Evolutionary study of *HSP* 90AA1gene among Nigerian zebu breeds of cattle revealed shared ancestry

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ABSTRACT

Heat shock protein (*HSP*) 90AA1 gene is a member of *HSPs* sub-family that act as molecular chaperons whenever animals come under the assaults of thermal stress, they fulfill essential roles of providing cellular protection, immune response, inhibitory apoptosis and adaptation to thermal assault. A total of ninety (90) adult bulls from Nigerian Zebu cattle breeds comprising of White Fulani (25), Sokoto Gudali (21), Red Bororo (21) and Ambala (23) sampled from northern parts of Nigeria. We reported that rooted evolutionary study based on Neighbour-joining dendrogram of *HSP* 90AA1 sequences of White Fulani, Ambala, Sokoto Gudali and Red Bororo Nigerian Zebu breeds of cattle revealed that *HSP* 90AA1 sequences of four Nigerian cattle breed showed shared homology which is suggestive of common ancestral lineage. Similarly, nucleotide sequences of *HSP* 90AA1 gene in four Nigerian *B. Indicus* and those of goat, sheep, yak, buffalo, camel, horse and taurine were also constructed. Our results showed that sequences of Nigerian cattle breeds and those of goat, sheep, yak, buffalo, camel, horse and taurine at *HSP* 90AA1 gene locus suggested that these animals had shared ancestry and common evolution. Therefore, the extent of relatedness detected among Nigerian breeds of cattle and those of selected mammalian species indicated that *HSP* 90AA1 gene is conserved among wide range of animals and as such it can be used as potential bio-marker for thermo-tolerance in wide range of livestock animals under assaults of thermal stress.

Keywords: *HSP* 90AA1 gene, thermal stress, phylogenetic analyses, cattle

INTRODUCTION

Thermal stress is major issue in the era of climate change occasioned by global warming as this affect adaptability, survivability and ability of livestock animals to cope under the assault of thermal condition (Onasanya et al, 2017). Climate change is a major threat to the sustainability of livestock production systems across the world (Sailo *et al*., 2015). Elevated temperature is detrimental to production,

reproduction, metabolic, health and immune response in livestock (St-Pierre *et al.*, 2003; Rosenzweig *et al.*, 2007). Phylogenetic study reveals patterns and evolutionary history of the genes, it give insight into the extent of relatedness between animals. For example, genes under a single/well-defined clade appear to descend from a common ancestor with shared homology (Doyle and Gaut, 2000). Animals whose genes share similar cluster suggest evolution from common ancestral lineage and such gene is known to be conserved (Kumar *et al.*, 2015). Such candidate gene can be used as bio-marker for selection program. Therefore, this study intends to determine extent of relatedness and ancestral relationship between Nigerian zebu cattle breeds and some selected mammalian species at *HSP* 90AA1 gene.

MATERIALS AND METHODS

Sampling regions and experimental animals

A total of ninety adults (90) bulls from Nigerian Zebu cattle population were samples; White Fulani (25), Sokoto Gudali (21), Red Bororo (21) and Ambala (23) sampled from northern parts of Nigeria were examined in this present study. The animals originated from different herds and research institutions and were raised under the extensive system of management.

Skin tissue sample collection for genomic DNA isolation

About 200 g of skin tissue was excised from each of the 90 adult bulls before slaughtering in abattoir, each of the 200 g of skin tissue was sliced into less than 1 mg weight and were quickly preserved in 0.5 ml RNA*later* reagent and at -20ºC until further analyses. All genomic analyses were performed in the Biotechnology Center Laboratory of Post Graduate Research Institute in Animal Science, Tamil Nadu University of Veterinary and Animal Sciences, Chennai, India.

DNA extraction of *HSP* 90AA1 gene

DNA extraction was performed according to HiPurA™ Multi-Sample DNA Purification procedure (MolBio™Himedia®, Mumbai, India). From the preserved skin sample, 25 mg was fetched for DNA extraction process, tissue homogenization, incubation and pre-washing and final washing using HiElute Miniprep spin column to generate pure DNA. Proteinase K was used to achieve pure DNA elutes. Incubation was performed using ACCUBLOCK™ digital dry (Labnet International, Edison, New Jersey, USA) to aid complete tissue digestion. All centrifuges were performed using Thermo Scientific Nanofuge (MCROCL 21/21R) micro-centrifuge (Waltham, USA). The quality and quantity of DNA was estimated by Thermo Scientific-NanoDrop 2000 spectrophotometer (Shimadzu co-operation, Japan) with absorbance ratio between OD_{260} and OD_{280} ($OD_{260/280}$ (DNA purity) and this was observed for each sample. DNA sample with absorbance ratio of 1.6 to 1.9 was considered good for further analysis.

Polymerase chain reaction and amplification condition for *HSP* 90 gene

Polymerase chain reaction (PCR) was performed according to standardized procedure to suit our study. Primers used for our study (F-GCGTCATCACGTGTCATCTT and R-CCTCCTTTGGGGTTCCAGT) were published earlier by Kumar *et al.* (2015) and target region span through 450 bp within exon 3 of *HSP* 90AA1. Both Optimization and amplification were performed in a TaKaRa Thermal Cycler Dice™ version III (Takara Bio Inc., Shiga, Japan) to suit conditions of our study.

HSP90	1	2	3

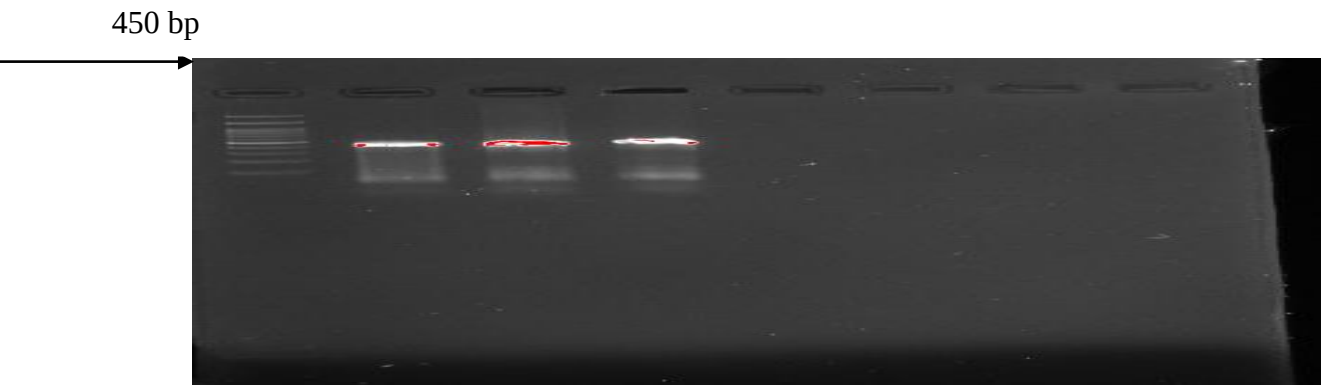

Plate 1 Amplified fragment of *HSP90* gene (450 bp), Lane 1:Molecular Marker; Lanes 1-3: Amplified fragment of *HSP90* gene

Phylogenetic analysis of *HSP* 90AA1 gene of Nigerian Zebu cattle breeds and some selected mammalian species

Rooted dendrogram was constructed from nucleotide sequences of *HSP* 90AA1 gene of four Nigerian Zebu breeds of cattle (White Fulani (WF), Ambala (AM), Sokoto Gudali (SG) and Red Bororo (RB) and GenBank-downloaded sequences of some selected mammalian species using MEGA 5.2 software to evaluate the degree of relatedness and ancestral evolution of *HSP* 90 nucleotide sequences of four Nigerian *B. indicus* and that of selected mammalian species (Tamura *et al.*, 2011). The PCR products were purified and subsequently sequenced using an automated ABI DNA Sequencer (Eurofins Genomics Pvt. Ltd., Bangalore, India).

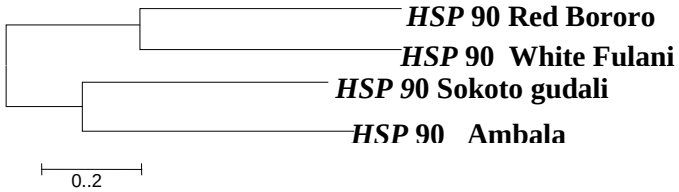

Figure 2: Neighbour-joining dendrogram of *HSP90* sequences of White Fulani. Ambala, Sokoto Gudali and Red Bororo cattle breeds

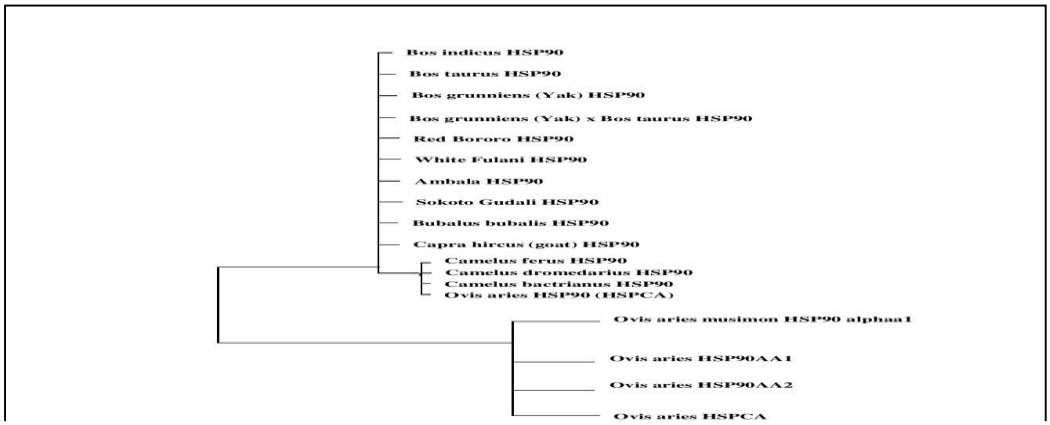

Figure 3: Neighbour-joining dendrogram of *HSP90* sequences of Nigerian Zebu breeds of cattle (White Fulani, Ambala, Sokoto Gudali and Red Bororo) and selected mammalian species

DISCUSSION

The rooted evolutionary study based on Neighbour-joining dendrogram of *HSP* 90AA1 sequences of White Fulani, Ambala, Sokoto Gudali and Red Bororo Nigerian Zebu breeds of cattle revealed that *HSP* 90AA1 sequences of four Nigerian cattle breeds showed shared cluster. Similarly, sequences of *HSP* 90AA1 in four Nigerian *B. Indicus* and those of goat, sheep, yak, buffalo, camel, horse and taurine demonstrated common clade architecture, therefore suggesting evolution from a common ancestor. This confirmed the earlier works of Gade *et al*. (2010) who reported that *HSP* 90AA1 gene of mammalian species showed high degree of relatedness. Pelham (1982) reported that *HSPs* were highly conserved both in protein-coding sequence and in regulatory sequence with common homology. The degree of relatedness of nucleotide sequences of *HSP* 90AA1 detected within four Nigerian breeds of cattle and those of selected mammalian species suggested that *HSP* 90AA1 is conserved among wide range of animals (Pelham, 1982; Sodhi *et al*., 2012; Kapila *et al*., 2013; Wang *et al*., 2015) and as such it can function as molecular bio-marker for thermo-tolerance selection of animal under assaults of thermal stress.

Conclusion

The shared homology of *HSP* 90AA1 gene among four Nigerian cattle breeds indicated common ancestral lineage. Similarly, shared cluster architecture between *HSP* 90AA1 of Nigerian *Bos indicus* and some selected mammalian species e.g. goat, sheep, camel, horse, yak and buffalo suggested common evolution and ancestry. Therefore extent of relatedness of nucleotide sequences of *HSP* 90AA1 detected among four Nigerian breeds of cattle and those of selected mammalian species indicated that *HSP* 90AA1 is conserved among Nigerian zebu cattle breeds and wide range of animals, therefore it can serve as potential bio-marker for selection for thermo-tolerance in wide range of livestock animals under assaults of thermal stress.

Acknowledgements

The Government of India funded the main analyses of this study in a form of visiting scholarships through the Research Training Fellowship for Developing Countries Scientists (RTFD-CS). New Delhi.

We thank the management of slaughter houses in Nigeria for giving us permission to sample from the animals in their custody.

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