decrease along with the increase in temperature. The difference between band patterns of test samples and control could be because of induced Hsp's. Hsp70 is conserved protein vital for survival at elevated temperature.

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Table 1: Heat Shock.

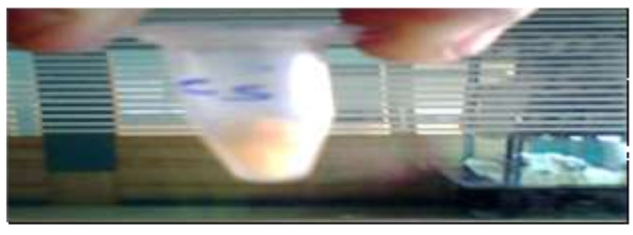
Sample	Test1	Test2	Control
Temperature& time required for treatment	Heat shock at 42ºC for 15min	Heat shock at 42ºc for 30 min	Keep at room temperature
	Heat shock at 45ºC for 15min	Heat shock at 45ºC for30min	Keep at room temperature

heat shock protein sample as against control sample.

Table 2: Heat shock protein samples against control sample.

Heat shock temp/Time Position of Band Sample Lane no./ Band no.	Band comparision with molecular weight marker	Conclusion
Heat shock at 42°C for15min Test 1 : 1/3	~ 70kDa	Hsp70was obtained
Heat shock at 42°C for30min Test 2 : 2/3 Test 2 : 2/13	~70 kDa ~33 kDa	Hsp70 was obtained Hsp35 was obtained
Heat shock at 45°C for15min Test 1 : 3/3	~ 70kDa	Hsp70 was obtained
Heat shock at 45°C for30min Test 2 : 4/3	~80kDa	Hsp80 was obtained

Maintainance of Culture



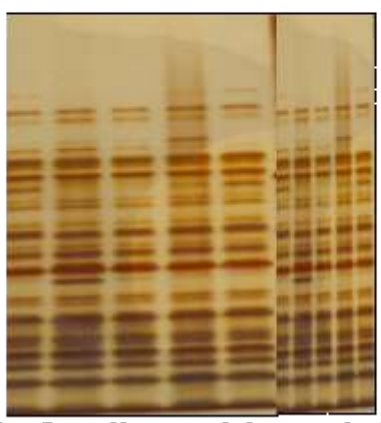
Supernatant of Housefly Control Protein Extract

Supernatant of Housefly Protein Extract Of Test Sample1 (42°C for 15 min)

Supernatant of Housefly Protein Extract of Test Sample2 (42°C for 30 min)

Supernatant Of Housefly Protein Extract Of Test Sample1 (45°C for 15 min)

Supernatant Of Housefly Protein Extract of Test Sample2 (45°C for 30 min)



Gel After Silver Staining Technique
97kDa 66kDa 43kDa 29kDa

Fig. 1: silver staining technique.

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