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Polymorphism of 5′ flanking region of lactoferrin gene in khuzestan buffaloes

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ABSTRACT

Lactoferrin is a glycoprotein with molecular weight 80 kDa iron-binding bond, which is composed of 690 amino acids. In most mammalian body fluids such as sweat, semen, tears, and saliva and milk neutrophil granules there. bovine lactoferrin gene be associated with susceptibility/resistance to mastitis and even with some economically important production traits. This study was carried out to detect polymorphism in lactoferrin gene in 85 Bufallo to 4 location khuzestan province were selected randomly. After extracting DNA, amplification of 367 bp of lactoferrin gene was performed using specific primers. In this research four SSCP pattern for AA, BB, EE and FF lactoferrin gene in Bufallo were found in Khuzestan Province. The results showed that lactoferrin gene is so polymorphism in studied population. Gene frequencies for A, B, E and F alleles were 0.341, 0.259, 0.118 and 0.282 respectively. Therefore, the results confirmed that SSCP technique can be used to identify different genotypic variation in these breeds and is a useful tool for selection programs based on marker-assisted selection.

Key words: Khuzestan buffalo, Lactoferrin gene, Polymorphism, PCR-SSCP

INTRODUCTION

Lactoferrin is an iron (Fe3+) binding glycoprotein belonging to serumtransferrin gene family. It is found in a variety of body secretions of mammals, notably in milk (Sorenson and Sorenson, 1939). Lactoferrin is also secreted in the secondary granules of activated neutrophils (Cramer et al.,1985) and from differentmucosal surfaces (Masson et al., 1966). Lactoferrin has a variety of physiological roles, the foremost being its antimicrobial properties. Other biological functions of lactoferrin include transporting iron through fetal intestine (Davidson and Lonnerdal, 1988), promoting DNA synthesis (Nichols et al., 1987), modulating the immune system(Lu et al., 1991; Zimecki et al., 1991) and growth promotion (Joslin et al., 2002). Lactoferrin was initially thought to act as a bacteriostatic agent due to its strong iron binding capacity, depriving growing micro organisms of their demand for ferric ions. However, it also has a mechanism by which it can disrupt or possibly penetrate bacterial cell membranes. The N-terminal basic peptides of this protein, which are released following proteolysis are more potent than the intact protein and are bactericidal in nature (Bellamy et al., 1992a,b). The well documented antibiotic action of

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lactoferrin makes it a candidate gene for increasing resistance against infections of themammary gland, especially in dairy cattle breeding (Seyfert et al., 1994). Lactoferrin is found in the milk of most mammals, however the concentration is quite variable across different species. Human milk has the highest lactoferrin level in comparison with milk from other species (Masson and Heremans, 1971). The average lactoferrin concentration in themilk of dairy cattle is 0.1 mg/ml which is only about one-tenth of that found in human. Also this concentration is dependent on age of cow, stage of lactation and infection status of the animal. The relative paucity of lactoferrin in dairy cattle may be due to lack of some sequence motifs for transcriptional enhancers which are reported in human andmouse lactoferrin promoter (Seyfert et al., 1994). Lactoferrin gene is organized into 17 exons with its size varying from 23 to 35 kb across different species. Lactoferrin has been mapped to human chromosome 3p21.3 (Kim et al., 1998), mouse chromosome 9 (Teng et al., 1987) and bovine chromosome 22 (Schwerin et al., 1994). Lactoferrin gene is differentially regulated in different tissues or cell types and its expression in different tissues appears to be controlled by different pathways. Lactoferrin expression inmouse uterus is regulated by estrogen (Pentecost and Teng, 1987), C/EBP in neutrophils (Verbeek et al., 1999) and the mammary gland expression is controlled by prolactin (Green and Pastewka, 1978). The presence of multiple regulatory elements within the lactoferrin promoter could contribute to this differential regulation of gene expression. Extensive characterization of lactoferrin promoter has been carried out in bovines (Seyfert et al., 1994), mice and humans (Teng et al., 1992). Polymorphisms within lactoferrin promoter region have been found to be associated with some milk performance traits in Polish Holstein–Friesian cows (Kaminski et al., 2006). However, very little information is available regarding riverine buffalo lactoferrin gene promoter (Das et al., 1999) which is one of the important dairy animals in the khozestan. Hence the present study was undertaken with the objective of examining the 5′ flanking region of bubaline lactoferrin gene (Bubalus bubalis) to gain insight into the polymorphisms existing in this region across different khozestan buffalo breeds.

MATERIAL AND METHODS

DNA samples

Blood samples of 85 unrelated individuals from four different location of khuzestan buffaloes were selected randomly (Bubalus bubalis) were utilized to extract DNA in this study. Blood was collected from jugular vein into EDTA containing vacutainer tubes. DNA was extracted using a kit DIATOM (Boom et al., 1989).

PCR-SSCP analysis

PCR amplification: The LTF genotypes were identified with the PCR-SSCP technique. Oligonucleotide primers were designed to amplify andsequence the 5 ′ regulatory region of the bubaline lactoferringene based on the published bovine sequence (Kathiravan et al.(2009) ; GenBank accession No. L19985). Sequence of the oligonucleotide primers, their annealing temperature and expected product sizes are listed in (Table 1).By manufacturer's instructions primers diluted with water and were stored at -20 degrees Celsius. The PCR reaction was performed in a 25 µl reaction volume containing about 50-100 ng genomic DNA, each primer (10 pmol), 1X buffer (including 1.5 mM MgCl2), 200 µM dNTPs and 1 units of Taq DNA polymerase. Initial denaturation at 95℃ for 5 min; 35 cycles of denaturation at 95°C for 30 s; annealing at 62°C for 30 s, extension at 72°C for 45s, then holding at 72°C for 10 min. The PCR products were tested by horizontal submarine PAGE gel (8%, free from DNAse and RNAse) electrophoresis in 1×TAE buffer at 160 V.

Single-stranded conformation polymorphism: Aliquots of 2 µl PCR products were mixed with 8µl denaturing solution (0.04g Sucrose, 0.025% xylenecyanole and 0.025% bromophenol blue), incubated at 98°C for 10 min and then chilled on ice. Denatured DNA was loaded on 8% PAGE gel (80 mm×73 mm×0.75 mm) in 1×TBE buffer and constant voltage 160 V for 3 h. Patterns and genotyping was performed using comparison functions.

RESULTS

In this experiment, PCR-SSCP method was utilized as a useful approach for LTF genotype marking and it could be considered as selection criterion in Khuzestan Buffaloes population. Single strand conformation polymorphism analysis (LtfP4) of the 5′flanking region revealed a total of 4 distinct patterns Khozestan Buffalo. The representative gel pictures showing the band patterns for different SSCP variants are presented in (Fig. 1). Four distinct SSCP variants (LtfP-A, LtfP-B, LtfP-E and LtfP-F) were observed in fragment LtfP (-299 to +68), The overall frequency of these four variants revealed more or less equal distribution with frequencies of 0.341, 0.259, 0.118 and 0.282 respectively. In association with genotypes highest and lowest frequencies, respectively AA and EE, as well as Shannon index (I), allele real (Na) and effective (Ne), respectively, 1.326, 4 and 3.611 were presented.

DISCUSSION

The result of this study showed a polymorphism on lactoferrin gene which caused by a point mutation. Looking for candidate genetic markers of potential value in Marker Assisted Selection in dairy cattle is lately very popular. Among many different candidates, lactoferrin seems to be directly associated with udder health, SCC, mastitis and even with some economically important production traits. By now, several mutations have been identified in Bovin Lactoferrin gene. Bovine Ltf gene is highly polymorphic. Investigation of the polymorphism for this gene first reported by Seyfert and Kuhn. (1994) that found two alleles A and B with frequencies of 0.755 and 0.24 respectively. Seyfert et al. (1994) also found 16 polymorphisms in Ltf gene. Lately, Li et al. (2004) found 3 new SNPs in the promoter region, and also 4 SNPs in exons 4, 8 9, 15 and one in intron 4. Kaminski et al. (2006) giving the following frequency of genotypes: 0.628, 0.313 and 0.059 for GG, GC and CC, respectively also A single SNP in position +32(C/ G) located in the promoter and was assessed its associations with milk performance traits, including SCC. Kathiravan et al. (2009) four distinct SSCP variants (LtfP4-A, LtfP4-B, LtfP4-C and LtfP4-D) were observed in fragment LtfP4 (- 299 to + 6 8), with frequencies of 0.258, 0.299, 0.237 and 0.206 respectively in India Buffaloes. The results of this study concorred with the results reported. They showed that the lactoferrin gene mutations are in Buffalo.

CONCLUSION

In summary, The results of this experiment showed that lactoferrin gene polymorphic behavior than shown and can be used in breeding programs. It should be specifically tested for the mutation, sequencing done.

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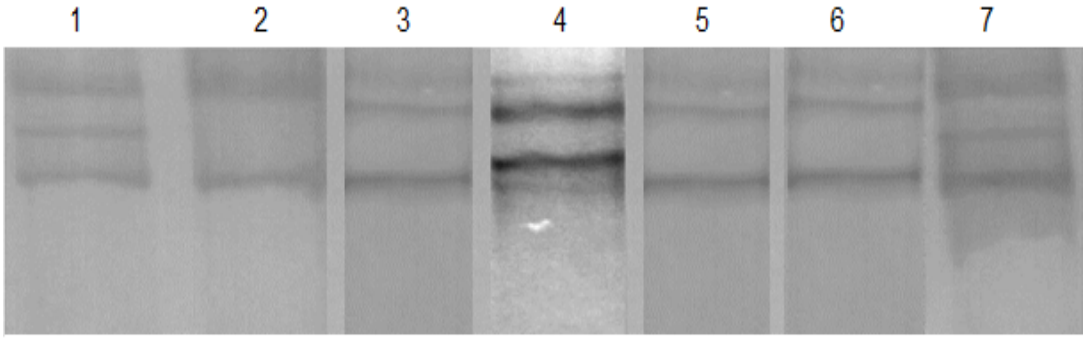

Figure 1. SSCP analysis of PCR products of primer. Lanes 2, AA genotype; laneS 3, 5, 6, BB genotype; lane 1, 7, EE genotype; Lanes 5-6, AA genotype and lane 4, FF genotype.

Table 1. Oligonucleotide primer designed for amplifying different fragments of 5′ flanking region of bubaline lactoferrin gene

5′ flanking region	Oligosequence	Annealing Product No. of Temperature	size	Observed SSCP patterns
LTF-P4	F :5′gggctgcggacaagtgggaaagaa3'	62 °C	367bp	4
(−299 to +68)	R :5′gacagcagggcggggacgaagag3′			