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Single nucleotide polymorphism (SNP) identification and sequence analysis of 5′ flanking region of lactoferrin gene in Indian buffaloes *(Bubalus bubalis)*

P. Kathiravan *, R.S. Kataria, B.P. Mishra

Buffalo Genomics Lab, National Bureau of Animal Genetic Resources, P.B. No. 129, Karnal – 132001, Haryana, India

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ABSTRACT

Lactoferrin, an iron (Fe^{3+}) binding glycoprotein, found in a variety of body secretions of mammals, is a potential candidate gene in dairy cattle breeding for increasing resistance against infections especially in the mammary gland. The present study was undertaken with the objective of examining the 5′ flanking region of bubaline (*Bubalus bubalis*) lactoferrin gene to gain insight into the polymorphism existing in this region across different buffalo breeds. The 5′ flanking region of lactoferrin gene was analyzed by single strand conformation polymorphism (SSCP) in five different Indian buffalo breeds, viz. Murrah, Jaffarabadi, Marathwada, Toda and Pandharpuri. A total of 10 distinct SSCP patterns were observed which further revealed 13 polymorphisms with 8 transitions, 4 transversions and one deletion mutation upon sequence analysis. A total of 1056 bp 5′flanking region of bubaline lactoferrin gene was sequenced which was found to have 96% homology with *Bos taurus*, 91% with *Capra hircus*, 95%, *Bos indicus*, 92% with *Ovis aries,* 57% with *Sus scrofa* and 28% with *Homo sapiens*. From the polymorphisms identified in the 1056 bp 5′ flanking region, polymorphism at nucleotide position −209 was found to lie within a potential SP1 transcription factor binding site, while SNP at −19 was found in the vicinity of the TATA box. Two SNPs identified at −57 and −61 positions were found to be present in the vicinity of putative estrogen response element. Another SNP at −636 position was found to be present in the vicinity of putative recognition sequence for IRF-1 which serves as a transcriptional activator for interferon genes and interferon inducible genes. The SNPs identified in the lactoferrin promoter region may serve as potential candidate genetic marker (s) in buffaloes for disease resistant traits.

1. Introduction

Lactoferrin is an iron (Fe^{3+}) binding glycoprotein belonging to serum transferrin 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 different mucosal 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 lactoferrin makes it a candidate gene for increasing resistance against infections of

* Corresponding author. Tel.: +91 9896 428823; fax: +91 184 2267654.
E-mail address: kathirvet@yahoo.co.in (P. Kathiravan).

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

5′ flanking region	Oligo sequence	Annealing temperature	Product size	No. of observed SSCP patterns
LTF-P1 (−988 to −758)	F — 5′ gtctgaacctacacatgctg 3′ R — 5′ tcctcagtacacaggctgac 3′	58 °C	231 bp	1
LTF-P2 (−732 to −433)	F — 5′ gttcctgtctcccacctcata 3′ R — 5′ gggaaccagttaagacagacg 3′	57 °C	300 bp	2
LTF-P3 (−511 to −271)	F — 5′ gcagcgggccctctttca 3′ R — 5′ tgctctttctttcccacttgtcc 3′	58 °C	241 bp	3
LTF-P4 (−299 to +68)	F — 5′gggctgcggacaagtgggaaagaa3′ R — 5′gacagcagggcggggacgaagag 3′	62 °C	367 bp	4
Gap Filling (Between I and II) (−873 to −645)	F — 5′ctgcgtgggagttgttgtgcttca 3′ R — 5′ggctgtctcctggtccctcatttg 3′	57 °C	229 bp	–

the mammary 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 the milk 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 and mouse 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 in mouse 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 Indian sub-continent. 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 Indian buffalo breeds.

2. Material and methods

2.1. DNA samples

Blood samples of 150 unrelated individuals from five different breeds of Indian riverine buffaloes *(Bubalus bubalis)* namely Murrah (30), Jaffarabadi (27), Marathwada (35), Toda (30) and Pandharpuri (28) were utilized to extract DNA in this study. Blood was collected from jugular vein into EDTA containing vacutainer tubes. DNA was extracted from whole blood following standard phenol–chloroform extraction

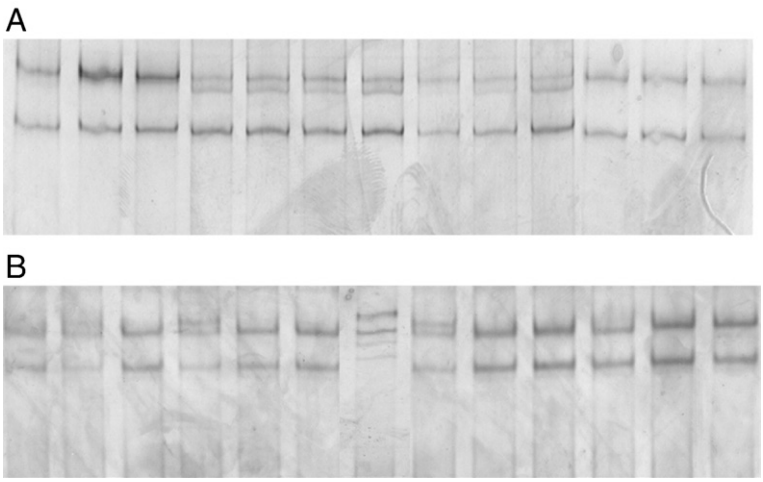

Fig. 1. A. Resolution of LtfP-2 fragment by 8% non-denaturing PAGE (Lanes 1–3 and 11–13: Variant LtfP2-B; 4–10: Variant LtfP2-A). B. Resolution of LtfP-3 fragment by 8% non-denaturing PAGE (Lanes 1, 3, 5, 6, 9–13: Variant LtfP3-A; 2, 4 and 8: Variant LtfP3-B; 7: Variant LtfP3-C).