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Characterization of insulin like growth factor-1 (IGF-1) partial gene in mithun

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Insulin like growth factor -1 (IGF-1), a protein hormone similar to insulin in molecular structure (Blundell *et al.*1978), is produced by liver and target tissues and the hepatocytes are main site for its synthesis (Schwander *et al.*1983). IGF-1 is considered as a major hormone, which is involved intricately in the growth of animals. The gene for IGF-1 extends over > 45 kilo base pairs, and contains 5 exons which are separated by 4 large non-coding introns ranging in size from 1.9–40 kb (Rotwein *et al.*1986). Exon 1 and 2 encode portions of the signal peptide whereas exon 3, 4 and 5 code for mature IGF1 molecule. Although there are several reports regarding the polymorphism in IGF-1 gene in different species, viz. cattle (Ge *et al* 1997), swamp buffaloes (Dierkes *et al.*1999), goats and buffaloes (Mishra 2005) and dogs (Sutter *et al.* 2007) are available but no reports are there on mithun - an important beef animal of NE India, which plays a significant role in the economic, social and cultural life of tribal people. Due to high meat value of this animal, the growth is the most desirable parameter for improvement. The present study is an attempt to characterize and ascertain polymorphism in the exon 5 of IGF1 gene of mithun.

Unrelated mithun (90) maintained at National Research Centre on Mithun, Medziphema and Porba farm, Nagaland from all 4 types, viz. Arunachalee, Nagamee, Manipuri, Mizoram were selected for this study. 15 ml of intravenous blood was collected from each animal in sterile 15 ml polypropylene centrifuge tube containing 0.5 ml of 2.7% EDTA as anticoagulant. DNA isolation was done by phenol-chloroform extraction method (Sambrook *et al.* 2001) and the precipitated DNA was dissolved in 200 µl of TE buffer. The quality of DNA was checked by performing agarose gel electrophoresis. The purity and concentration were evaluated by spectrophotometry. The sample that showed an OD ratio (OD_{260} /OD_{280}) in the range of 1.7–1.9 were assessed to be of good quality. Concentrations of DNA samples were in the range of 16–20 µg/ml of blood. A set of primers (5' ATG TCA CTT TTT CTC GCT TAT T3' as forward primer and 5' CAT GCA TTT GTG GCT CTT G 3' as reverse primer) was designed on the basis of cattle genomic sequences (AF210383S5), to amplify a fragment (396 bp) corresponding to part of intron 4 (27 bp), exon 5 (60 bp) and part of 3′ flank (309 bp) of IGF1 gene. The stock was reconstituted by DNAse free milipore water to the tune of 300 pmoles//µl. The working primer solution was then prepared by further 10 fold dilution of stock solution to have a final concentration of 30 pmole/µl. The reaction mixture and PCR programme were optimized to achieve the satisfactory level of amplification in a final volume of 25 µl containing 1µl of genomic DNA (80–100 ng), 2.5 µl of 10 X PCR buffer (1.5 mM), 2.5 µl of dNTP mix (0.2 mM), 1.5 µl of $MgCl_2$ (1.5 mM), 1 µl each of forward primer and reverse (30 pmol/µl), 0.2 µl of Taq DNA polymerase (5U/µl). Samples were amplified for 35 cycles with initial denaturation at 94°C for 3 min, cyclic denaturation 94°C for 45 sec, cyclic annealing at 60°C for 45 sec, cyclic extension at 72°C for 45 sec followed by final extension at 72°C for 10 min. It was digested with *Msp*I (*Hpa*II) restriction enzyme, which cuts at the recognition site of 5′…C↓CGG…3′. The RE digestion was carried out in 15 µl reaction mixture carrying 7 µl of autoclaved triple distilled water, 2 µl of 10X assay buffer for RE, 1 µl of RE enzyme (10U/µl) and 15 µl of PCR product. The incubation was carried out at 37°C for overnight for complete digestion. Restriction fragments were resolved in 2.5% agarose gel stained with ethedium bromide in 1X TBE buffer and visualized under UV light. The amplicon of IGF-1 gene was eluted and sequenced. The obtained sequence was then aligned with other available sequences of different species from GenBank (www.ncbi.nlm.nih.gov).

A portion of IGF-1 gene corresponding to 27 bp of intron 4, complete exon 5 having 60 bp length and 309 bp of 3´ flanking region constituted the amplicon of 396 bp length and its digestion with *Msp*I (C↓CGG) produced 2 bands i.e.

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of 273 and 123 bp (Fig. 1), which was confirmed by the presence of only one cutting site in the sequence. All the bands were clearly visible in the gel. This type of banding pattern suggested that amplified fragment contained only one RE site. No polymorphism was found with respect to *Msp*I gene and genotype frequencies for this fragment were calculated to be 1.00. Similar studies on a fragment encompassing exon 3 and 5 of IGF-1 gene of mithun also revealed monomorphism with respect to *Ava*II and *Hind*III restriction enzymes (Panigrahi *et al*. 2009a, 2009b). Though there was no PCR-RFLP report till date for this particular fragment, nevertheless, the other sites of IGF-1 were shown to have polymorphism with respect to different restriction enzymes, viz. Ge *et al*. (2001) reported polymorphism at *Sna*BI restriction site of a 5´ flanking region of bovine IGF-1 gene with allele frequency of 0.55 and 0.45. They also reported PCR--SSCP in this region with allele frequencies of 0.566 and 0.434 for alleles A and B respectively in Angus cattle. The same fragment was analyzed PCR-SSCP by Mishra (2005) observed monomorphic pattern in buffaloes whereas polymorphic in case of goats showed 3 different genotypes AA, AB and BB with allelic frequencies 0.49 and 0.51 for A and B alleles respectively. Association studies with phenotypic parameters of mithun could not be resorted due to lack of polymorphism whereas significant association of IGF-1 RFLP genotypes with the weight gain during the first 20 days after weaning was found by Ge *et al*. (2001). Similarly, Condie *et al*. (2001) reported association of micro satellite located at 5′ flanking region of the IGF1 gene with birth weight in Nellore cattle. These studies clearly indicated that the IGF system has a significant relationship with birth weight. On the other hand, our reports indicated a monomorphic pattern, which obviated the association studies.

The sequencing results revealed that the amplicon of mithun was 396 bp, which was submitted to GenBank (Accession no. EF686016). Alignment reports indicated that the genomic length of 396 bp was similar to that of cattle. The amplified fragment contained only 1 *Msp*I restriction site at 123rd nucleotide position respectively. Only 2 bands corresponding to the restriction enzyme were obtained in all the individuals showing no mutant allele in mithun under study. Only 2 single nucleotide differences existed in mithun sequences when compared to that of cattle, which were identified at 290th (T to C) and 342nd (G to T) positions, respectively, and homology between them was 99.5%. Despite the differences, the deduced amino acid sequences showed 100% homology, indicating that mutations in these nucleotides occurred in the intronic regions and did not affect the amino acid coding with respect to exon-5. There was 96.7% homology, which could be confirmed from the phylogenetic tree. Mithun had maximum deviation from mouse with respect to nucleotide as well as amino acid sequences.

Further, if any polymorphism in these genes is identified in future, then association of these polymorphs with economic traits like milk yield, fat %, weight gain and carcass traits could be used as a genetic marker for selection programme. It was also observed that the regions of IGF-1, which possess nucleotide variations, can be considered as candidates for marker identification in mithun. More number of individuals belonging to different types should also be screened with more number of restriction enzymes to find out the allelic variants in other exonic and intronic regions of this gene. Attempts should also be made at studying the polymorphism of other growth associated genes.

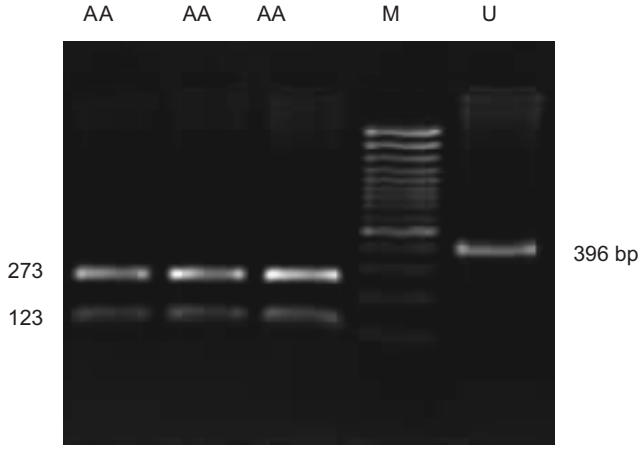

Fig. 1. Digestion of 396 bp fragment of IGF-1 gene of mithun with *Msp*I restriction enzyme. M,100 bp Marker; U,undigested; AA, *Msp*I genotypes.

SUMMARY

A 396 bp fragment of insulin like growth factor-1 (IGF-1) gene, which included complete exon 5, was amplified in mithun (*Bos frontalis).* The PCR–RFLP analysis showed the absence of polymorphism in this fragment with respect to *Msp*I restriction enzyme and showed 1 restriction site, which produced 2 fragments of 273 and 123 bp. Only 2 single nucleotide differences existed in mithun sequences when compared to that of cattle. The genomic sequence homology between mithun and cattle was 99.5%, whereas the deduced amino acid sequences showed 100% homology. The sequence of the wild allele, which was the first report on exon 5 of mithun IGF-1, was submitted to GenBank (Accession number EF686016).

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