

GENOTYPING OF PANDHARPURI BUFFALO FOR *k*-CASEIN USING PCR-RFLP

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ABSTRACT

The objective of the study was to genotype Pandharpuri buffalo breeds for *k*-casein type by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). A 379-bp *k*-casein gene fragment was amplified using primers K1 (5'-CAC GTC ACC CAC ACC CAC ATT TAT C-3') and K2 (5'-TAA TTA GCC CAT TTC GCC TTC TCT GT-3'). DNA from 20 Pandharpuri buffaloes were extracted by phenol chloroform method, PCR was carried out in a final reaction volume of 15 µl and the reaction mixture was subjected to standard PCR protocol. PCR products (379 bp) were digested with *Hinf* I and *Hind* III (REs). *Hinf* I analysis yielded two fragments of 288-bp and 91 bp only for all the DNA samples hence, all the samples were typed BB. *Hind* III yielded two fragments of 225 and 154 bp for all DNA samples. Thus PCR-RFLP using *Hinf* I and *Hind* III revealed all the samples to be monomorphic for this locus.

Key words: *k*-casein, Pandharpuri buffalo, *Hinf* I, *Hind* III,

INTRODUCTION

A decade ago, milk produced by cows in the Western World had a lower fat to protein ratio than consumers wanted. This excess milk protein was disposed of on world markets in the form of dried skim milk. Today's trend in dairy production is to focus on more production of milk proteins. As pricing systems increasingly include payment for the amount of protein in the milk, breeding decisions have focused on protein production. Two protein genes have been identified that affect milk protein production. It is now relatively simple to determine the genotypes of animals for these protein genes.

Caseins amount to nearly 80% of the protein output in cow milk. caseins are biologically important proteins and they are also a raw material for the cheese making industry (Fox, 1989). The bovine casein genes (alpha S1, alpha S2, beta and kappa) reside in a region of less than 200 kb on chromosome 6 and form a strong gene cluster (Threadgill and Womack, 1990).

Polymorphisms of all casein genes are known (Eigel *et al.*, 1984). Out of 5 known *k*-casein genetic variants, the A and B are the most common in the majority of cattle breeds (Erhardt, 1989). These variants are differing by point mutations in the nucleotide sequence encoding amino acid 136 and 148. At position 136, threonine (ACC) is changed

into isoleucin (ATC) and at position 148, asparagine (GAT) is changed into alanine (GCT) for A and B variants, respectively (Alexander *et al.*, 1988). In many investigations, the *k*-casein B variant was found to be associated with protein content in milk and to exert a significant influence on some cheese making properties of milk. The *genotype* BB yields more protein than AB, which yields more than AA (Schaar, 1984).

There are two types of effect of *k*-casein genotype on cheese processing. The first is that the B variant causes much faster renneting and a firmer cheese curd. The second, less precisely defined, is that the B variant causes enhanced cheese yield from milk of a given fat and protein concentration. Early results estimated a very substantial increase of 8 to 12% in cheese yield.

Since the frequency of the *k*-casein B allele in dairy breeds is low (20%), it would be worthwhile to have breeding programs that emphasize increasing the frequency of the B allele in the dairy cattle population. Some countries have called for national breeding programs for increasing the frequency of the B allele in their dairy herds. Because of AI (Artificial Insemination) technology, a bull's genotype is of particular interest.

As Polymerase Chain Reaction (PCR) is very specific, it is possible to amplify segments of genome containing allelic variations that can be identified by Restriction Fragment Length Polymorphism (RFLP). The PCR-RFLP techniques have been used to study the frequency of *k*-casein alleles in bovines (Kim *et al.*, 1997).

Although India posses the world's largest buffalo population but only very few studies have been conducted on buffaloes in this important area. Hence the present study was undertaken with the following objectives.

Detection of polymorphism if any at *k*-casein locus in buffaloes by PCR-RFLP technique using PCR amplification by restriction

digestion with *Hinf I*, *Hind III* restriction enzymes (REs).

MATERIALS AND METHODS

The of blood samples from 20 Pandharpuri buffaloes from NARP Buffalo Research Station Shenda Park Kolhapur of MPKV Rahuri. Genomic DNA were isolated from whole blood samples of buffaloes as described by John *et al.*, (1991) using phenol chloroform method.

The DNA concentration was determined and the samples were diluted to 30 ng / μ l of autoclaved HPLC water at least 24 hours prior to the reaction. 2 μ l of DNA was used as template for PCR reaction. The *k*-casein gene specific primers (concentration 10 pM) K1 (5' CAC GTC ACC CAC ACC CAC ATT TAT C-3') and K2 (5' -TAA TTA GCC CAT TTC GCC TTC TCT GT-3') were custom synthesized and supplied by Bangalore Genei .

PCR was carried out in a final reaction volume of 15 μ l. Each reaction volume contained 1.5 μ l of 10X PCR buffer, 1.2 μ l of dNTPs (0.3 μ l each), 1.6 μ l of primer (0.8 μ l of each), 0.2 μ l of Taq DNA polymerase (one unit), 2.0 μ l of template DNA and 8.5 μ l of sterile distilled water. The reaction mixture was subjected to initial denaturation at 94°C for 4 minutes, annealing at 56°C for 1 minute, and extension at 72°C for 1 minute followed by 35 cycles of 94°C for 1 minute, 56°C for 1 minute, 72°C for 1 minute and final extension step of 72°C for 10 minutes.

The amplification of PCR product is confirmed by 5 μ l of the PCR product electrophoresed on 1% agarose gels containing ethidium bromide at constant voltage of 10 V/ cm for 15 minutes using 1X TBE buffer. The 100-bp ladder was used as molecular size marker. The amplified product was visualized as single band under UV light and

photographed by gel documentation system.

The PCR products (10 µl) from each tube were digested with 5 units of the REs (*Hinf I* and *Hind III*) in final reaction volume of 25 µl. The reaction mixture was incubated at 37°C for at least 3 hours in Mini Cycler. After restriction digestion, the PCR products were electrophoresed on 2.5% agarose gel. The 100 bp ladder was used as molecular size markers. The bands were visualized under UV light and photographed with gel documentation system. The band size were judged by comparing with molecular size markers and recorded.

RESULTS AND DISCUSSION

The primers K1 and K2 are amplified

with the DNA fragment which is used as template for PCR reaction. The PCR amplification was confirmed by running 5µl of PCR product along with 100bp DNA ladder in 1% agarose gel. The amplified PCR product of 379 bp was visualized as a single band of expected size under the U.V. with ladder.

The amplified PCR product was digested with *Hind III* enzyme and after restriction digestion the PCR products were electrophoresed on 2.5% agarose gel containing ethidium bromide 1% @ 5µl/100 ml by submarine gel electrophoresis apparatus at constant voltage of 80 V for 60 to 90 minutes. When length of each fragment compared with the marker lane and fragment size was estimated. All the samples revealed two fragments of 225-bp and 154-bp. Only 'B' allele with BB genotype was found in Pandharpuri buffaloes,

Fig. 1

RE digestion of PCR product of Pandharpuri buffalo by *Hind III*.

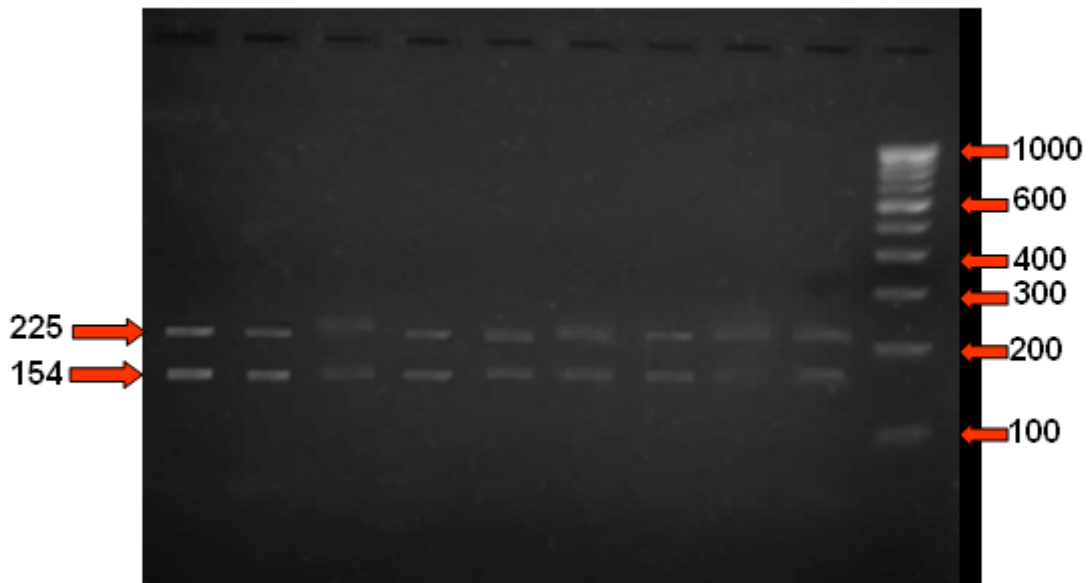
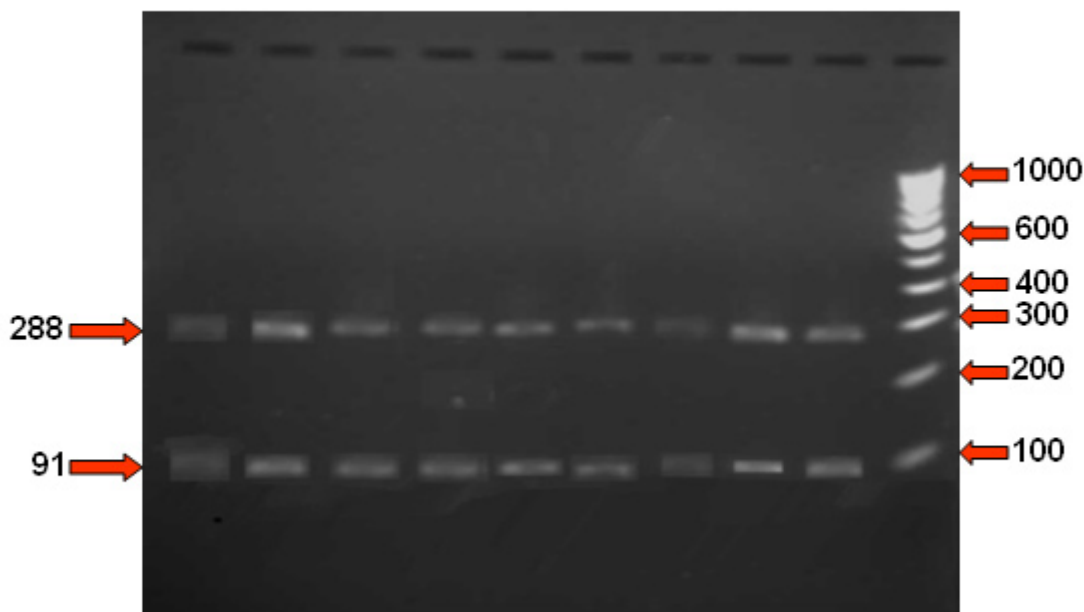


Fig. 2

RE digestion of PCR product of Pandharpuri buffalo by *Hinf I*.



The amplified PCR product was digested with *Hinf I* enzyme and after restriction digestion the PCR products revealed two fragments of 288-bp and 91-bp. Only 'B' allele with BB genotype was found in all Pandharpuri buffaloes. Hence all the samples were typed BB (figure-2). The present findings were in concurrent with Mitra *et al.*, (1998), who reported monomorphism for *k*-casein gene showing similar band pattern of 225 bp, 154 bp and 288 bp, 91 bp by using restriction endonuclease *Hind III* and *Hinf I* respectively in Murrah and Nili-Ravi buffalo breeds.

Lama and Zago (1996) scanned probably the different area of *k*-casein gene in buffalo also could not revealed any polymorphism. Thus monomorphic pattern of *k*-casein gene may be a characteristic feature for buffalo.

Genotypic and allelic frequencies at *k*-casein locus in Pandharpuri buffalo was 0.00 and 1.0 respectively (Table 1). Similarly allelic frequency of allele B of *k*-casein gene in cattle (Sahiwal) is 0.16 according to Mitra *et al.* (1998) was much lesser than the Pandharpuri.

The results of present study can be verified by typing of milk samples from these buffaloes for *k*-casein protein. Any variant form of *k*-casein if obtained will indicate the overestimation of frequency of BB genotype by molecular method using only *Hinf I* or *Hind III* enzymes. Hence, the observed frequency distribution of *k*-casein 'B' allele in Pandharpuri buffaloes confirmed the monomorphism

Table-1
Genotypic and allelic frequencies of k-casein locus for Pandharpuri buffalo

Breed	Number of buffaloes analysed	The frequencies of				
		Genotype			Alleles	
		AA	AB	BB	A	B
Pandharpuri	20	0.00 (0)	0.00 (0)	1.00 (20)	0.0	1.0

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