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COMPARISON OF HAEMOLYMPH PROTEIN PROFILES BETWEEN TWO SILKWORM BREEDS UNDER TEMPERATURE STRESS

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INTRODUCTION

Rising temperatures and other environmental factors influenced by global climate change can cause increased physiological stress for many species and lead to range shifts or regional population extinctions. To advance the understanding of species' response to change and establish links between individual and ecosystem adaptations, physiological reactions have to be compared between populations living in different environments. An experiment was conducted using a simple and reliable technique to compare the cellular stress response to temperature shock by two breeds of silkworms originating in two different climatic zones. The study results indicate the potential of the species as a candidate experimental animal for future laboratory experiments towards deeper understanding of animal stress response to climatic change.

The mulberry Silkworm *Bombyx mori* L. has a history of more than 4000 years of domestication. Long term care and selective breeding targeting only at enhancement of quantitative traits (with reference to silk production) has deprived the animal of many of its attributes for self defence against predators, pathogens and extreme climatic conditions. Thus the silkworm races of tropical and temperate countries differ in their adaptive capabilities to variations in temperature. While 'polyvoltine breed' silkworms of tropical origin can withstand higher temperature the 'bivoltine breed' silkworms of temperate origin are extremely susceptible to it. Hence comparison of cellular stress response to temperature stress by these two breeds, by way of stress induced protein synthesis is hypothesised to be indicative of the differential adaptive responses by the breeds.

OBJECTIVE

These fluctuations in temperature have an adverse effect on the survival and pupation of silkworms, especially the bivoltine breeds (which have the capacity to produce higher quantities of high quality silk), incurring heavy loss to the industry. However, the Indian polyvoltine breeds of *Bombyx mori* (*Pure Mysore*, *C. Nichi* and *Nistari* for example) are more tolerant to high temperatures. Thus it is necessary to understand 1) the mechanism of temperature tolerance in silkworm, 2) identification of various families of HSPs synthesized and the threshold temperature which induce their expression and, 3) the differential expression pattern of various HSPs in bivoltine and polyvoltine races. The objective of the paper is to analyze the varying response of a tolerant and non tolerant silkworm breed to high temperature by simple techniques to study the difference if any, in haemolymph protein levels, subjecting them to SDS- PAGE analysis.

METHODOLOGY

A temperature tolerant polyvoltine silkworm breed, *Nistari* and a non-tolerant bivoltine breed, *CSR2* were used as experimental animals. Four thousand eggs (10 Disease-Free Layings) of each race were reared on mulberry leaves (V1 variety) up to cocooning by following the

standard method with recommended temperature and humidity condition. High temperature exposure at constant relative humidity of 80%±1%was given in *sericatron*, an environment chamber with precise and automatic control facilities. Worms of III, IV and V instars were exposed to temperature levels of 36°C and 40°C for durations of 1 h and 6 h and subsequently returned to the room temperature and reared. Haemolymph collected immediately after each treatment and at specific intervals of recovery periods viz., 1 h, 6 h, 24 h and 48 h was subjected to SDS PAGE analysis in both 12% and 8% gels to see the differential accumulation of polypeptides of varying molecular weight. The protein profiles were visualized under visible trans-illuminator and photographed.

RESULTS

The protein profiles of both the races under various treatments when compared with control indicated changes mostly between the 68 and 97 kDa size regions apart from the case of new bands. The effect of short and long exposure differed in the two races. In the third instar larvae of CSR2, 1 h exposure at high temperature seems to disrupt or stop the normal protein synthesis and hence the protein level of treated larvae is less than control. During recovery period of 1 h and thereafter when the larvae were brought to room temperature, an increased protein level was observed. Perhaps this much time is needed for the larvae to induce HSP and chaperone the normal protein.

The differential susceptibility of fifth instar to high temperature was more pronounced in females, especially for 6 h duration. The initial hike in protein content immediately after the treatments always indicated a sign of response to temperature. New bands in the region between 97 and 205 kDa size appeared only in fourth instar CSR2 larvae during recovery period of the treatment. These high molecular weight HSPs are supposed to have role in protein folding and their consistent expression induced under heat shock indicates these may be specific to CSR2 breed. The Nistari race which is comparatively tolerant might constitutively possess some other HSPs and hence, no expression of new protein. When the treated larvae were reared up to cocooning, the larvae treated at 40°C for 6h showed severe mortality than the others that pupated successfully. This indicates that the larvae could overcome the stress of high temperature (1h at 40°C and or 6h at 36°C) by induction of new HSPs or increasing the already existing ones.

CONCLUSIONS

The results indicate that the silkworm *Bombyx mori* L. is capable of eliciting temperature induced stress response by way of synthesis of specific proteins. The silkworm breeds of tropical and temperate origins elicit a differential stress response which is clearly discernible by visual observation of SDS PAGE gels. These breeds exhibit dichotomy in their capacity to recover after temperature exposure which is also manifested in their cellular response by way of transcription. These breeds are also capable of sex specific differential cellular response to temperature stress. These results indicate the possibility that *Bombyx mori* L could be an ideal candidate organism for future laboratory experiments towards better understanding of animal stress response to climatic change and their ecosystem adaptations.