Genomic Organization of Heat Shock Protein Genes of Silkworm Bombyx mori

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The Hsp 20.8 and Hsp 90 cDNA sequence retrieved from NCBI database and consists of 764 bp and 2582 bp lengths respectively. The corresponding cDNA homologous sequences were BLAST searched in Bombyx mori genomic DNA database and two genomic contigs viz., BAAB01120347 and AADK01011786 showed maximum homology. In B. mori Hsp 20.8 and Hsp 90 is encoded by single gene without intron. Specific primers were used to amplify the Hsp 20.8 gene and Hsp 90 variable region from genomic DNA by using the PCR. Obtained products were 216 bp in Hsp 20.8 and 437 bp in Hsp 90. There was no variation found in the six silkworm races PCR products size of contrasting response to thermal tolerance. The comparison of the sequenced nucleotide variations through multiple sequence alignment analysis of Hsp 90 variable region products of three races not showed any differences respect to their thermotolerance and formed the clusters among the voltinism. The comparison of aminoacid sequences of B. mori Hsps with dipteran and other insect taxa revealed high percentage of identity growing with phylogenetic relatedness of species. The conserved domains of B. mori Hsps predicted, in which the Hsp 20.8 possesses α-crystallin domain and Hsp 90 holds HATPase and Hsp 90 domains.

Key words: Genomic organization, Hsp, Bombyx mori, Domains, Phylogeny, Exon

Introduction

Silkworm B. mori is a lepidopteron, piokilothermic and monophagous insect. The insect is reared in both tropical and temperate climates. The temperature, an abiotic factor affects its growth and silk yield. The heat shock response of organism’s habitating tropical climate is likely to be different from those of temperate climate. The native Indian polyvoltine races of B. mori exhibit more tolerance to high temperature, than the exotic bivoltine races of temperate origin (Krishnaswami et al., 1977; Pershad et al., 1986).

The heat shock response is a universal physiological phenomenon exhibited by both prokaryotes and eukaryotes (Glover 1982). Ritossa (1962) studied the heat induced a characteristic pattern of puffing in salivary gland chromosome of Drosophila larvae. It is normally manifested by the appearance of a set of new proteins or an increase in the quantity of certain specific pre-existing proteins in response to exposure of an organism or a cell to thermal stress and other form of stresses (Lindquist 1986; Nover 1991).

Heat shock response was studied in different cell types of Drosophila and other model systems (Tissiers et al., 1974; Dean and Atkinson 1983; Nath and Lakhotia 1989; Joplin and Denlinger 1990). The Hsp are presumed to ensure survival under stressful conditions by involvement in damage protection or damage repair due to their action as molecular chaperons (Hightower 1991). Generally heat shock response depends on the magnitude at temperature elevation and duration of exposure, and is relative to the environmental temperature at which the organism normally survives (Nath and Lakhotia 1989). Cells that have been pre-exposed to thermal stress and such a thermo tolerance is caused by an enhanced resistance of proteins against thermal denaturation and aggregation (Kampinga 1993).

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The Hsp 90 family is a very highly conserved group displaying higher than 50% identity among eukaryotes and ubiquitously expressed in prokaryotes to eukaryotes (Lindquist and Craig, 1988). The presence of Hsp 90, Hsp 70 and other heat shock proteins are required for protecting the functional domains of the denatured protein. The protection provided by the heat shock protein binding helps refolding of denatured protein (Becker and Carig, 1994). The Hsp 90 function as folding of non native proteins (Weich et al., 1992), peptide translocation (Brugge et al., 1981), regulation of nuclear receptors and kinases (Holley and Yamamoto, 1995; Pratt and Toft, 1997), centrosome structure (Lange et al., 2000), membrane biogenesis (Deshaies et al., 1988) and anti apoptotic pathway (Pandey et al., 2000). Landais et al., (2001) identified the Hsp 90 is a unique gene in both S.frugiperda and B.mori genomes.

Small heat shock proteins (sHsps) were 15-30-kDa heat-shock proteins monomers, consist of a conserved α-crystallin domain of approximately 90-100 amino acid residues bordered by variable amino and carboxy terminal extensions (de Jong et al., 1993; Jakob et al., 1993). Small heat shock proteins (sHSPs) were associated with nuclei, cytoskeleton and membranes, and as molecular chaperones. They bind partially denatured proteins, thereby preventing irreversible protein aggregation during stress (Sun and Mac Rae 2005). The multiple sHsps occur in living cells and number of genes encoding sHsps is not identical (Narberhaus et al., 2002; Haslbeck et al., 2005). The six putatively encoding small heat shock protein (sHsp 19.9, 20.1, 20.4, 20.8, 21.4 and 23.7) genes was characterized in silkworm B. mori (Sakano et al., 2006). Small Hsps and Hsp 20.8 having a similar domain to that of α-crystallin proteins and occur ubiquitously in a variety of organisms and involved in various phenomenon such as apoptosis protect against heat stress. In this paper, we report the genomic organization and phylogenetic relationship of heat shock protein genes (Hsp 90 and Hsp 20.8) in silkworm B. mori. These sequences differences could be used to discriminate racial differences existing in the silkworm races. The information generated from the present study would be useful to clone, characterize and to analyze polymorphic patterns of the genes in silkworm germplasm, and the polymorphic studies could be correlated with thermotolerance level of silkworm.

Materials and Methods

Silkworm strains selected
A total of six silkworm strains viz., Pure Mysore, Nistari, PMX, NB4D2, CSR 18 and CSR 19 were utilized in the present work. The first three strains are polyvoltine in nature and the other three strains are bivoltine silkworm strains. Among the polyvoltine silkworm races, Pure Mysore and Nistari were found to be hardy races, tolerant to thermal stress, while PMX is a susceptible breed. CSR 18 and 19 showed higher thermo tolerance than NB4D2 among bivoltine strains.

Identification of Hsp 90 and Hsp 20.8 gene and genomic contig
The cDNA of Hsp 20.8 (AF315317) and Hsp 90 (AB060275) genes was already identified and deposited by Sakano et al., 2006 and Landais et al., 2005 respectively. The gene sequence was BLAST searched with B. mori genomic DNA database for identification genomic contig homologous sequence to corresponding gene sequence. The genomic contig DNA sequences showing homologous sequence to B. mori Hsp 20.8 and Hsp 90 gene was identified and subsequently translated to determine putative amino acid sequence. The amino acid sequence was further analyzed through conserved domain search for the presence of domain in Hsp 20.8 and Hsp 90 gene using the programme conserved domain search (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml).

Selection of primers
The up and down gene specific primers were designed for Hsp 20.8 and Hsp 90 genes in the B. mori genomic contigs using the software program of primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3). Based on the software programme, the primer binding site and the PCR product size were determined.

PCR amplification and analysis of amplified product
The genomic DNA isolated from silkworm using standard protocol (Nagaraja and Nagaraju 1995) and used as template in PCR reactions. The PCR reaction was performed for Hsp 20.8 and Hsp 90 variable region reaction in a 20 µl final volume, each reaction mixture containing 50-100 ng of genomic DNA as template, 2.0 µl of 10X PCR buffer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 66 ng of gene specific (each forward and reverse primer) and 0.3 U of Taq DNA polymerase (MBI fermentas). The PCR schedule was 94°C for 3 min followed by 30 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 2 min and a final extension of 7 min at 72°C.

The PCR products were resolved on 1.5% agarose gel in Tris-Acetic acid/EDTA buffer with a constant voltage of 80V in parallel with standard markers. Gel was stained with ethidium bromide (0.5 μg/ml) and photographed with gel documentation. The PCR amplified products were purified through Gel-spin column (Bangalore