Short communications

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Coding variations in bovine α-lactalbumin in Kashmiri cattle *vis-a-vis* exotic breeds

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Alpha-lactalbumin is a major whey protein of bovine milk and a potential quantitative trait locus of dairy cattle. Expression of α- lactalbumin is restricted to the mammary gland. However, its expression is diverse among various breeds leading to differential lactogenesis and hence milk yields. Differential expression of bovine alpha-lactalbumin gene is attributed to its genetic polymorphism in various regions of the gene such as promoter and other upstream regulatory elements (Dayal *et al*. 2005a,b). Limited work has been done to examine the polymorphism in coding region of alpha-lactalbumin. Therefore in this study, Holstein, Jersey and indigenous Kashmiri cattle breeds were screened for variations in the coding region of alpha-lactalbumin gene.

Blood samples were collected from 180 lactating cows belonging to Holstein, Jersey and local Kashmiri breeds with 60 samples from each. Cows were selected on the basis of being genetically 100% pure and lactating. Blood samples of Jersey and Holstein were collected from the organised herds maintained at Cattle Research Station SKUAST-K, Manasbal and Military Dairy Farm, Srinagar respectivel; while as those of local Kashmiri cattle were collected from the animals maintained by the farmers in the areas of the Kashmir Valley not covered by the Artificial Insemination services of Animal Husbandry Department Kashmir.

Genomic DNA was extracted by phenol chloroform extraction method (Blin and Stafford 1976). The concentration, purity and quality of genomic DNA was checked using absorption coefficient and spectrophotometry. For amplification of the fragment containing exon 1 and 2, primers were designed by aligning cattle and sheep whole gene sequence with software. The pair of primers used to amplify this fragment was, forward 5'-CTCTCTTGTGATCCTCTTC-3' and reverse 5' GGTTATCCCAGGAGTAGGT-3'. For amplification of the fragment, PCR reaction was performed in a total volume of 50µl with 100ng of genomic DNA, 0.30 pico moles of each primer, 0.2 mM of each dNTP, 1× PCR reaction buffer and 0.03 units of Taq DNA polymerase. DNA was initially denatured for 5 min at 94°C, then 35 cycles of denaturation for 1 min at 94°C, annealing for 30 sec at 50°C and extention for 45 sec at 72°C. PCR product was purified by gel cut purification method (Vogelstein and Gillespie 1976).

Before sequencing, purified PCR product was

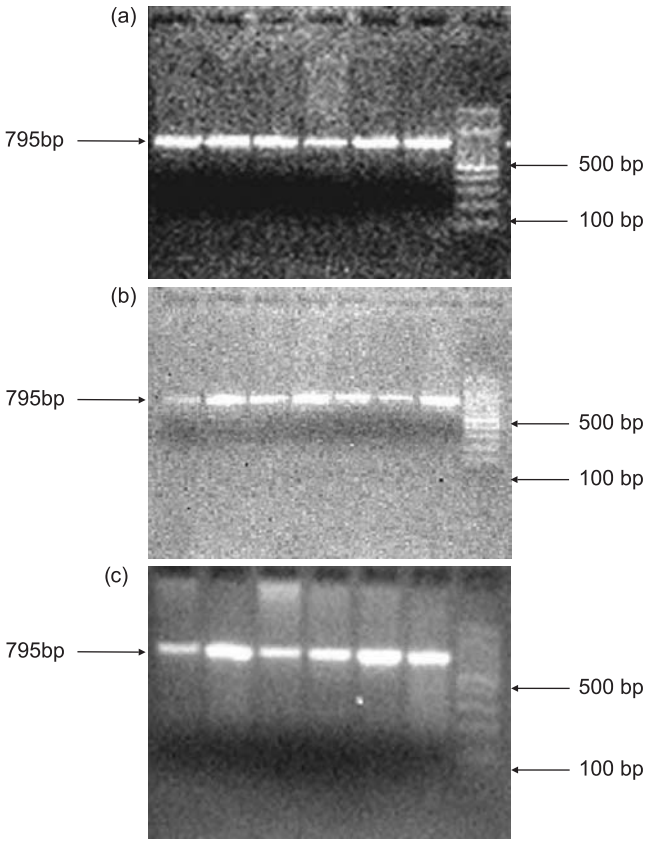

Fig. 1. PCR amplification of bovine alpha lactalbumin with each ampicon conataining exon 1 and 2 generated from various samples of: a. Holstein; b. Jersey; c. local Kashmiri breeds.

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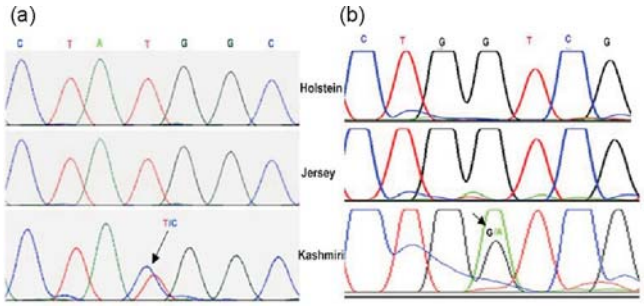

Fig. 2. Chromatopherogram of bovine alpha lactalbumin gene, exon 2 of Holstein, Jersey and Kashmiri breed. Arrow indicates : a. The position of A/G polymorphism at 178th bp in Kashmiri breed; b. the position of C/T Polymorphism at 249th bp in Kashmiri breed.

quantitatively estimated by band intensity comparison after running them on 2% low melting agarose gel and the desired product was eluted from the gel using gel elution kit. The concentration of purified PCR product was adjusted in the range of 20– 50ng/µl by proper dilution using milli Q water before sequencing. The sequencing of purified PCR product was commercially done.

The 795 bp fragment containing exon1 and exon 2 of bovine alpha lactalbumin of the 3 breeds is given in Fig. 1. The results of sequence alignment analysis of different breeds reported 2 exonic polymorphisms in bovine alpha lactalbumin within the Kashmiri breed (Fig.2 a, b). Among the 2 exonic polymorphisms, the first single nucleotide polymorphism (SNP) was found at 178th nucleotide position of mRNA while as the second SNP was found at its 249th position of mRNA. However both these allelic variants were localized to exon 2 of the gene. First SNP caused A→G conversion leading to conversion of isoleucine to valine and second SNP caused C→T conversion, which results in no change in aspartic acid in the alpha lactalbumin protein. Since the first SNP caused the substitution of one hydrophobic residue for another hydrophobic residue and the second SNP resulted in no change of the amino acid, therefore, the first mutation is missense but synonymous whereas the second one is silent. The details of both these polymorphisms are given in Table 1. The representative diagram showing DNA sequence of alpha lactalbumin gene with translation and position of various SNPs identified is shown in Fig 3.

Since alpha-lactalbumin, is an important gene having strong correlation with production traits of the cattle, therefore polymorphism in this gene is reported to be significantly associated with milk production (Bleck and Bremel 1993). Thus screening of various bovine breeds varying markedly in their milk production potential for genetic polymorphism in alpha-lactalbumin could provide important insight into the genetic mechanisms governing milk yield. Our results reflect a positive correlation of genetic

Fig. 3. DNA Sequence of alpha lactalbumin gene with translation. Blue lines indicate position of SNPs within exonic region.

Table 1. Details of SNPs identified in the Bovine alpha lactalbumin gene of Kashmiri breed

Wild type nucleotide	Base pair change observed	Nucleotide position in mRNA	Exon position	Amino acid position	Codon change	Amino acid change
A	A to G	178	44	60	AUA to GUA	Ile to Val
C	C to T	249	116	83	GAC to GAU	Asp to Asp

polymorphism with decreasing milk production among local Kashmiri cow, which shows 5- times decreased milk production than Holstein breed and 3- times reduced milk production than Jersey cows. Polymorphism at +15, +21, +54 and –1689, –1466 positions in 5' flanking region of alpha-lactalbumin gene were reported in Holstein cattle (Bleck and Bremel 1993, Voelker *et al*. 1997) out of which polymorphism at +15 was found associated with milk production (Bleck and Bremel 1993). Zhang *et al*. (2007) could however locate only 2 nucleotide transitions in 5' flanking region of alpha-lactalbumin gene but none was found associated with milk production.

If mutations occur in coding regions of the gene, it may influence biological activity through changing amino acid composition in the polypeptides and ultimately affect various economic traits (Chianese *et al*. 2004). In present study we have seen some novel SNPs within the coding regions of the gene causing amino acid changes which may affect the protein activity by affecting its binding capacity while interacting with ß-galactosyltransferase during lactose synthase complex formation which catalyses lactogenesis.

SUMMARY

Bovine alpha lactalbumin, a major milk protein, acts as a regulatory component of lactose synthase complex during lactogenesis within the mammary gland epithelial cells. Differential expression of this gene in various cattle species is attributed to its genetic polymorphism. Holstein, Jersey and indigenous Kashmiri cattle populations were screened for allelic variations. Among the 2 exonic polymorphisms observed within the exon 2 of indigenous Kashmiri breed, first SNP caused A'™G conversion leading to conversion of isoleucine to valine and second SNP caused C'™T conversion, which results in no change in the protein. The first mutation was found to be missense but synonymous whereas the second one was silent.

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