The Pharma Innovation Journal 2023; SP-12(11): 495-498

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 495-498
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www.thepharmajournal.com
Received: 07-09-2023
Accepted: 10-10-2023

Phylogenetic tree analysis of HSP4A gene across important livestock taxa

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Abstract

Climate change in the recent century has caused serious economic impact on production and economics of livestock species. Heat stress is known to impact the overall systems biology of livestock. Response of animals in response to thermal stress is produced with the increased production of certain heat stress proteins and chaperones. Heat shock proteins gene expression is upregulated during period of heat stress. HSP4A gene is one of the important gene which is expressed under heat stress in different livestock species. Phylogenetic tree is an important bioinformatics tool to ascertain evolutionary relatedness of a particular gene across various taxa. Phylogenetic tree constructed through Molecular evolutionary Genetic analysis revealed that gene is more closely related across various Bovidae family species as compared to Caprine, Ovine and other livestock taxa.

Keywords: HSP4A, phylogenetic tree, MEGA, neighbour joining method

1. Introduction

Climate change has severely impacted the production, health and well-being of livestock animals. Heat stress is a consequence of climate change which causes significant economic loss to livestock animals all over the world (Ferreira *et al*., 2016) [4]. There has been found that heat stress affects wide range of functions including feed intake, production parameters, reproduction and overall systems biology (Collier *et al*., 2017) [1]. Heat stress leads to release and more gene expression of certain molecules such as proteins and several other chaperones. One of the proteins released during heat stress include heat shock proteins (HSPs) which affect physiology of animal under heat stress. Heat stress response is more importantly exhibited a wide range of heat shock proteins (Moseley *et al*., 1993) [6]. Among heat shock proteins, HSP4A is one of the important HSPs which has increased gene expression during heat stress. The expression of HSP4A gene is found to be upregulated under heat stress in indicine cattle (Khan *et al*., 2021) [5]. There was also increased of HSP4A gene in Pig ovary under seasonal heat stress response (Pennarossa *et al*., 2012) [7]. Phylogenetic analysis is one of important analysis for deciphering the descend of a particular gene across various taxa. Its importance increases because of simplicity in analysis and interpretation (Roy *et al*., 2014) [8]. A phylogenetic tree is constructed to establish relationship across various species derived from its ancestors. Most of the phylogenetic trees are constructed either based on gene sequences or protein sequences of different organisms. These phylogenetic trees are used to predict interactions across species based on underlying relatedness. With the advancement in bioinformatics, phylogenetic analysis has been improved with the use of several graphical user interface tools such as MEGA, Geneious and SeaView. However, Molecular Evolutionary Genetics Analysis (MEGA) is one of such tools which relatively more updated and provide a wider range of accessibility for phylogeny analysis (Tamura *et al*., 2021) [11]. Heat stress has significantly affected productivity of livestock sector. The expression of Heat stress proteins under stress is significantly established. However, relatedness of HSP70 family across various livestock taxa has not been analysed for HSP4A gene. So, the objective of this study is to construct phylogenetic tree across different livestock species through different algorithm such as neighbour joining method, maximum likelihood method.

2. Materials and Methods

2.1 Data Collection

Genome sequences for most common HSP70 family i.e., HSP4A gene were retrieved from NCBI gene repository in fasta format (www.ncbi.nlm.nih.gov) from various livestock animals.

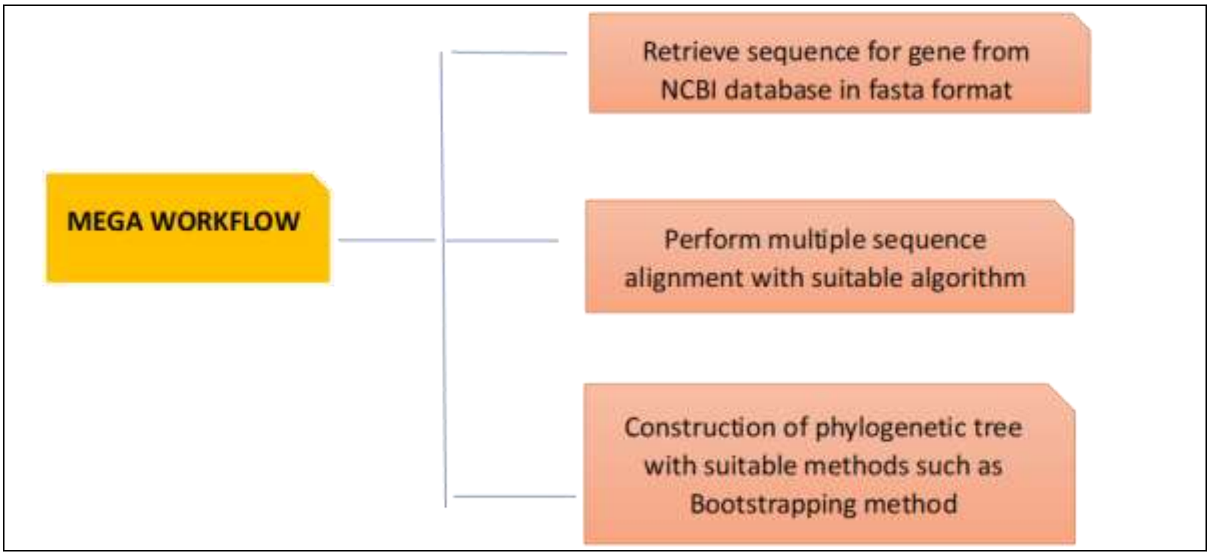

Fig 1: Workflow of Molecular Evolutionary Genetic Analysis (MEGA) Software

2.2 Data Processing

The whole analysis is carried out using MEGA software (Molecular Evolutionary Genetic Analysis) which was downloaded in suitable format as per operating system (www.megasoftware.net). Figure 1 depicts the workflow of MEGA software.

2.2.1 Multiple Sequence Alignment

The collected data is analysed after downloading of data in fasta format. The DNA sequence for HSP4A gene in Taurine cattle, Indicine cattle, Sheep, Goat, Pig, Dromedarian camel, Bactrian camel, Yak and Bison. The multiple sequence alignment was performed by CLUSTAL-W and MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithm. MUSCLE algorithm is found to be better than other algorithm such as CLUSTAL-W and T-Coffee.

2.2.2 Phylogenetic tree construction

Phylogenetic tree was constructed using Neighbour joining method (Saitou and Nei, 1987) [9]. The bootstrapping test of phylogeny was used which involves the number of times same kind of phylogenetic relation is repeated when applied for a particular clade in applied condition (Felsenstein, 1985) [3]. p-distance method was used during phylogeny tree construction. The distance between taxa was measured on a suitable scale.

3. Results and Discussion

The phylogenetic traceback of heat shock protein gene was performed to trace the differences in ancestry of heat stress response in different livestock species. Multiple Sequence alignment was performed with two different alignment programs Clustal W and MUSCLE program. Clustal W is based on penalty for gap opening and is based on iterative algorithm (Thompson *et al*., 1994) [12] This iteration process is likely to be uncorrected in further steps. MUSCLE program is based on progressive algorithm where re-optimization occurs during the sequence alignment (Edgar, 2004) [2]. It is also much faster in alignment especially in case of larger set of sequencing data. After multiple sequence alignment, phylogenetic tree was constructed using different methods such as neighbour joining method and maximum likelihood method. Neighbour joining method is more commonly used distance based phylogenetic tree construction method. It is based on polynomial-time phylogeny construction with bottom-up clustering (Saitou and Nei, 1987) [9]. The phylogenetic tree with neighbour joining method with Clustal W and MUSCLE program are depicted in Figure 2 and Figure 3. Maximum likelihood method is other method for phylogeny analysis which relies on likelihood of a particular dataset (Sullivan, 2005) [10]. Maximum likelihood method based phylogenetic tree are depicted in Figure 4 and Figure 5. Phylogenetic analysis depicted that HSP4A gene is more closely related in Indicine and Taurine cattle followed by their distant relatives Bison and Yak. The nodes for sheep and goat are different from Bovidae family ancestral roots. Moreover, origin of HSP4A gene is different for Dromedary camel and Bactrian camel. One of the subtrees from camel leads to ancestral node of pigs.

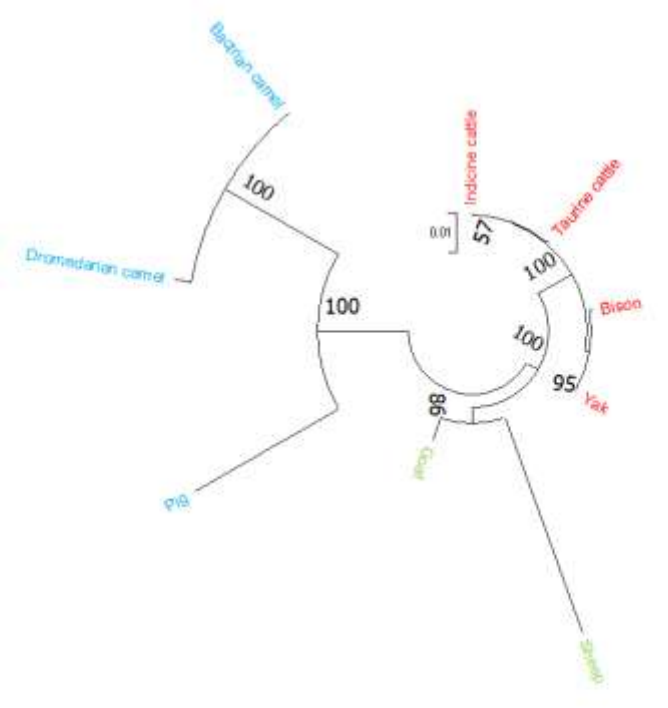

Fig 2: Phylogenetic tree constructed by Neighbour joining method with sequence alignment using MUSCLE program

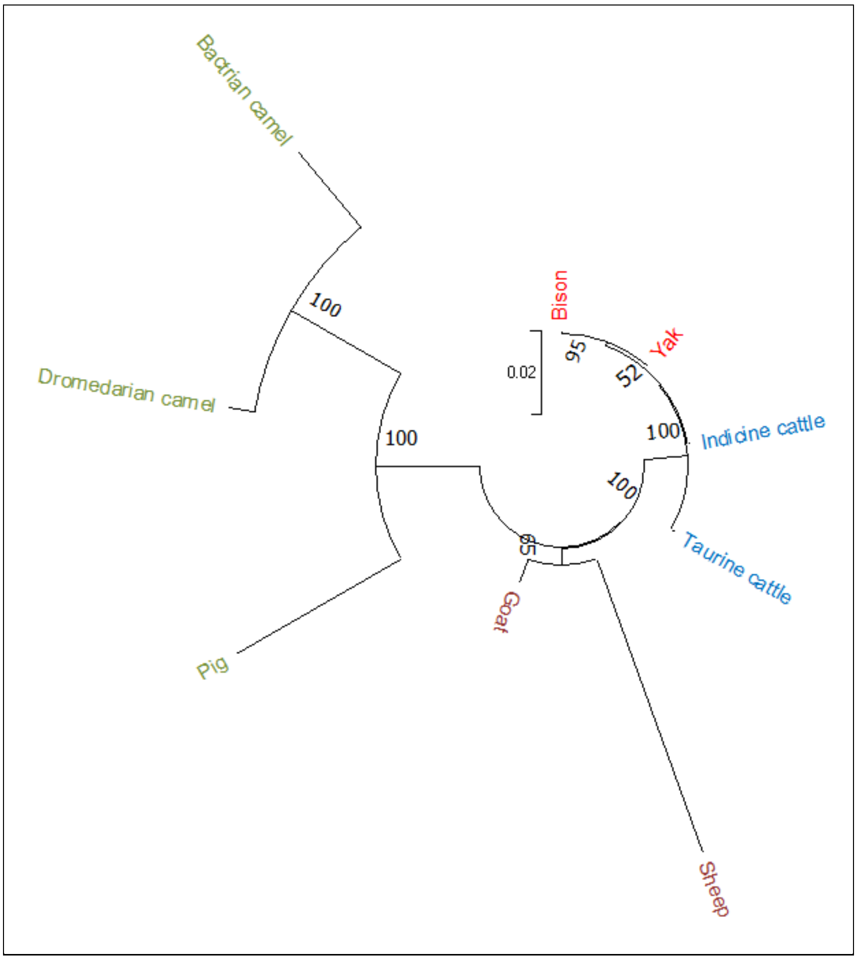

Fig 3: Phylogenetic tree constructed by Neighbour joining method with sequence alignment using Clustal W program

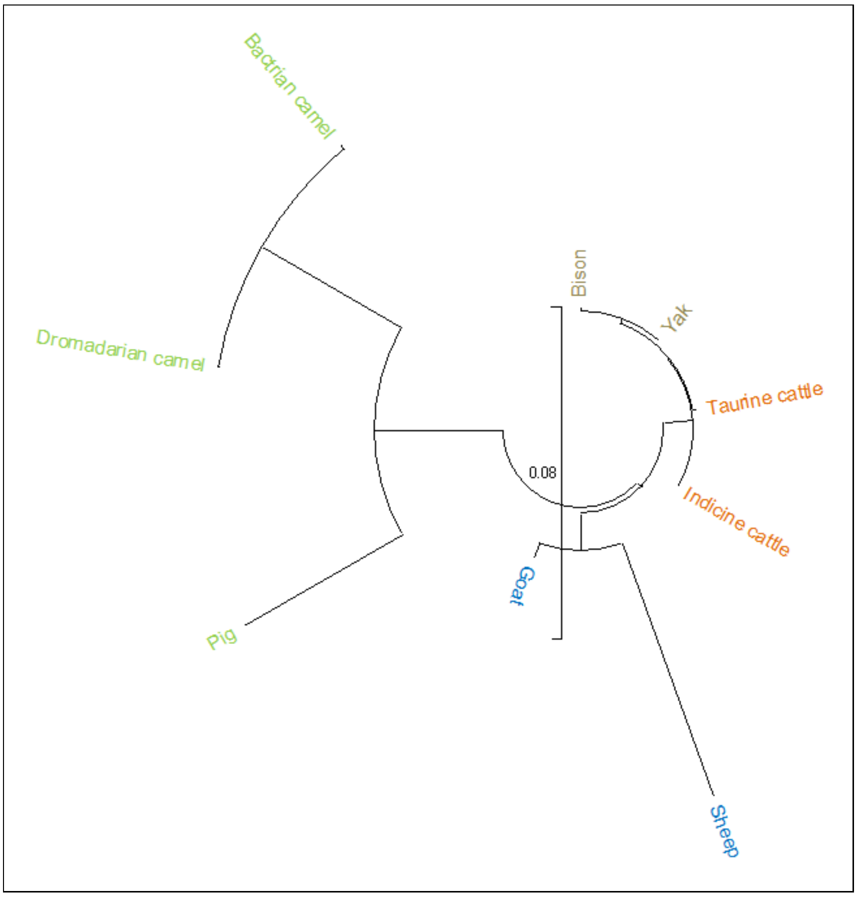

Fig 4: Phylogenetic tree constructed by Maximum likelihood method with sequence alignment using MUSCLE program

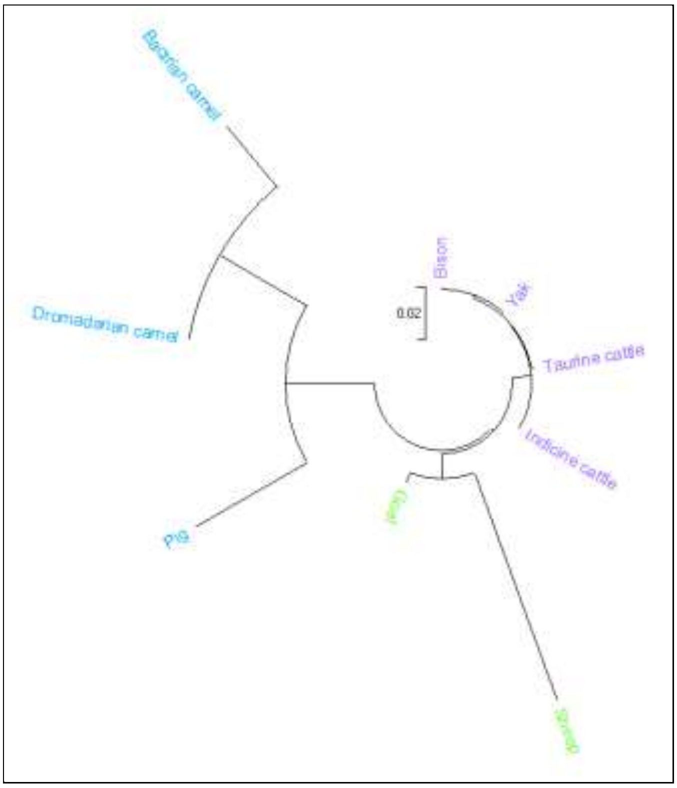

Fig 5: Phylogenetic tree constructed by Maximum likelihood method with sequence alignment using Clustal W program

4. Conclusion

In the wake of climate change, detailed study of heat shock proteins along with tracing their phylogenetic root is necessary to better understand the biology and interactions of heat shock proteins across various livestock species. Besides providing ancestry of a particular gene, phylogeny also confirms biological relatedness of various species. This will ultimately lead to better understanding evolutionary adaptation mechanism to heat stress in current day livestock taxa.

6. Availability of Data and Materials

The data is publicly available and retrieved from https://www.ncbi.nlm.nih.gov.

7. Ethics approval and consent

Not applicable

8. Declaration of competing interest

The authors have no financial and personal interest involved in writing of this manuscript.

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