Int. J. Indust. Entomol. Vol. 15, No. 1, 2007, pp. 17~21

International Journal of *Industrial Entomology*

Evaluation of Thermo Tolerance of 'Nistari' an Indigenous Strain of Multivoltine Silkworm, *Bombyx mori* L.

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(Received April 24 2007; Accepted July 16 2007)

An indigenous multivoltine silkworm, Nistari was evaluated for their thermo tolerance by exposing the larvae to various temperature regimes for eight hours. Among different temperature exposed, this strain has significant tolerance at 32°C. Analysis of heat shock protein revealed the expression of 70 kDa and 64 kDa polypeptides in fat body and midgut tissues. Interestingly esterase isozyme pattern in midgut showed characteristic expression of Est-1 and Est-3 at different temperatures signifying role in heat and cold shock.

Key words: Nistari, Thermo tolerance, Heat shock proteins, Esterase.

Introduction

Insects has adapted to varied environments including inhospitable polar regions and deserts (Wolfe *et al.*, 1998). Many insects overcome heat stress behaviorally by avoidance (May, 1985) and larger insects rely on evaporative water loss for thermoregulation (Wolfe *et al.*, 1998). Extensive literature is available on the biochemical mechanism used by insects to protect against low temperature (Lee *et al.*, 1987; Denlinger *et al.*, 1991; Storey and Storey, 1992) and also high temperature (Lindquist, 1980; 1986; Joplin and .Denlinger,1990; Lohmann and Riddiford, 1990; Watanabe *et al.*, 2002). All organisms synthesize a few number of heat shock proteins (Hsps) in response to thermal and certain other stresses and it functions as molecular chaperons that protect cellular components from injuries due to thermal and other stresses. Many groups of Hsps were reported depending on their sizes (Singh and Lakhotia, 1999).

The silkworm, *Bombyx mori* L is a poiklothermic and attained optimum grwth in the temperature range of 23°C to 28°C. Nistari an indigenous multivoltine strain being reared throughout year by the farmers of Eastern India. This is well acclimatized to local climatic conditions, where maximum temperature goes above 45°C during summer and minimum temperature touches below 10°C during winter. Nistari has the capability to combat the environmental stress. Most of the studies on thermo tolerance in insects are allied with high or low temperature separately, relatively few studies are address on combined study of both cold and heat shock in same insect (Neargarder *et al.*, 2003; Garcia *et al.*, 2003). The present study was undertaken to evaluate the both heat and cold shock on the multivoltine silkworm, Nistari and their effect on changes in the protein and esterase pattern in different tissues.

Materials and methods

Multivoltine strain, Nistari was reared in the laboratory conditions at 25 - 27°C with 70 - 85% humidity. Fifth day of Vth instar silkworm larvae were exposed to 0, 15, 25, 32 and 40°C for eight hours. For each treatment 100 numbers of larvae were used. After the treatment the larvae were kept at 25 ± 1°C till pupation. The survival % was assessed by the number of larvae successfully spin a cocoon subsequently in to a adult. For analysis of protein profiles and esterase isozyme, fat body and midgut was dissected out from the treated larvae. The SDS-PAGE was analyzed following Lammellli (1970) and esterase by Harris and Hopkinson (1970).

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Table 1. Mean performance of multivoltine strain, Nistari during different seasons of the year (Mean of five years)

Season	Fecundity (No)	Larval period (days)	Pupation %	Yield / 10000L (wt-kg)	SCW (g)	SSW (g)	Shell %
Wet summer (July-September)	401 ± 19.99	21.65 ± 0.808	84.45 ± 2.225	7.959 ± 0.243	0.957 ± 0.023	0.129 ± 0.003	13.44 ± 0.062
Dry summer (April-June)	378 ± 18.694	20.54 ± 0.416	80.74 ± 2.175	6.808 ± 0.397	0.852 ± 0.045	0.109 ± 0.005	12.81 ± 0.161
Favourable (October-March)	404 ± 13.465	22.58 ± 0.468	94.25 ± 2.077	10.129 ± 0.341	1.098 ± 0.015	0.152 ± 0.002	13.84 ± 0.101
Mean	394	21.59	86.48	8.299	0.969	0.130	13.36
CD at 5%	NS	2.4	6.49	2.052	0.091	0.012	0.338
CV%	12.119	6.75	6.21	8.829	5.713	4.74	2.51

*Values are presented in mean ± standard error

Results and Discussion

Thermal tolerance describes an organism's ability to maintain physiological functions at extreme temperature (Denlinger *et al.*, 1991). In general, Nistari, multivoltine silkworm is characterized with high survival, shorter larval period and low silk content. Data presents in the table 1 shows that stability in survival character irrespective of the season. During dry summer (April- June) the survival is 80.74%, in Wet Summer (July-September) 84.45% and during favourable season (Oct-Mar) 94.25%. In this region the rearing room temperature touches ~18°C during winter and ~34°C during summer along with low humidity and high humidity respectively. However this strain sustains these extreme environmental conditions. Survival % data presented in the Fig. 1 after thermal shock indicates this strain has the capability to tolerate the temperature of 32°C. At 15°C, 46% of the larvae able to form healthy pupa and metamorphosed into adult. However exposure at 40°C for eight hours proved to be lethal as no single larva able form a healthy pupa. This was supported by the findings of Omana joy and Gopinathan (1995), they observed that survival of the silkworm *Bombyx mori* after exposure to 41°C for two hours was reduced. When C.aeneicollis were exposed to a range of temperature near the upper limit (33-41°C) and lower limit (−3 to 17°C), they differed substantially in ability to tolerate extremes of heat, but differed less in cold tolerance (Neargarder *et al* ., 2003).

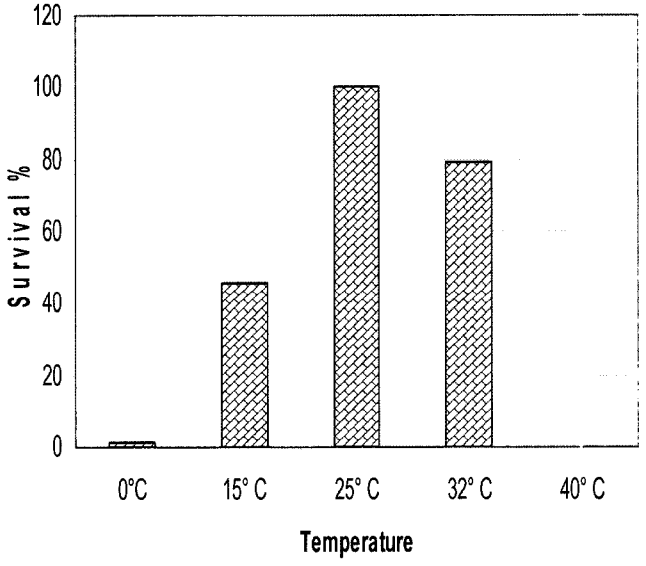

Fig. 1. Effect of cold and heat shock in survival of multivoltine silkworm strain, Nistari. 5th Instar 5th day larvae were exposed to 0°C, 15°C, 25°C, 32°C and 40°C for eight hours. Survival % was calculated by number of larvae successfully form healthy pupa and subsequently into an adult.

High temperature cause protein unfolding and malfunction, eventually resulting in cell death. Heat shock proteins (hsps) counter the effects of heat by serving as molecular chaperones that assist in the refolding of denatured proteins or proteases that degrade and remove the denatured proteins (Samad *et al.*, 2005). The synthesis of proteins in fat body and midgut tissues induced by heat shock at different temperature was analyzed. A total of 14 protein bands were observed in the fat body and midgut tissues after exposures to different temperatures (Fig. 2). In fat body polypeptides with apparent molecular weighs of 14 kDa to 98 kDa were observed. Of which the major Hsps corresponded to apparent molecular weight are 96 kDa, 70 kDa, 64 kDa, 52 kDa and 36 kDa. Of these 96 kDa visible at 15°C, 25°C and 32°C treated larvae and 70 kDa, 64 kDa, 52 kDa and 36 kDa appeared at 0°C, 15°C, 25°C and 32°C. At 40°C (fat body) nearly complete inhibition of protein synthesis is noticed. Polypeptides with apparent molecular weight of 70 kDa, 64 kDa and 39 kDa were observed in 0°C, 15°C, 25°C, 32°C and 40°C in midgut. Omana Joy and Gopinathan (1995) reported the presence of 93 kDa, 89 kDa and 70 kDa in the fat

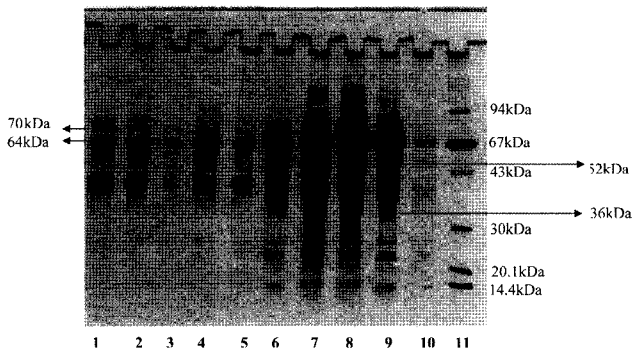

Fig. 2. Protein profiles of Nistari strain exposed to various temperature regimes for eight hours.
Midgut (1) 0°C, (2) 15°C, (3) 25°C, (4) 32°C, (5) 40°C.
Fat body (6) 0°C, (7)15°C, (8) 25°C, (9) 32°C, (10) 40°C, (11).Marker.

body of multivoltine and bivoltine silkworm and 84 kDa, 62 kDa, 60 kDa, 47 kDa and 33 kDa was reported in the bivoltine silkworms (Chavadi *et al.*, 2006) on heat shock. Interestingly we observed that in both the tissues 70 kDa was present even at 25°C. Presence of 70 kDa at control temperature in Silkworm, C.Nichi (Omana Joy and Gopinathan, 1995) and in cockroaches (Singh and Lakhotia, 1999) was reported. It is also interesting to observe that 70 kDa and 64 kDa was present in both midgut and fat body in all temperature range. Good expression of the 70 kDa and 64 kDa polypeptide without heat shock was observed in different tissues of cockroaches and appears to be resulted from the effect of the pollutants present in the drains (Singh and Lakhotia, 1999). So in addition to 70 kDa; 60 kDa family may also play an important role during thermal shock in silkworm.

Relationships between temperature, protein polymorphism and some aspects of physiological performance or fitness have been recorded in number of organisms (Kochn, *et al.*, 1980; Watt *et al.*, 1983; Watt, 1992; Dahlhoff and Rank, 2000). Esterase isozyme pattern in fat body and midgut tissues collected from the larvae exposed to different temperature revealed five bands and eight bands respectively. No remarkable difference in esterase pattern was observed in fat body (Fig. 3B). While interesting expression of protein was observed in midgut where eight bands were observed(Fig. 3A), of which characteristic band Est-1, Est-3 change their expressions depending upon exposure temperature. Expression of Est-1 was most active at 32°C followed by 0°C, 25°C and 15°C. Similarly expression of Est-3 was maximum at 0°C. But lower expression was observed at both 32°C and 15°C. However it was not detected at 25°C. This esterase pattern proves and justifies the inherent tolerance capacity of this strain during thermal shock. Variation in thermal tolerance linked to phosphogluco isomerase (PGI) was studied by Dahlhoff and Rank (2000) in *Chrysomela aeneicollis*. They reported PGI genotypes differ tolerance

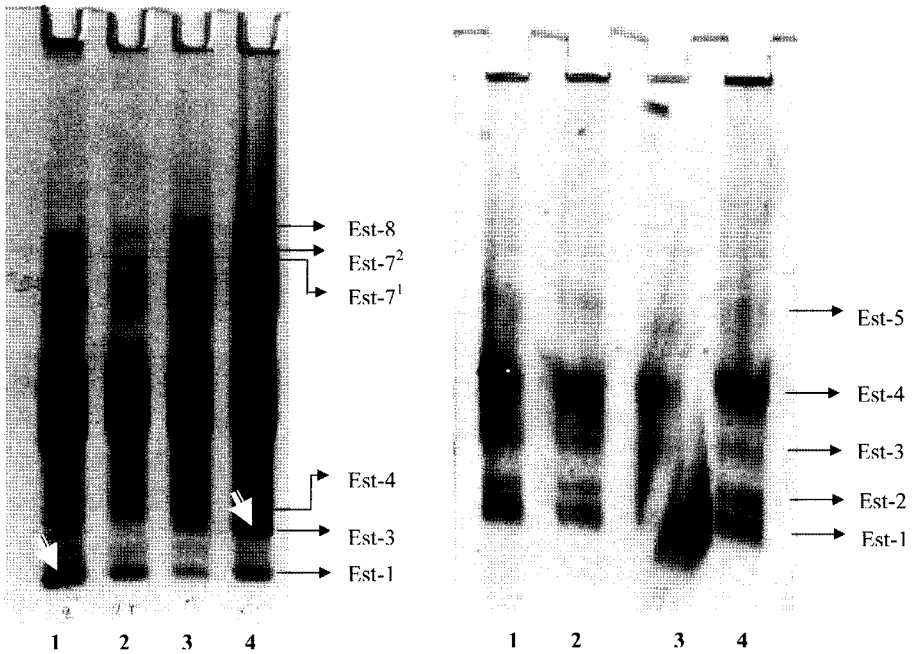

Fig. 3. Esterase isozyme pattern in Nistari strain exposed to various temperature regimes for eight hours.
3A. Midgut (1)32°C, (2) 25°C, (3) 15°C, (4) 0°C
3B. Fat body (1) 32°C, (2) 25°C, (3) 15°C, (4) 0°C

of thermal extremes and some times differ in expression of a stress inducible 70 kDa heat shock protein (Hsp 70) in the field and PGI allozymes has distinct, temperature dependent kinetic properties. Reports are also available in relation between thermo tolerance and esterase in silkworm (Lie *et al.,* 1984; Wu and Hou, 1993) and *Drosophila melanogaster* (Cochrane, 1976). Wu and Hou (1993) reported that in midgut of silkworm, heat stable band, Est-5 was most active and varies in different strains. It is significant in the thermo tolerance of silkworm and closely related to digestive functions. Further this enzyme seems to be induced by rearing under high temperature. Hence study of esterase in relation to thermal tolerance is appropriate one to understand genetic mechanism in silkworm. The heat shock response in organism has evolved in relation to the environmental conditions both internal and external of cells (Krebs *et al.,* 1998; Feder and Hoffman, 1999). Therefore specific difference may exist in the pattern of Hsp induction among various cell types in the same organism (Singh and Lakhotia, 1999).

For insects temperature variation has the major impacts on an individual's fitness (Hoffmann *et al.*, 2003; Rohmer *et al.*, 2004) and its behavioral activities (Gilchrist and Huey, 1999; David *et al.*, 2003). Thus, variation in environmental temperature will generally impose stress upon the organism, which may result in the evolution of adaptive genetic mechanisms to cope up with temperature extremes in nature (Hoffmann and Parsons, 1991). In *Drosophila*, naturally segregating genetic variances for resistance to high- and low-temperature extremes has been documented by response to artificial or natural laboratory selection (Morrison and Milkman, 1978; Tucic, 1979; Cavicchi *et al.,* 1995). Moreover heatshock response is basicaly an adaptive cellular response that is linked to the ecologiocal conditions in which a given organisms liives (Bijlsma and Loeschecke, 1997). Since the silkworm (Nistari) which being reared in this region for several decades and were exposed to variable environmental conditions which compelled the insect to adapt in this region and developed a mechanism to overcome this environmental vagaries. However it warrants in depth study to understand the molecular mechanism underlying in this silkworm.

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