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Kappa casein gene in water buffaloes using polymerase chain reaction and sequence specific oligonucleotide probes (PCR-SSOP)

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ABSTRACT

Polymorphism of kappa casein gene was studied in genomic DNA samples isolated from peripheral blood lymphocytes of 24 animals of Murrah breed of buffaloes using polymerase chain reaction and sequence specific oligonucleotide probes. The primer and probes for the amplification and hybridization of the variable fragments of the CASK gene was designed from the published nucleotide sequence of the cattle. PCR amplification of buffalo DNA samples and probe hybridization confirmed the homology between the 2 species at nucleotide level. In spite of homology, buffalo DNA samples did not show any polymorphism, instead monomorphism was observed at CASK gene. However, in kappa casein gene an interesting hybridization pattern was observed. The kappa casein gene shows monomorphism but it is like CASK-A allele of cattle at codon 136 i.e. ACC (Thr) and like B allele of cattle at codon 148 i.e. GCT (Ala) at the nucleotide level confirming the earlier report on protein sequence.

Key words: κ-casein, Milk protein polymorphism, Murrah, PCR-SSOP.

Caseins constitutes major fraction of the milk proteins. Four major types of casein genes i.e. αS1 (CASAS1), αS2 (CASAS2), ß (CASB) and κ caseins (CASK) are reported in cattle. Comparisons of the caseins of buffalo and cow showed that ß and κ caseins were of comparable electrophoretic mobilities and that each of the main protein components of cow's milk has its counterpart in buffalo's milk (Aschaffenburg and Sen 1963). Four alleles of CASK have so far been identified in cattle, viz. A, B, C and E. While CASK-A and CASK-B alleles are frequently found in cattle, CASK-C and CASK-E alleles are rare. The B allele of CASK has been shown to give higher cheese production. Cheese produced from the CASK-BB genotyped bovine milk reportedly have a higher protein content, higher yield and better quality than those produced from CASK-AA milk (Medrano and Anguilar-cordove 1990). CASK-BB genotype is also reported to be responsible for firmer curd formation and higher first lactation milk yield (Lin *et al*. 1989). Considering the importance, kappa casein gene of buffaloes was studied at molecular level using polymerase chain reaction (PCR) followed by hybridization with sequence specific oligonucleotide probes (PCR-SSOP).

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MATERIALS AND METHODS

Blood samples were collected from 24 animals of Murrah breed of water buffaloes (*Bubalus bubalis*) maintained at farm of the Institute (IVRI).

Extraction of genomic DNA: Genomic DNA was isolated from the blood samples using standard phenol chloroform extraction method (Andersson *et al.* 1986) with slight modifications. Briefly, 15 ml fresh blood collected with anticoagulant (0.5 M EDTA) was centrifuged first with TE (tris EDTA, 10:1) buffer at 4°C for 15 min at 3000 rpm. The pellet was resuspended in lysis buffer (1% Trition X-100, 0.32 M sucrose, 5 mM Tris, pH 7.5, 5 mM $MgCl_2$) and centrifuged at 3000 rpm for 15 min at 4°C. The pelleted buffy coat was incubated overnight at 37°C in nuclease buffer (70 mM sodium chloride, 1% SDS and 1 mM EDTA, pH 8.0) along with proteinase–k (0.2 mg/ml) and than phenol chloroform extraction was done on the following day.

PCR-SSOP method: Primers were designed for amplification of 202 bp variable fragment of κ casein gene (Table 1) using the sequence of cattle κ-casein (Stewart *et al.* 1984 and Kang and Richardson 1988). Synthesis of primers and oligonucleotide probes were carried out at CSIR Centre for Biochemical Technology, New Delhi, India. PCR was carried out in 200 ng of genomic DNA in 50 µl of PCR

mix containing 10 µl of 10X PCR buffer, 3 µl of 10 mM $MgCl_2$, 10 µl of 1X dNTP's, 25 pmoles of each of the 2 primers, 0.25 µl of Taq DNA polymerase (5 units/µl) was allowed to amplify in thermocycler for 30 cycles. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 55°C for 50 sec and extension at 72°C for 1 min and 30 sec. This was followed by a final extension at 72°C for 5 min and the amplified samples were stored at 4°C until ready to use. The amplified samples were run on 0.8% agarose gel to confirm amplification.

Hybridization: Dot blot were prepared from 2 µl of amplified DNA samples on Xeta probe membrane. The membranes were dipped in 0.4 N NaOH for 5 min and then in 2X SSC for 10 min and exposed to UV light for 5 min to cross link the amplified DNA on the membrane.

Four oligonucleotide probes were designed (Table 1) in such a way that the nucleotide substitutions giving rise to the variable region at codon 136 and 148 for kappa casein gene could be covered.

Membranes were placed in prehybe buffer (15% formamide, 0.1% Denhardt's solution, 5X SSPE, 5% dextran sulphate, 1% SDS and 0.2 mg/ml denaturated salmon sperm DNA) and incubated for at least 1 h and then hybridised with P^{32} labelled probes overnight. After washing twice with 3X SSC at room temperature for 10 min, membranes were washed with 2X SSC at 42°C for 10 min and then exposed to X-ray and kept at –70°C overnight.

RESULTS AND DISCUSSION

The variable fragments of 202 bp for κ-casein gene was amplified by polymerase chain reaction from the genomic DNA samples of Murrah buffaloes using primers derived from sequences of bovine CASK gene. The successful amplification of κ-casein fragments in buffaloes showing the homology of kappa gene in cows and buffaloes. The amplified products were hybridized with the 4 probes designed for κ-casein. The hybridization with different allele specific (cattle) probes showed monomorphism in κ-casein gene as earlier observed in starch gel electrophoresis (Jairam and Nair 1979). Hybridization with allele specific probes for κ-casein showed very interesting results. While A1 probe (specific for CASK-A allele) hybridized, A2 probe, which is also specific for A allele did not show any hybridization. The probes for CASK-B allele showed preferential hybridization with only B2 probe showing that the buffalo gene, which is monomorphic has ACC (Thr) at codon 136 and GCT (Ala) at codon 148. The observation at the nucleotide level is in agreement with the amino acid sequence of buffalo κ-casein reported by Mercier and Chobert (1976). However, they reported variability at amino acid position 135, which could be either Thr or Ile. Our probe A1 has ACC at codon 135 (Thr) (Table 2), and all the samples showed hybridization with this probe indicating that the Indian water buffaloes have Thr at amino acid position 135 as well as 136, which is like A allele of the cattle and have GCT (Ala) at amino acid position 148, which is like B allele of cattle. The present results explain that it could be due to homology of buffalo κ-casein gene with bovine CASK-A at codons 135 and 136 and with bovine CASK-B at codon 148. Contrary to this, Jairam and Nair (1979) found only single band corresponding to B allele of cattle using starch gel electrophoresis. Similar findings were observed at DNA level in Jaffarabadi, Mehsana, Surti and Pandharpuri (Pipalia *et al.* 2001), Murrah and crossbred buffaloes (Otaviano *et al.* 2005) as well as in South Kanara, Surti and Murrah buffaloes (Gangaraj *et al.* 2008). However, the present study was in agreement with those reported by Majumdar and Ganguli (1970) where both the κ-casein variants (CASK-A and CASK-B) were observed in all the samples analysed by starch gel electrophoresis and at DNA level by Mukesh *et al.* (2006) in riverine buffaloes, Singh *et al.* (2005) in Murrah and Bhadawari breeds and Patel *et al.* (2007) in Murrah, Surti and Pandharpuri breeds. The present study, however suggested that a point mutation took place either in codon 136 or 148.

The monomorphism in the casein genes of buffalo is not surprising because there has been a lot of inbreeding in Indian buffaloes. Average proportion of polymorphic loci (P) observed for buffalo is significantly lower than cattle. More

Table 1. Primers and probes designed to study the polymorphism of κ-casein

Oligo	Sequences 5'-3'	Nucleotide position	Amplification/ hybridization
5'-Primer	ATCATTTATGGCC-ATTCCACCAAAG	322-346	202 bp
3' Primer	TTAGACTGC-AGTTGAAGTAAC	503-523	fragment
Probe A1	CACCTACCACC*-GAAGCAGT	411-429	A allele
Probe B1	CACCTACCATC* GAAGCAGT	411-429	B allele
Probe A2	CTCTAGAAGAT* TCTCCAGA	447-465	A allele
Probe B2	CTCTAGAAGCT* TCTCCAGA	447-465	B allele

*Variable codons differentiating the alleles.

Table 2. Hybridization pattern of κ-casein oligonucleotide probes

Alleles	Probes for codon 136		Probes for codon 148	
	A1 ACC (Thr)	B1 ATC (Ile)	A2 GAT (Asp)	B2 GCT (Ala)
Cattle:				
CASK-A	+	-	+	-
CASK-B	-	+	-	+
Buffalo	+	-	-	+

detailed molecular studies with respect to the protein systems and diversified buffalo breeds/genetic groups would through a light in the genetic conversation in buffaloes.

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