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Nucleotide sequence variation in exons 9 and 10 of lactoferrin gene in Murrah buffalo

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Abstract

The study was undertaken with the objectives to characterize and identify single nucleotide polymorphisms in exons 9 and 10 of lactoferrin gene in Murrah buffalo. Genomic DNA was isolated from 200 lactating Murrah buffaloes and exons 9 and 10 of lactoferrin gene were amplified using specific sets of primers, which resulted in amplicons of 332 and 209 bp respectively. Comparison of nucleotide sequence of exonic region of lactoferrin gene in Murrah buffalo with that of *Bos taurus* cattle revealed a total of 16 mutations comprising of 11 transition and 5 transversion type. Conceptualized translation of nucleotide sequence of exonic region revealed six amino acid changes (five in exon 9 and one in exon 10). The BLAST analysis revealed that exons 9 and 10 of lactoferrin gene in Murrah buffalo were 94 to 99 % identical with other species. The identified SNPs may serve as marker for selection of Murrah buffaloes for improved resistance to mastitis.

Key words: Lactoferrin, Murrah buffalo, SNP.

Mastitis is one of the most expensive and devastating diseases of dairy animals causing huge economic losses. In India, about 1 to 10% and 5 to 20% of buffaloes are affected with clinical and subclinical mastitis respectively every year (Joshi and Gokhale, 2006). Selective breeding of buffaloes for increased resistance to mastitis is difficult, being a polygenic trait with low heritability. Earlier, mastitis was considered purely a managemental disease, but at present many candidate genes for mastitis have been identified. Lactoferrin is one of the important candidate genes for mastitis resistance. It is a minor whey non-heme iron binding protein with molecular weight of 80 kDa containing a single polypeptide chain of 708 amino acids. The gene is located on *Bos taurus* autosome 22 and spans 34.5 kb consisting of 17 exons and 16 introns (Seyfert and Kuhn, 1994). It is a potent activator and regulator of various immunological functions such as granulopoiesis, cytokine production, antibody synthesis in vitro, natural killer cell cytotoxicity, lymphocyte proliferation, complement activation and production of interleukins (Sanchez *et al*., 1992; Kimber *et al*., 2002). However, little information is available with respect to exonic region of lactoferrin gene in Murrah buffalo except for its promoter and 5' flanking regions (Kathiravan *et al.* 2009). Hence, the present study was undertaken with the objectives to characterize and identify single nucleotide polymorphism in exons 9 and 10 of lactoferrin gene in Murrah buffalo.

Materials and Methods

The experimental animals for the present study were taken from dairy herd of Murrah buffaloes maintained at Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal, India. 10 ml of venous blood was collected from the jugular vein of each buffalo in a 15 ml polypropylene centrifuge tube containing 0.5 ml of 0.5M EDTA solutions, as an anticoagulant. Genomic DNA was isolated from 200 buffaloes using phenol-chloroform extraction method, as described by Sambrook and Russell (2001)

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with minor modifications. Primers for exon 9 were designed using Primer 3.0 software and those for exon 10 were taken from published literature Kathiravan *et al*., 2009. (The details of oligonucleotide sequence, annealing temperatures and amplicon size are presented in Table 1). The PCR amplification was carried out in programmed thermal cycler (PTC 200, MJ Research) comprising final reaction volume of 25 µl containing 3 µl (100 ng) genomic DNA, 12.5 µl 2X PCR Master Mix (Fermentas), 0.5 µl of each primer and 8.5 µl nuclease free water. Amplification was performed using initial denaturation at 95°C for 2.5 minutes followed by 35 cycles of 94°C for 30s, respective annealing temperature for 30s and extension for 72°C for 1 minute, with a final extension for 5 minutes at 72°C. PCR amplified products of exons were sent to SciGenom Labs Pvt. Ltd. (Kochi, India) for purification and sequencing. Nucleotide sequence of the two exons of lactoferrin gene was deduced from the raw sequence data which were obtained for each of exons were edited to reduce overlapping sequences by using BioEdit software of DNASTAR. For determining the single nucleotide polymorphism in exons 9 and 10 of lactoferrin gene in Murrah buffalo, the available sequence in the NCBI for *Bos taurus* (Accession number: 000179.1) was compared and aligned with the edited sequences of Murrah buffalo using ClustalW software. Basic Local Alignment Search Tool (BLAST) analysis was performed to find out sequence identity of lactoferrin gene of Murrah buffalo with other species.

Results and Discussion

The primer for exons 9 and 10 of lactoferrin gene were amplified successfully which yielded amplicon size of 332 and 209 bp respectively. For determining the SNPs in exons and flanking intronic regions of lactoferrin gene in Murrah buffalo, the sequences in NCBI Accession numbers (AC_000179) for *Bos taurus* were compared and aligned with the edited sequences of Murrah buffalo by ClustalW software. Comparison of nucleotide sequences of exons 9 and 10 with that of *Bos taurus* cattle by ClustalW multiple alignments revealed a total of 16 mutations (14 mutation in exon 9 and 2 mutation in exon 10). Out of them 11 were transition and 5 were transversion (Table 2). Similarly Li *et al.* (2004) found 3 SNPs in the promoter region and also 4 SNPs in exons 4, 8, 9, 15. Raja (2007) reported one SNP in each exons 8, 9 10, 12 and 15. Kathiravan et al.(2009) found a total of 34 nucleotides changes in exons 2, 5, 10, 11, 14 and 16 of bubaline lactoferrin gene as compared to Bos taurus which indicated 9 C-T and 16 G-A transition, 3 G-C and 3 C-A transversion and 3 deletion.

The coding sequences were translated into amino acid sequence by using ExPAsy translate tools and the resulting amino acid sequence was aligned with corresponding sequence of *Bos taurus* by ClustalW. Conceptualized translation of nucleotide sequence of exon 9 and 10 were non synonymous in nature affecting the sequence of amino acid and resulted in 6 amino acid changes in Murrah buffalo as compared to that of *Bos taurus* cattle. Amino acid changes observed in exon 9 were five viz. Lysine to Glutamine (358), Tyrosine to Arginine (361), Threonine to Alanine (362), Asparagine to Isoleucine (387) and Valine to Alanine (401).Whereas in exon 10 only one amino acid change (Asparagine to Serine) was observed at 412 position. In a similar study, Li *et al*. (2004) reported that mutation occurring in exon 4 led to the amino acid substitution (isoleucine to valine), while other mutations were silent. However, O'Halloran *et al.* (2009) identified 47 polymorphism in lactoferrin coding sequences, out of these 18 SNPs were synonymous causing no change to the amino acid sequence, while 27 SNPs were associated with amino acid changes. In another study, Kathiravan *et al*.

Table 1. Sequence of the primers for amplification of lactoferrin gene in Murrah buffalo

Primer		Sequence (5'-3')	Annealing Temp.	Amplicon size (bp)
Exon 9	F	GGGAAGTGAGAGGTATGAAGAC	53.5 °C	332
	R	TAGACACTCTGGAGGACAGAAA		
Exon 10	F	GGCACCTGACGTCCGTTCTCTTAG	63 °C	209
	R	GCCAGGGGGTACTCTTCTCCACTT		

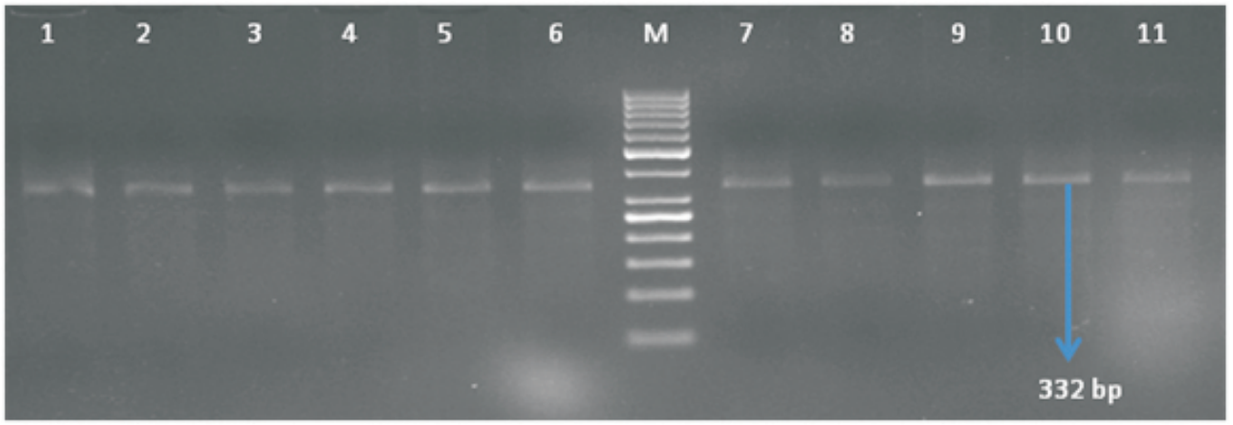
(2009) observed nine amino acid changes in exons *2, 5, 10, 11, 14 and 16 of* buffalo lactoferrin gene as compared to *Bos taurus*.

The BLAST results were used to check percent homology of Murrah buffalo lactoferrin gene with that of other species. The sequence homology of exon 9 was 94% with *Bubalus bubalis*, 93% with *Bos taurus,* and *Capra hircus* and 92% with *Ovis aries*. Similarly, the percent homology of exon 10 was 99% with *Bubalus bubalis*, 97% with *Bos taurus,* 96% with *Capra hircus* and *Ovis aries.* It revealed that exonic region of lactoferrin gene was 92 to 99% identical with several species. This is consistent with the finding on exonic region of the bubaline lactoferrin gene as reported by Kathiravan *et al*. (2009). A similar homology (65-100%) in a gene sequence among different mammalian species was reported by Teng, (2002).

Table 2. Summary of nucleotide change in exons 9 and 10 of lactoferrin gene in Murrah buffalo

S.No.	Exon	Base change	Nucleotide position
1	Exon 9	A>C	16861
2		G>A	16866
3		T>C	16870
4		A>G	16871
5		A>G	16873
6		C>G	16875
7		T>C	16890
8		C>G	16893
9		T>C	16902
10		A>T	16949
11		G>C	16968
12		T>C	16977
13		C>T	16980
14		T>C	16991
15	Exon 10	A>G	18969
16		A>G	18980

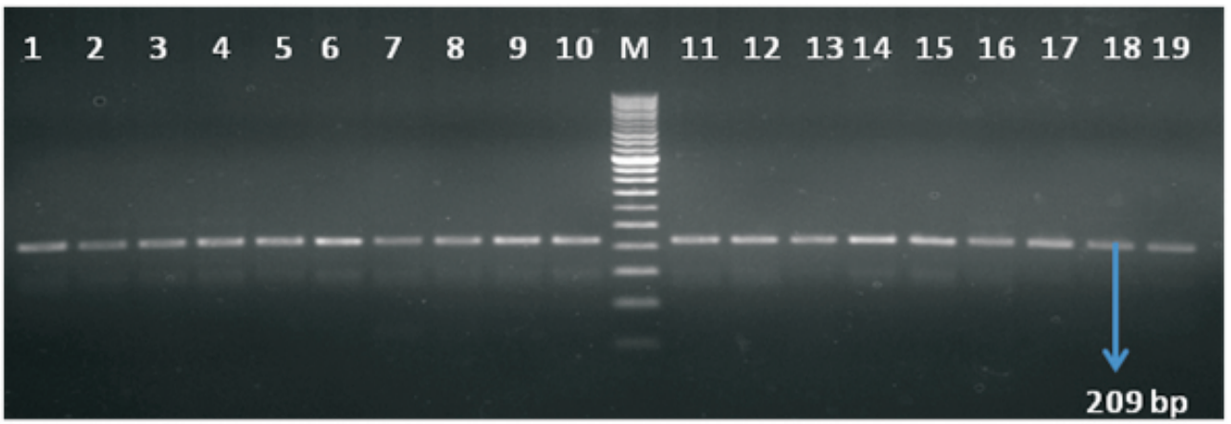
Fig. 1. PCR product of exon 9 of lactoferrin gene in Murrah buffalo



Lane 1-11 : PCR product (332 bp)

M : 50 bp DNA ladder

Fig. 2. PCR product of exon 10 of lactoferrin gene in Murrah buffalo



Lane 1-19 : PCR product (209 bp)

M: 50 bp DNA ladder

Conclusion

The study revealed 16 mutations in Murrah buffaloes, out of which 11 were transition and 5 were transversion. The SNP in exonic region were found to be non synonymous. The number of amino acid changes was five in exon 9 and one in exon 10. The BLAST revealed that exonic region of lactoferrin gene in Murrah buffalo was 94 to 99% identical with other species. These SNPs found in exonic region may be associated with incidence of mastitis which can serve as marker for selection of Murrah buffaloes.

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