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SEQUENCE ANALYSIS AND PHYLOGENETIC RELATIONSHIP OF MYOSTATIN GENE OF BIKANERI CAMEL (*Camelus dromedarius*)

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ABSTRACT

The draught potential of the Bikaneri camel depends on the gene affecting the muscular growth. Myostatin (*MSTN*) or growth and differentiation factor (*GDF8*) gene is the major regulator of myogenesis and skeletal muscle growth in mammals. Genomic *DNA* was isolated from whole blood of randomly selected Bikaneri camels (n=6) through spin column method. The *MSTN* exon-2 region of 375 bp was amplified using primers designed from homologous regions of *MSTN* gene sequence (GenBank accession number. DQ167575). The *PCR* amplified fragments of all the animals were sequenced through Sanger dideoxy chain termination method. The sequence of *MSTN* exon-2 gene was submitted to *NCBI* GenBank database to which an accession number KX863740 was assigned. A Neighborhood Joining (NJ) phylogenetic tree was constructed based on the lowest Bayesian Information Content (BIC) value. Sequence comparison of *MSTN* exon-2 gene of Bikaneri camel with homologous regions of goat, sheep, Algerian camel, buffalo, horse and pig revealed more than 95% homology. The sequence information generated for *MSTN* gene of Bikaneri camel would help in better understanding of growth traits and could support in conservation of dwindling camel population in Rajasthan.

Key words: Bikaneri camel, growth, myostatin gene, sequence analysis

The one humped Bikaneri breed of camel is the heaviest camel breed (Khanna *et al,* 2004) that is well known for its load carrying capacity and is primarily used for transport (Faye, 2015). Myostatin (*MSTN*) or GDF8 (growth and differentiation factor 8) gene is considered as candidate gene with functional and positional role in the regulation of muscular growth in different parts of the body (Tahmoorespur *et al,* 2011). The *MSTN* gene functions as a negative regulator of skeletal muscle growth in mammals (Peng *et al,* 2013). Quantitative trait loci (QTL) studies showed that myostatin gene affect the muscular development and muscle depth through alteration in amount and composition of muscle fibres (Zhang *et al,* 2012). The gene functions as a "chalone" and helps to maintain a global balance in tissue growth (McPherron and Lee, 1997). Molecular analysis of the *MSTN* gene in different species has shown that it consists of 3 exons and 2 introns (Kurkute *et al,* 2011). Mutations in the *MSTN* gene could alter its expression and may affect muscle fibre development and may cause dramatic muscularity (Mirhoseini and Zare, 2012). Endogenous myostatin mutations present in the breeding herds are difficult to detect due to highly conserved nature of myostatin gene across livestock species.

The sensitivity of earlier methods, such as single-stranded conformation polymorphism analysis (SSCP) (Kunhareang *et al,* 2009) to detect single nucleotide polymorphism (SNPs) are low (70% to 80%) and require considerable skill and labour. Direct gene sequencing is a powerful method for identifying nucleotide sequence variation in amplified DNA and is considered as the gold-standard approach for genotyping analysis and expected to have almost 100% sensitivity (Laurie and George, 2009). Sequence analysis of *MSTN* gene of Bikaneri camel and its comparison with different species or breeds would help in better understanding of muscle development and differential growth mechanisms. Such knowledge will be helpful in selection and mating strategy, development of knockout technology and understanding the structure, function and evolution of the gene. The characterisation of *MSTN* gene through gene sequence analysis would also help in the establishment of current status of Bikaneri camel.

Till now, few studies (Muzzachi *et al,* 2015) have been conducted in camel to investigate polymorphism at different myostatin loci. Thus the sequence analysis

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of *MSTN* exon-2 region in Bikaneri camel (*Camelus dromedarius*) has been undertaken in the present study.

Materials and Methods

Bikaneri camels (n=6) of unknown pedigree were randomly selected from different regions of Bikaner district of Rajasthan. The blood samples (2ml) were collected from jugular vein in vacutainer tubes containing EDTA as an anticoagulant. The genomic DNA was extracted by spin column method as per manufacturer's protocol. The quality and the concentration of DNA were checked on 0.8% agarose and nano drop spectrophotometer, respectively. Amplification primer pair was designed based on the caprine *MSTN* gene sequence (GenBank accession number. DQ167575) to amplify 375 bp fragment of exon-2 region (F-5'AAAAACCCAAATGTTGCTTCTTTA3'; R5'CAGTCCTTCTTCTCCTGGTCTGG3'). Amplification reactions for each sample was done by using the following constituents in a final volume of 25 µl containing 5X PCR buffer, 1 unit of Taq DNA polymerase, 0.2 mM each of dNTPs, 1.5 mM $MgCl_2$, 75 pMol of each primer and 100 ng of template DNA. Amplification was performed in a thermal cycler with the following program; after an initial denaturation step at 95°C for 5 min, 35 cycles were programmed as follows: 94°C for 30s, 54°C for 60s, 72°C for 60s and final extension at 72°C for 10 min. The amplified DNA fragments were stained with ethidium bromide and visualised on 1.5% agarose gel under gel documentation system.

Sequencing and Sequence Analysis of Amplicons

The amplicons for each sample were initially purified and then sequenced through Sanger dideoxy chain termination method by X celris Genomic Services (Ahmedabad, India) in both directions. Forward and reverse sequences of each gene fragment was assembled against the most closely related reference sequence of respective gene to obtain total sequence length and similarity was looked in to the non-redundant database of GenBank with BLAST algorithms (http://www.ncbi.nlm.nih.gov/BLAST/). The nucleotide sequence of the amplified fragment was submitted to NCBI database. Pair wise sequence analysis and alignment was carried out using Clustal X2 and Bioedit (v 7.0.7.1) bioinformatics tools to identify each nucleotide substitution between different samples.

Phylogenetic Analysis

Sequence analysis was further validated by multiple sequence alignment of query sequence of Bikaneri camel with *MSTN* gene sequence of different species in FASTA format using Clustal X2. Phylogenetic tree was constructed using Neighbourhood Joining (NJ) method of bootstrap test of phylogeny in MEGA7 (Kumar *et al,* 2016) to evaluate the evolutionary relationships of Bikaneri camel *MSTN* exon-2 with the *MSTN* gene of other farm animals. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and implemented with bootstrap test involving simple stepwise addition.

Results and Discussion

The present study reports for the first time the nucleotide sequence of the exon-2 region of *MSTN* gene for Bikaneri camel (*Camelus dromedarius*). The amplification band of 375 bp of *MSTN* exon-2 coding region was obtained from all the samples of Bikaneri camel (Fig 1). The sequence generated in the present study was submitted to the GenBank NCBI database and accession number KX863740 was obtained. The sequence alignment of the respective amplified products from all the 6 randomly selected camels revealed monomorphism and generated similar sequence information for 375bp fragment. The highly conserved nature of *MSTN* exon-2 gene observed in the present study in Bikaneri camel is suggestive to compare the observed sequence with other camel breeds or species to reveal the presence of any intra and inter species polymorphism.

The *MSTN* exon II gene of Bikaneri camel was compared with homologous regions of *Bubalus bubalis* (KJ123755), *Sus scrofa* (HM241657), *Capra hircus* (HM462259), *Ovis aries* (JN856459), *Equus caballus* (NM_ 001081817) and *Camelus dromedarius* Algerian population (KJ847811) which revealed that *MSTN* exon-2 region is highly conserved among livestock species. More than 95% homology of Bikaneri camel *MSTN* exon-2 with that of the buffalo, pig, goat, sheep, horse and Algerian camel sequences published in the NCBI GenBank database, was observed.

The Tamura 3 parameter with gamma distribution model (T92+G) having lowest BIC (Bayesian Information Criteria) value of 617.97 was selected for the construction of phylogenetic tree (Fig 2). The optimal NJ tree was constructed with the summed branch length of 0.3382104. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000

replicates) was shown next to the branches. Sequence of the *MSTN* exon-2 gene obtained in the present study showed 98% homology towards *Camelus dromedarius* Algerian (KJ847811), *Capra hircus* (HM462259) and *Ovis aries* (JN856459). A 96% homology was found between obtained *MSTN* exon-2 gene sequence and *Sus scrofa* (HM241657). A relatively lower homology (95%) was documented between the obtained *MSTN* exon-2 gene sequence and *Bubalus bubalis* (KJ123755) and *Equus caballus* (NM_ 001081817).

A closer relationship between Tylopoda and Suiformes was observed in the present study which is in line with previous reports (Muzzachi *et al,* 2015). Bikaneri camel and goat shared the same cluster with 98% homology, indicating that these 2 species have had a small number of mutations in this gene. The lowest homology observed between Bikaneri camel and *Bubalus bubalis* is in agreement with Muzzachi *et al* (2015). The high level of sequence conservation among all myostatin orthologs suggests the importance and conservation of its function in vertebrates (Karim *et al,* 2000). Absence of polymorphism in *MSTN* exon-2 gene of Bikaneri camel is in agreement with the reports on different *Camelus dromedarius* breeds (Shah *et al,* 2006; Muzzachi *et al,* 2015). The absence of *MSTN* diversity observed in Bikaneri camel reflects the evolution of camel from low variable wild ancestor population.

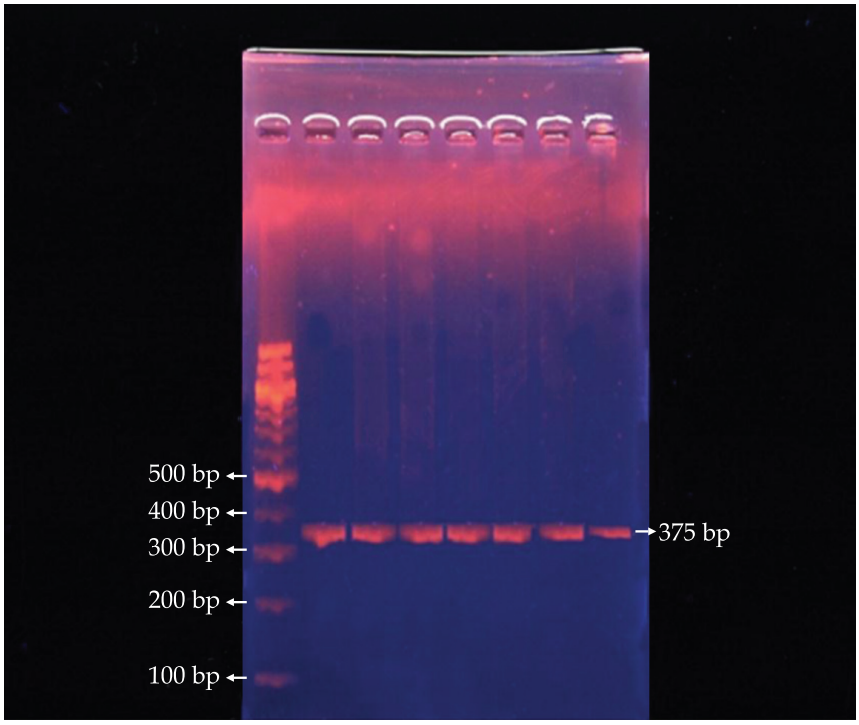

Fig 1. PCR Amplification of myostation (*MSTN*) Exon-2 Gene of Bikaner camel legends lane 1: Molecular weight marker lane 2:-8: PCR amlpcion of mystation (*MSTN*) Exon-2 Gene.

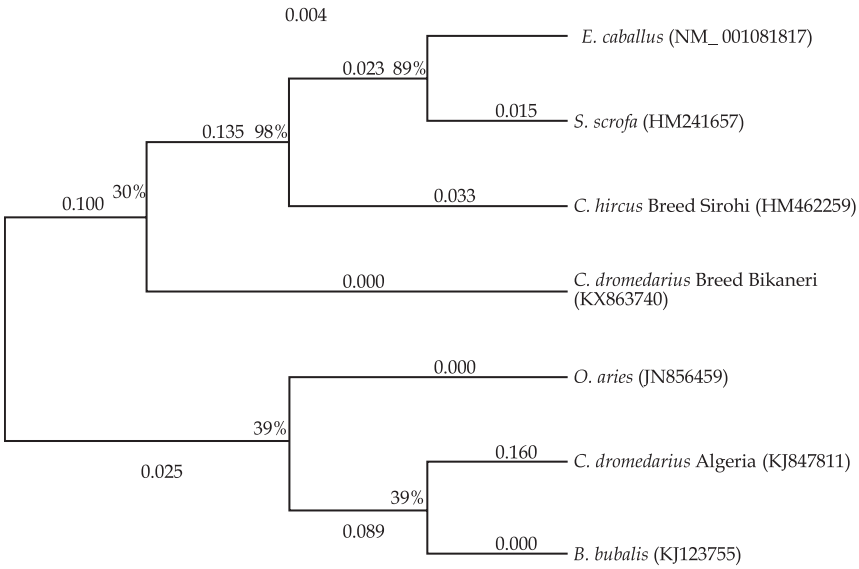

Fig 2. Phylogenetic tree showing common ancestry at *MSTN* exon-2 Gene by NJ method.

The sequence information generated for *MSTN* exon-2 gene of Bikaneri camel would help in better understanding of growth traits and provides clues for investigation of other regions of myostatin gene in Bikaneri camel for the conservation of dwindling camel population in Rajasthan (Livestock Census, 2012). Such knowledge will be helpful in further breeding and selection strategy. The sequence comparison of *MSTN* exon-2 gene of Bikaneri camel with other species could also elucidate the mechanism of diseases associated with emaciation in camels such as Trypanosomosis as over-expression of myostatin gene was

observed in muscle wasting and atrophy (Ma *et al*, 2003).

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