STRATEGY FOR GENETIC CONSERVATION OF BIKANERI CAMEL (*CAMELUS DROMEDARIUS*) FOR MYOSTATIN GENE USING PCR-RFLP

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Received revised on: 29.06.2018
Accepted on: 12.09.2018

ABSTRACT

The present study was undertaken to characterize the Bikaneri breed of camel through PCR-RFLP genotyping for growth affecting gene myostatin (*MSTN*). A total of 56 blood samples from unrelated Bikaneri camel were randomly collected from different regions of Bikaner (Rajasthan), India. Genomic DNA was extracted from whole blood using blood DNA isolation kit based on spin column method as per standard protocol. The quality of extracted genomic DNA was checked on 0.8% agarose gel. The exon 2 of *MSTN* gene was amplified using *MSTN* primers designed from GenBank. The amplified 375 bp region of exon 2 of camel *MSTN* gene was digested with *Hae* III restriction enzyme at 37ºC for 3 hours. The genetic variability in exon 2 of *MSTN* gene in Bikaneri camel was assessed on 8% polyacrylamide gel electrophoresis to detect the different genotypic pattern. All the digested samples showed 'm' allele with two fragments of 88 and 287 bp. The study concluded that all the animals were monomorphic and genotyped homozygous 'mm' for the locus studied. The result showed the suitability of PCR-RFLP technique for evaluating genetic variability in *MSTN* gene of camel and the information could be utilized in formulation of breeding plan to conserve this genetic resource.

Key words: Bikaneri camel, myostatin, PCR RFLP

Introduction

Camels, popularly known as the "ship of the desert" support the survival of million of people in the arid and semi arid regions and are one of the important sources of draught power in Rajasthan (Singh *et al*., 2013a; Singh *et al*., 2013b).The traction power of the camel reflects its body weight (Saini *et al*., 2013) which ultimately depends on the muscular growth and conformation. The draught potential of the Bikaneri camel depends on the gene affecting the muscular growth. Significant differences exist between the breeds of the camel (*Camelus dromedarius*) in their draught capability (Rai *et al*., 1992). 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 fibers (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 three exons and two introns (Kurkute *et al*., 2011). Mutations in the *MSTN* gene could alter its expression and may affect muscle fiber development and may cause dramatic muscularity (Mirhoeini 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.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is a powerful method for identifying sequence variation in amplified DNA and can detect single base substitutions in enzymatic restriction sites (Amie Marini *et al*., 2012). Till now, few studies (Muzzachi *et al*., 2015) were conducted in camel to investigate polymorphism at different myostatin loci. Thus the PCR-RFLP analysis of *MSTN* exon-2 region in Bikaneri camel (*Camelus dromedarius*) has been undertaken in the present study.

Materials and Methods

Bikaneri camels (n=56) of unknown pedigree were randomly selected from different regions of Bikaner district of Rajasthan. The blood samples (2 ml) 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 *MSTN* gene sequence (GenBank accession number DQ167575) to amplify 375 bp fragment of exon-2 region (F-5'AAAAACCCAAATGTTGC TTCTTTA3'; R-5'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 programme; 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 visualized on 1.5% agarose gel under Gel Documentation System.

About 10 µl of PCR amplified product was digested with 10 units of restriction enzyme *Hae* III at 37ºC for 3 hours. After digestion, the enzyme was inactivated by increasing the incubation temperature to 80°C for 30 minutes. The polymorphism in exon-2 region of *MSTN* gene in Bikaneri camel was assessed on 2.5% agarose gel electrophoresis with 100 bp DNA ladder in a gel

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documentation system. The gene and genotypic frequencies were calculated through direct counting method.

Results and Discussion

The present study represented genetic characterization of the myostatin gene in the Bikaneri breed of camel and reports for the first time the genetic variation in exon-2 region of *MSTN* gene in Bikaneri camel (*Camelus dromedarius*) through PCR-RFLP. The amplification band of 375 bp of *MSTN* exon-2 coding region was obtained from all the samples of Bikaneri camel after *in vitro* amplification of genomic DNA using heterologous oligonucleotide primers designed from the publicly available database. The polymorphism in camelid *MSTN* exon-2 gene of Bikaneri camel was detected by PCR-RFLP technique in the present study. The *Hae III* digests the 'm' allele, but not 'M' allele. The digestion of the 'm' allele produced two fragments of 88 and 287 bp. The amplified products from all the fifty six randomly selected camels revealed monomorphism and generated similar restriction pattern information (mm) for 375bp fragment (Fig. 1). As a result, the polymorphic nature of 'm' allele was not observed in the present study. The genotype 'MM' and 'Mm' were not observed in any of the animal studied.

The nucleotide sequence information submitted by Agrawal *et al*. (2016) for the *MSTN* exon-2 gene in Bikaneri camel (GenBank accession number KX863740) also revealed the monomorphic nature of *MSTN* exon-2 gene in Bikaneri camel. 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). Shah *et al*. (2006) did not observe any polymorphism in the myostatin gene, which is known in other species to harbor functional mutations (Baron *et al*., 2012).

The absence of genetic variation in *MSTN* exon 2 gene as compared to other livestock species might be due to the limited geographical distribution of the Bikaneri camel and the evolution of camel from low variable wild ancestor population. The highly conserved nature of *MSTN* exon-2 gene observed in the present study in Bikaneri camel is suggestive to compare the observed information with other camel breeds or species to reveal the presence of any intra and inter species polymorphism. Genotyping information on Bikaneri dromedary myostatin would help in better understanding of muscle growth and differentiation mechanisms 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 selection strategy.

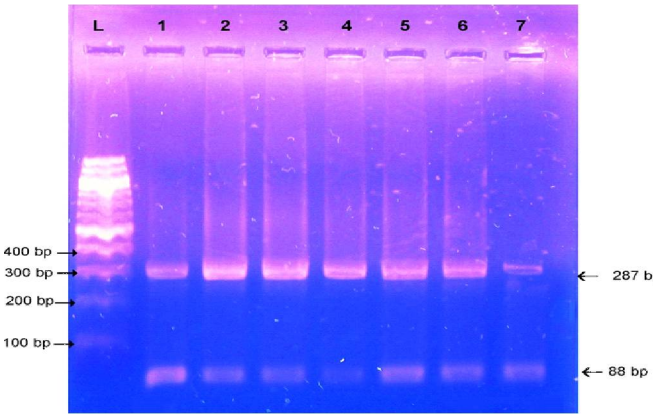

Fig.1: RLFP genotyping of myostatin (*MSTN*) exon-2 gene in Bikaneri camel; Lane 1: Molecular weight marker (1Kb ladder); Lane 2-7: Genotypic pattern (mm)

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