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Full Length Research Paper

Molecular characterization of leptin (*obese*) gene in domesticated Yak (*Bos grunniens*)

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Leptin is obesity gene involved in production and reproduction traits in domestic animals. In the present study complete leptin gene was sequenced in Yak hitherto uncharacterized. The complete leptin gene was sequenced using twelve sets of overlapping primers and submitted to the GenBank. BLAST analysis was performed with other livestock species revealed 98, 98, 97, 95, 95% homology with *Bos indicus*, *Bos taurus*, *Bubalus bubalis*, *Capra hircus* and *Ovis aries*, respectively. Also, 54 Yak samples from two geographically different regions of India were used for SNP identification in the coding regions of the gene. Both exon 2 and 3 was found to be highly conserved, the interestingly reported bovine R4C polymorphism was found to be fixed in Yak population.

Key words: Yak, leptin gene, SNP.

INTRODUCTION

In India, Yak (*Bos grunniens* or *Poephagus grunniens*) is found in Changecgenmo valley in Ladakh and Spiti valleys of Himachal Pradesh, Jammu and Kashmir (J & K) and the North East states, particularly in Arunachal Pradesh, Sikkim, and Nagaland and Uttarakhand (Nivsarkar et al., 1997). Yak is a multipurpose animal; it is raised mainly for milk production, meat, transportation, fur, hide as well as fuel in the form of dung (Dong et al., 1999). In India, there are no any specific breeds of Yak reported yet, but they are classified into different regions according to there origin; for example Ladakh YakSikkim Yak, Arunachal Yak and Himachal Yak. Leptin is a16 kDa protein synthesized by white adipose tissue and is involved in the regulation of feed intake, energybalance, fertility, Milk production, and immune functions (Campfield et al., 1995; Blache et al., 2000; Chilliard et al., 2000; Thomas et al., 2001; Zwierzchowski et al., 2002; Liefers et al., 2002, 2003a, 2003b; Nkrumah et al., 2005; Nkrumah et al., 2006). The total length of the gene is 3897bp (Dubey et al., 2008) in *Bos indicus* and 4067bp (Tellam, 2004) in *Bos taurus* cattle. The gene consists of three exons, of which the first exon is not transcribed into the protein product. So far leptin gene has not been characterized in Yak. The objective of present study was to sequence the leptin gene in Yak and to indentify SNPs in the Yak population.

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MATERIALS AND METHODS

Blood samples were collected from 54 unrelated Yaks covering two distinct regions of India, that is, Kargil District (Ladakh Region), Jammu & Kashmir and National Research Center on Yak, Arunachal Pradesh. DNA was isolated from blood using the protocol of Sambrook and Russel (2000). Twelve sets of overlapping primers reported for amplification of leptin gene in *Bos indicus*, LepII – LepXIII (Dubey et al., 2008) were used to amplify leptin gene in *Bos grunniens* (Table 1).

25 µl PCR master mix consisted of 1X *Taq* buffer, 200 µM dNTPs, 4 ng/µl of each primer, 0.75 unit of *Taq* DNA polymerase and PCR amplification was carried out in a PTC-200 machine (M J Research Inc., MA, USA) pre-programmed for the following conditions: initial denaturation for 5 min at 94°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s and extension at 72°C for 30 s. Amplified products were verified on

Table 1. Primers used for PCR amplification of leptin gene in Yak.

Fragment number	Primer Sequence 5' to 3'	Product size bp	Location
LEP2 F	ACATCCGTTGTTCACTGTG	340	422-761
LEP2 R	TGCAGGCATATCCCATAACC		
LEP3 F	AGGTACATTGTGGGGGATACA	378	706-1083
LEP3 R	GGGCCTGAAAACAGAAGAAA		
LEP4 F	GTGCCACGTGTGGTTTCTTC	210	1051-1260
LEP4 R	CCTCCCTACCGTGTGTGAGA		
LEP5 F	ACACACGGTAGGGAGGGACT	345	1245-1589
LEP5 R	GCTCAGTTACCAGGCAGGAA		
LEP6 F	GCATTCTTCCTGCCTGGTAA	323	1564- 1886
LEP6 R	ATGGGGTCTCAAAGAGTTGG		
LEP7 F	AAGCTAGTCAGGTTCCACAAG	416	1780-2195
LEP7 R	GGCACACACAGTTCTGGTTC		
LEP8 F	GTGACCCTCCCTGAATACCC	354	2151-2504
LEP8 R	GCCACCCACAGCAATCAAT		
LEP9 F	AGAGTGCCCCAACCTGTGT	361	2458-2818
LEP9 R	ATCTTTCTGCCCTTCCCAAA		
LEP10 F	AGGGAGTCATGGCTGGTTCT	310	2713-3022
LEP10 R	TTGGAGGAGACGGACTGCTA		
LEP11 F	GCTCTTGCTCTCCCCTTCCT	430	2977-3406
LEP11 R	GGTTTCTTCCCTGGACTTTGG		
LEP12 F	GGGTGCTGAAGCCTTGAA	346	3357 -3702
LEP12 R	CTTGCTTGATGGTCCAAAGG		

1.5% (w/v) agarose gel with loading dye (95% formamide, 0.25% bromophenol blue and 0.25% xylene cynol) in 1X TAE buffer using a 100 bp ladder as molecular weight marker. Gels were stained with ethidium bromide. All the PCR products were sequenced by ABI prism 3100 Big dye terminator sequencing kit ver3.1 (Applied Biosystems, USA) and then the obtained sequences were aligned using *Bioedit* software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) to obtain the complete leptin gene sequence of 3571 bp in Yak (*Bos grunniens*).

RESULTS AND DISCUSSION

The leptin gene sequence having full length open reading frame, intron-1, intron-2 and 3'-UTR region and was submitted to NCBI GenBank under the Accession No.EU603265, which is the first report of the leptin gene sequence of Yak. BLAST analysis of the leptin gene sequence revealed 98, 98, 97, 95 and 95% homology *Bos indicus*, *Bos taurus*, *Bubalus bubalis*, *Capra hircus* and *Ovis aries* respectively. The multiple sequence alignment revealed a total of 58 nucleotide changes between *Bos grunniens* and *Bos indicus* (Table 2). Of these, there were two insertions and one deletion in the intronic region and five nucleotide changes were found in the exonic region (four in exon 2 and one in exon 3). The multiple sequence alignment revealed a total of 60 nucleotide changes between *B. grunniens* and *B. taurus* (Table 3). Of these, there were two insertions and one deletion in the intronic region and five nucleotide changes were found in the exonic region (four in exon 2 and one in exon 3). Amino acid changes between *B. grunniens* and *B. indicus* were identified at position 987: 'C' in the Exon 2 of *B. indicus* was replaced by 'T' at position 672 in *B. grunniens.* The change in the amino acid was from phenylalanine (*B. indicus*) to serine (*B. grunniens*).

At position 1047, 'C' in the exon 2 of *B. indicus* was replaced by 'A' at position 732 in *B. grunniens.* The change in the amino acid was from serine (*B. indicus*) to arginine (*B. grunniens*). At other positions there is no change in the amino acid because of the degeneracy of amino acids. Amino acids changes between *B. grunniens* and *B. taurus* were also found at position 1120; 'C' in the Exon 2 of *B. taurus* was replaced by 'T' at position 672 in *B. grunniens.* The change in the amino acid was from phenylalanine (*B. taurus*) to serine (*B. grunniens*). At position 1180, 'C' in the exon 2 of *B. taurus* was replaced by 'A' at position 732 in *B. grunniens.* The change in the amino acid was from serine (*B. taurus*) to arginine (*B. grunniens*). At other positions there was no change in the amino acid. The uncommon nucleotide changes may be utilized as signature sequences to differentiate these species. SNP identification in the coding regions of the gene (exon 2 and exon 3) was done using two sets of

Table 2. Nucleotide changes in *B. grunniens* vis-à-vis *Bos indicus* (EU313203: Dubey, 2008).

Region	Insertion	Deletion	Variation	Total
Exon 2	NIL	NIL	4	58
Exon 3	NIL	NIL	1	
Intronic region	2	1	53	

Table 3. Nucleotide changes in *B. grunniens* vis-à-vis *Bos taurus* (BTU50365 : Tellam, 2004).

Region	Insertion	Deletion	Variation	Total
Exon 2	NIL	NIL	4	60
Exon 3	NIL	NIL	1	
Intronic region	2	1	55	

primers (LepIV and LepXI). In the present study exons 2and 3 of leptin gene was found to be highly conserved and no SNP could be identified. Well known and speculated R4C mutation(C/T) of others reported *Bos* genera viz *indicine* (Dubey, 2008) and taurine (Liefers et al., 2003; Konfortnov et al., 2006; Friedman and Halaas, 1998) in exon 2 were found to be fixed with T allele. The observed allele fixation might be due high altitude adaptation or bulky body selection by nature for its survival, but this needs more investigations. The polymerphisms in exon 3 reported in *B. indicus* (Dubey, 2008) were not found in the present investigation. It can be recommended from the present study that, for SNPs identification large number of samples and all intronic and UTR region should be covered. Even if any SNP could be found in non-coding region of leptin gene, in future it can be utilized for Marker Assisted Selection in Yak population.

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