GENOTYPING THE PROLACTIN GENE IN PANDHARPURI BUFFALOES BY PCR-RFLP

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ABSTRACT

The present study was undertaken to detect polymorphism at exon I of the prolactin locus using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) in the Pandharpuri buffalo breed. DNA from 50 Pandharpuri buffaloes was extracted by the phenol - chloroform method. A pair of bovine specific primers (forward 5'-ATTATCTCTCTCAT TTCCTTTTTACA-3' and reverse 5'-ACTCTGC TGTCACTGTCTGTATT-3') were used for amplification of PRL gene exon I. PCR was carried out in a final reaction volume of 25 µl and the reaction mixture was subjected to standard PCR protocol. The PCR product digested with *Hae* III (RE). Digestion with *Hae* III revealed an intact product of 857 bp. The results of PCR-RFLP using *Hae* III show a different band pattern from cattle and previously observed studies in Jaffarabadi buffaloes. This indicated there might be absence of *Hae* III restriction sites in this buffalo breed. However, this would require confirmation by sequencing of amplified region to find the exact sequence variation.

INTRODUCTION

Lactation is under the physiological influence of the endocrine system. The milk protein and hormone genes are excellent candidate genes for linkage analysis with quantitative trait loci (QTL) because of their biological significance on the quantitative traits of interest. Among several hormones that regulate lactation and reproduction in bovines, prolactin is an important anterior pituitary hormone (Ladani *et al*., 2003).

The polypeptidic hormone prolactin is responsible not only for triggering lactation but also for mammary gland growth and lactogenesis (Tucker, 1981; Collier *et al*., 1984). It also plays an important regulatory function in expression of milk protein genes. Therefore, the bovine prolactin gene (*bPRL*) seems to be an excellent candidate for linkage analysis with quantitative trait loci (QTL) affecting milk production traits (Brym and Kaminski, 2005). Considering the importance of the prolactin hormone gene, the present study was undertaken. The objective, was to investigate polymorphism within prolactin gene using PCR RFLP technique in Pandharpuri buffalo.

MATERIALS AND METHODS

Experimental material for the present study comprised of blood samples from 50 Pandharpuri buffaloes. The animals were unrelated and selected at random.

Genomic DNA was extracted by the phenol-chloroform method as described by John *et al.* (1991) with minor modification. The quality and quantity of DNA were checked and quantitation done by UV spectrophotometry and agarose gel electrophoresis. The DNA samples were diluted 1:50 by dissolving 2 µl of DNA in 98 µl of autoclaved

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distilled water and used for spectrophotometry. DNA samples with an OD 260/280 ratio of 1.8 to 2.0 were further subjected to electrophoresis as a quality check; this was done on the 0.8 percent agarose in 0.5 X TBE buffer.

A pair of bovine specific primers (forward 5'-ATTATCTCTCTCAT TTCCTTTTTACA-3' and reverse 5'-ACTCTGCTGTCACTGTCTGTA TT -3') (Zhang *et al*., 1994) were used in the present study to amplify PRL gene exon 1. PCR was carried out in a final reaction volume of 25 µl. Each reaction volume contained 200 µM of each dNTP, 10 pmole of each primer and 0.65 unit of Taq. polymerase and 90 ng of template DNA in 1X PCR buffer. The reaction volume was subjected to 36 cycles comprising of denaturation at 94°C, annealing at 56°C for 1 minute and extension at 72°C for 1 minute followed by a final extension step at 72°C for 10 minutes.

The PCR products were digested with 10 units of *Hae* III at 37°C for 6 h in a final reaction volume 25 µl. The RE digests were electrophoresed on agarose along with 100 bp DNA ladder.

RESULTS AND DISCUSSION

The primers amplified an 857 bp fragment from prolactin gene in all the Pandharpuri buffalo DNA samples. The PCR amplification was confirmed by running 5 µl of PCR product along with 100 bp DNA ladder in 0.8% agarose gel. (Plate1) The amplified PCR product of 857 bp was visualized as a single compact fluorescent band of the expected size under the U.V. transilluminator and was confirmed by comparing its distance from the well with that of the ladder and documented by the gel documentation system.

The amplified PCR product was digested with *Hae* III enzyme and after restriction digestion the PCR products were electrophoresed on 2.5 percent agarose gel containing ethidium bromide

181	aatgactgct	ataattttat	agttcctcta	actcaaacta	gtctccagat	etcaccatc**{a**
241	**ttatctctct**	**catttccttt**	**cag**tctaatt	aatcaaaatc	cttcctagat	gttcatttct
301	ggtcagtatg	…….	……	……..	……	…….
481	aggacgagag	cttcctggtg	aagtgtgtgt	cttgaaatca	teaccaccat	ggacagcaaa
541	ggttcgtcgc	agaaaggtat	gtacagcage	ttgtggagtt	gttgggtttt	atccatgttc
601	caatgggggc	…….	……	……	……	……
781	ggttgtctta	atatggggca	ggggtgcaac	tggtgccagg	gggtagagaa	cagagatatt
841	gctaagcata	ctacaatgca	caagttagcc	cccagaacaa	gtacttatcc	agctcagggt
901	**gg↓cc**aatcat	gtcaaagttg	agaaaactta	gactggaata	agaccaaaaa	tgtctctgag
961	tccaattcat	cacaactcca	gaaggtagaa	acaaacattt	tctagttacc	aagattctta
1021	tgtggtgtgg	ctaagatgag	tcagtctgat	gaaactttta	actctgaagc	tat**aatacag**
1081	**acagtgacag**	**cagagt}**agtc	teetacaata	ctttgttgac		

↓ *Restriction sites for Hae* III *in cattle*

Figure 1. Nucleotide sequence bovine prolactin gene 5' flanking region and exon 1 (Camper *et al*., 1984).

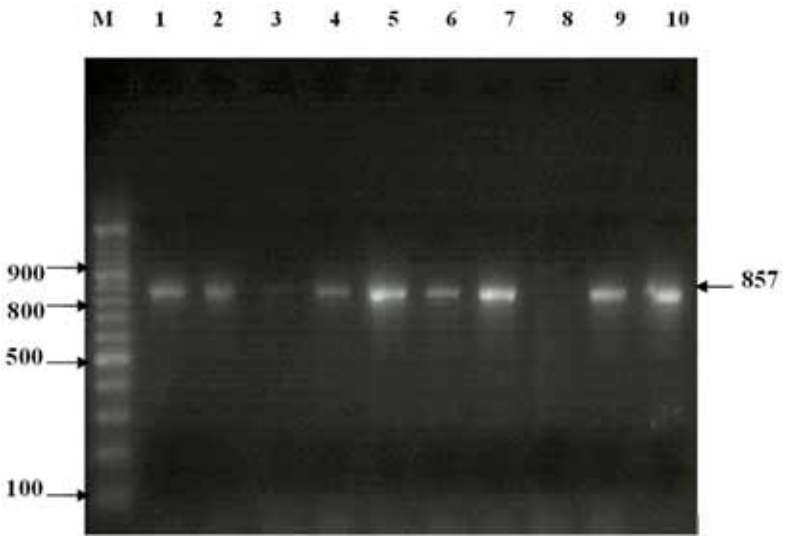

Plate 1. PCR product of Pandharpuri buffaloes.

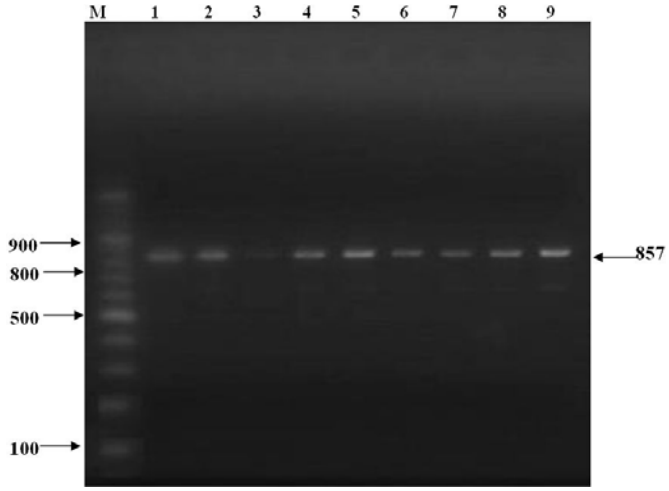

Plate 2. RE digestion of the PCR product of Pandharpuri buffaloes by *Hae III.*

Well No.	**Sample No.**
M	100 bp ladder
1	undigested PCR product
2	A
3	B
4	C
5	D
6	E
7	F
8	G
9	H

1 percent 5 µl/100 ml with a submarine gel electrophoresis apparatus at a constant voltage of 80 V for 90-120 minutes. The length of each fragment compared with the markers lane and fragment size was estimated. However, in all samples, an intact product of 857 bp was revealed (Plate 2). This suggests that a restriction site might be absent/abolished and reveals variation in nucleotide sequence of the PRL gene in Pandharpuri buffalo.

The present findings are in agreement with Ladani *et al*. (2003) who reported that *Hae* III has no restriction site for PRL gene (exon 1) and generates only one fragment of 857 bp in Mehsani and Surti buffaloes. However, they observed different restriction pattern in Jaffarabadi buffalo suggesting the presence of *Hae* III restriction sites.

The results obtained supports the existence of PRL sequence variation in Pandharpuri buffaloes that is different from other buffalo breeds. Comparison of above finding with published sequence of bovine 5' flanking region (Figure 1.) and exon I, it seems possible that point mutation (transition or transversion) could have abolished *Hae* III sites in this buffalo breed. However, this would require confirmation by sequencing of amplified region to find the exact sequence variation.

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