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Conservation of coding and untranslated regions of heat shock protein Beta-1 (*HSPB1*) gene and its expression pattern in heat stressed peripheral blood mononuclear cells of Indian native cattle (*Bos indicus*) and riverine buffaloes (*Bubalus bubalis*)

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1. Introduction

Heat shock proteins (HSPs) have been identified as major proteins that are induced during heat stress to regulate cellular homeostasis and folding-unfolding of damaged proteins (Lindquist and Craig, 1988; Multhoff, 2007; Hendrick and Hartl, 1993). HSPs are multi-gene families that range in molecular size from 10 to 150 kDa and are found in all major cellular compartments. These are highly conserved and can be grouped into five subfamilies based on molecular weight, these include: HSP100, HSP90, HSP70, HSP60, and small heat shock proteins (sHsps) such as HSP27, αB crystalline (Morimoto et al., 1994) and immunophilins (Bose et al., 1996; Duina et al., 1996; Pennisi, 1996). Several studies have shown elevation of HSPs for providing cyto-protection upon thermal stress (Collier et al., 2008; Horowitz, 2001; Sonna et al., 2002; Kapila et al., 2013).

The differential response of species/breed to heat stress has been documented mainly on the basis of anatomical differences and physiological parameters (Hansen, 2004.). Genetic components and alterations in gene sequences in heat stress responsive genes, though, play an important role in heat stress tolerance are not well understood. In recent past, studies are being carried out across different species/breeds to sequence characterize heat shock protein genes for identification of single nucleotide polymorphism (SNPs) and association of these SNPs with higher thermo tolerant ability has been reported in pig, sheep and poultry breeds (Schwerin et al., 2001; Zhang et al., 2002; Salces-Ortiz et al., 2013). In cattle, *HSP70A1A* gene is well studied across different types/breeds and several variants in promoter, coding and untranslated region have been identified across (Basirico et al., 2011; Sodhi et al., 2013). However, such information is very limited for other genes of heat shock protein family. Heat shock protein beta-1 (*HSPB1*) is one such gene that is known to be involved in providing cytoprotection and thermotolerance during stress conditions (Arrigo, 2007), but not well characterized. This gene helps in cell survival under stress, regulate apoptosis, cellular development, and differentiation. Another function of *HSPB1* is the activation of proteosome that further leads to activation of NF-kB pathway for regulating cellular processes like cell growth and inflammatory response (Parcellier et al., 2003). The cyto-protective properties of *HSPB1* result from its ability to modulate reactive oxygen species and to raise glutathione levels. It is a molecular chaperone with an ability to interact with a large number of proteins to aid in refolding of non-native proteins (Bryantsev et al., 2007). Further, it functions as an anti-apoptotic agent under conditions of chemical stress by interacting with both mitochondrial dependent and independent pathways of apoptosis. *HSPB1* is also involved in protection from programmed cell death by inhibition of caspase-dependent apoptosis.

In recent studies, the stress responsive nature of *HSPB1* has also

Abbreviation: HSPB1, Heat shock protein beta-1; HSP, Heat shock protein; sHsps, Small heat shock proteins; SNP, Single nucleotide polymorphism; UTR, Untranslated region; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cells; PBMCs, Peripheral blood mononuclear cells; SAC, Sahiwal cattle; RAC, Rathi cattle; LAC, Ladakhi cattle; HFC, Holstein Friesian cattle; GIC, Gir cattle; THC, Tharparkar cattle; KJC, Kankrej cattle; RKC, Red Kandhari cattle; DEC, Deoni cattle; DAC, Dangi cattle; KYC, Kangayam cattle; NMC, Nimari cattle; MGC, Malnad Gidda cattle; MUB, Murrah buffaloes; TRANSFAC data base, Transcription Factor database; CTR, Control; TRT, Heat treated; RPS9, Ribosomal Protein S9; B2M, Beta-2-Microglobulin; RPS15, Ribosomal Protein S15; HSD, Tukey's honestly significant difference; ORF, Open reading frame; ACD, Alpha crystallin domain; TFBSs, Transcriptional factor binding sites; Sp1, Transcription factor1; GATA-1, GATA-binding factor 1; Egr-1, Early growth response protein 1; NF-1, Nuclear factor 1; REB1, DNA-binding protein REB1; NF-muE1, Mouse B-cell nuclear factor; MEB-1, Membrane protein of endoplasmic reticulum body 1

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been established in dairy animals especially during heat and cold stress. Mohanarao et al. (2014) showed increased expression of *HSPB1* in both heat and cold stressed peripheral blood mononuclear cells (PBMCs) of goat. The heat responsive nature of *HSPB1* has also been reported for buffaloes (Kapila et al., 2013). But not much efforts have been made to sequence characterize the gene and unravel genetic variations across different Indian native cattle breeds. In the current study, the coding as well as 5′ and 3′ untranslated regions of *HSPB1* was characterized in Indian cattle breeds. In addition, expression profile of *HSPB1* in PBMCs of Sahiwal cattle (*Bos indicus*) and Murrah buffalo (*Buablus bubalis*) was also performed under heat stress condition.

2. Materials and methods

2.1. Animal included in the study

For the purpose of cloning and sequencing of *HSPB1* cDNA, peripheral blood mononuclear cells (PBMCs) purified from 15 whole blood samples including 3 each from Sahiwal (SAC), Rathi (RAC), Ladakhi (LAC), Holstein Friesian (HFC) cattle and Murrah buffaloes (MUB) were used. The PBMCs were isolated by density gradient centrifugation on Histopaque (1.077 density, Sigma-aldrich, USA) following the protocol established earlier in the lab (Kishore et al., 2014). For identification of genetic variations in exonic regions (coding and UTRs) of *HSPB1* gene, a total of 181 genomic DNA samples representing 12 different Indian native cattle breeds viz., Gir (GIC), Rathi (RAC), Sahiwal (SAC), Tharparkar (THC), Kankrej (KJC), Red Kandhari (RKC), Deoni (DEC), Dangi (DAC), Kangayam (KYC), Nimari (NMC), Malnad Gidda (MGC), and Ladakhi cattle (LAC) were utilized. The blood samples for all these breeds were collected either from fields or organized farms located in the breeding tract of individual breed. The breeds selected for this study were of different utility types (milch, dual and draft purpose breeds) and belonged to different agro climatic regions of India. In terms of their utility, GIC, RAC, SAC, THC are well known native milch/dairy breeds; KJC, RKC, DAC, DEC are of dual-purpose breeds while KYC and NMC are of draught type breeds. Among these, MGC and LAC are short statured cattle breeds; MGC is native to hilly and densely forested area of Western Ghat while LAC is adapted to high altitude of Trans-Himalayan region of Ladakh.

2.2. RNA isolation and cDNA synthesis

Total RNA was extracted from 15 PBMCs samples, using Trizol reagent (Invitrogen, USA) according to manufacturer protocol. RNA was purified using RNeasy Mini kit (Qiagen, Germany) and followed by on-column digestion with the RNase-free DNase (Qiagen, Germany). RNA was quantified using Nano drop ND-1000 spectrophotometer (Nano Drop Technologies). RNA integrity was confirmed by denaturing agarose gel electrophoresis. From the 5 µg purified RNA, cDNA was synthesized using Revert Aid First Strand cDNA Synthesis Kit (Fermentas, USA) following manufacturer instructions. The reaction was performed in an Eppendorf Gradient cycler using the program: 65 °C for 5 min, 42 °C for 60 min and 70 °C for 5 min. The primers (Table 1) to amplify *HSPB1* cDNA were designed based on *Bos taurus HSPB1* sequence with acc No.-NM_001025569. The amplification was performed with the following PCR conditions: 94 °C for 5 min followed by 35 cycles at 94 °C for 1 min, 57 °C for 1 min and extension at 72 °C for 1 min with a final extension at 72 °C for 10 min.

2.3. Cloning and sequencing of HSPB1 cDNA

The amplified cDNA was purified using AxyPrep™ DNA Gel Extraction Kit (Axygen, USA), and cloned into pGEM-T easy vector with T4 DNA ligase (Promega, USA). The ligated mixture containing *HSPB1* amplified products were individually transformed into *E.coli* DH5α. The colonies harboring the recombinant plasmids were inoculated into Luria Bertani broth and incubated at 37 °C overnight with horizontal shaking. The plasmid DNA was extracted from culture using AxyPrep™ Plasmid Miniprep Kit (Axygen, USA). The positive clones were confirmed by restriction digestion with *Eco*RI and sequenced using universal T7 primer and ABI Prism® Big Dye™ Terminator Cycle Sequencing kit (Applied Biosystem, Foster City, CA).

2.4. Analysis of HSPB1 cDNA sequences

The nucleotide sequences and deduced amino acid sequences of *HSPB1* gene were aligned and compared with several other mammalian species *Bos taurus* sequence (*Acc No.BT021550*) like *Ovis aries* (*Acc No.XM_012123070.2*), *Capra hircus* (*Acc No.JQ957566.1*), *Canis lupas familiaries* (*Acc No.*NM_001003295.2), *Homo sapiens* (*Acc No.AB020027*) using CLC Genomic Workbench 8.5. A phylogenetic tree was constructed using nucleotide and putative amino acid sequences of *HSPB1* from all mammalian species using neighbor joining method. The tree topological stability was evaluated by 1000 bootstrap resampling. The hydrophobicity profile of deduced protein sequence was determined using Kyte and Doolittle plots (Kyte and Doolittle, 1982).

2.5. Amplification of coding and untranslated regions

Genomic DNA from 181 animals representing 12 different Indian cattle breeds was utilized to amplify exonic regions (coding and untranslated) of *HSPB1* gene. The specific primers (Table 1) were designed based on taurine *HSPB1* sequence (*Acc No. ENSBTAG00000011969*). The PCR conditions were as follows: initial denaturation at 94 °C for 5 min followed by 35 cycles at 94 °C for 45 s, annealing at 62 °C (5′UTR + exon1)/63 °C (intron 2 + exon 2 and 3 + 3′UTR) for 45 s and extension at 72 °C for 45 s, with final extension at 72 °C for 10 min.

2.6. Sequence analysis

Purified PCR products were sequenced using the ABI Prism® Big Dye™ Terminator Cycle Sequencing kit (Applied Biosystems, USA) with the 3100 ABI automated DNA sequencer. The chromatogram of each sequence obtained was checked manually. The DNA sequence analysis was performed using the CLC genomic workbench 8.5 and SNPs across exonic region of *HSPB1* gene were identified by multiple sequence

Table 1
Details of primers employed for amplification and sequencing of *HSPB1* gene.

Abbreviation	Region covering	Primer sequence (5′-3′)	Amplicon size (bp)	Accession number
HSPB1-F	CDS region	CCGGCTGGAGACCCATAAAA	741	NM_001025569
HSPB1-R	(amplification)	GTGATGGCTACTTGTGGGCT		
HSPB1–5′F	5′UTR and exon 1	GAGACCTGAAACACCGCCTG	594	ENSBTAG00000011969
HSPB1–5′R	(sequencing)	CACTTTCCAGCGTTCCCCTC		
HSPB1–3′F	Exons 2, 4 and 3′UTR	CCAGATCCCTCCGTCAGTCT	663	ENSBTAG00000011969
HSPB1–3′R	(sequencing)	AGCAAACGTCAAACCAGCAC		
HSPB1-qR		GGACAGAGAGGAGGAGAC		

bp- base pairs.