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Effect of Butyrophilin Gene Polymorphism on Milk Quality Traits in Crossbred Cattle

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ABSTRACT : A genetic polymorphism study on butyrophilin gene was carried out to explore variability of this gene and to estimate effects of such variability on milk quality traits in crossbred cattle. Polymorphism was unraveled by conducting *Hae* III PCR-RFLP of this gene. Three genotypes such as AA, BB and AB and two alleles namely A and B were observed in crossbred population. The frequencies of genotypes and alleles were 0.78, 0.17 and 0.04 for AA, AB and BB genotypes, respectively, and 0.87 and 0.13 for A and B alleles, respectively. The nucleotides, which have been substituted from allele A to B, were observed as C to G (71st nucleotide), C to T (86th nucleotide), A to T (217th nucleotide), G to A (258th nucleotide), A to C (371st nucleotide) and C to T (377th nucleotide). The nucleotide substitutions at 71st, 86th and 377th position of the fragment were found as silent mutations whereas nucleotide changes at 217th, 258th and 371st positions were detected as substitution of amino acid lysine with arginine, valine with isoleucine, and leucine with proline from allele A to B. The genotypes had significant effects ($p \leq 0.05$) on total milk solid%, fat%, SNF%, while showing non-significant impact on total protein%. AA genotype produced highest average yield for all the traits. (**Key Words :** Butyrophilin, Crossbred Cattle, Polymorphism, Milk Quality Traits)

INTRODUCTION

Genetic variation is the main 'diet' for the improvement of animals and can be studied at the phenotypic as well as genetic level. As certain gene(s) is/are responsible for the expression of character, identification of that/those gene(s) and studying the underlying variation at genetic level responsible for the different degrees of expression in different animals can be a very good, fast and effective way of genetic improvement of animals. In this direction, candidate gene approach is gaining potential to identify the gene responsible for conferring significant variability of certain character and ultimately define the genetic marker for economic traits. Butyrophilin (BTN), a protein associated with milk fat droplets is directly involved in secretion of fat globules at the apical surface of mammary epithelial cells during lactation (Jack and Mather, 1990). Basically, butyrophilin is an acidic glycoprotein comprising more than 40% of total milk fat globule membrane protein in cow (Mather et al., 1980). This protein is normally sandwiched between plasma membrane and surface of fat droplets (Wooding and Kemp, 1975), and is insoluble in non-ionic detergent (Freudenstein et al., 1979) due to its hydrophobic property. This protein is integrated into the milk-fat-globule membrane during the budding and secretion of fat droplets into milk. Franke et al. (1981) suggested that butyrophilin is specific to mammary tissue and expressed extensively during lactation. However, butyrophilin may function as an integral receptor for cytoplasmic fat droplets and the budding of the droplets at the cell surface is initiated by interactions between cytoplasmic tail of BTN and other proteins like xanthine oxidase, fatty acid synthetase, GTP-binding proteins and lipids (Jack and Mather, 1990). In the process, formation of milk fat globule membrane is assumed to stabilize milk fat droplets after synthesizing and during storage and secretion from mammary gland. Therefore, a study was designed to detect polymorphism at this locus and estimate the effect of BTN polymorphism on milk quality traits in crossbred cattle.

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MATERIALS AND METHODS

Sample

Blood sample was collected from 114 Holstein Friesian ×Hariana crossbred cattle maintained at Cattle and Buffalo Farm of Indian Veterinary Research Institute, Izatnagar,

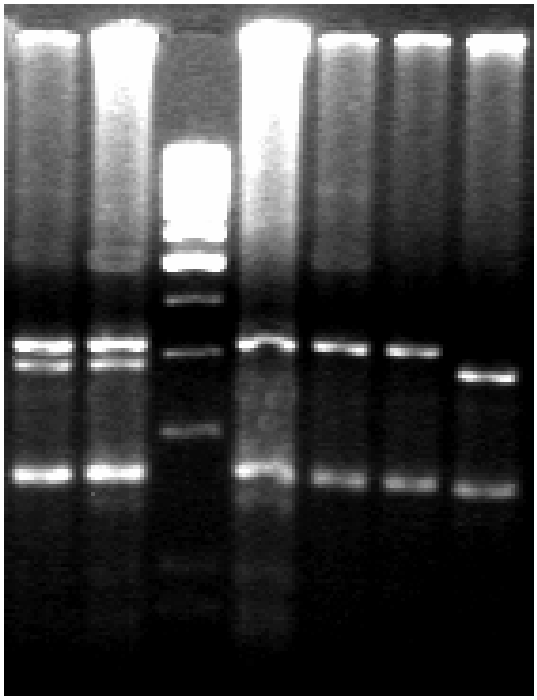

Figure 1. *Hae* III RFLP of butyrophilin gene of crossbred cattle. Lane AA, AB and BB are different genotypes. Lane M is DNA ladder marker.

India. Each sample was taken in 15 ml polypropylene vial containing 0.5 ml 0.5 M EDTA solution. Genomic DNA was extracted from frozen blood by phenol/chloroform extraction and ethanol precipitation technique (Bhattacharya et al., 2003). Finally, DNA was dissolved in TE buffer and quality as well as quantity was checked for each sample. Besides, 50 ml milk sample was collected from each animal for estimation of total solid%, Fat%, SNF% and total protein%.

Estimation

Fat percentage of milk samples was estimated by Gerber method (ISI, 1977) while total protein percentage was worked out by formaldehyde titration method (Pyne, 1932). Lactometer reading was taken of all milk samples and total solid% (TS%) and SNF% were calculated by using the formula,

$$TS\% = CLR/4+1.2F+0.14$$

$$SNF\% = CLR/4+0.2F+0.14$$

where CLR is Corrected lactometer reading which equals to lactometer reading+0.5+temperature difference from 84°F× 0.1 and F is fat%.

PCR-RFLP

A 501 bp butyrophilin gene fragment covering part of exon 8 was amplified with a pair of primer, 5'-TGGAGCTCTATGGAAATGGG-3' (forward) and 5' -TAC CCAACAGGAAGAAACAG-3' (reverse) (Taylor et al., 1996). The PCR amplification was performed in a total volume of 25 μl with 100 ng of genomic DNA, 60 ng of each primer, 2.5 mM of $MgCl_2$, 200 μM of each dNTP, 1× PCR reaction buffer and 1.25 U of *Taq* DNA polymerase (MBI Fermentus). DNA was denatured for 2 min at 95°C, then 39 cycles including 30 s at 95°C, 60 s at 65°C and 30 s at 72°C followed by final extension of 5 min at 72°C. The products were digested with *Hae*III enzyme to screen the population. The digested products were electrophoresed on 2% agarose gel and stained with ethidium bromide (0.5 μg/ml). The gel was visualized and documented on Gel-Doc system (Syngene, USA).

Sequencing

The PCR product belonging to different genotypes was run on 1% low melting agarose gel and the desired product was eluted from the gel using gel elution kit (GIBCO BRL) for purification. The purified PCR-products were sequenced using the automated dye-terminator cycle sequencing method with Ampli *Taq* DNA polymerase in ABI PRISM 377 DNA sequencer (Perkin-Elmer).

Statistical analysis

Gene and genotype frequencies were calculated according to Falconer (1998). The effect of genotype on milk quality traits was analyzed with ANOVA (Snedecor and Cochran, 1967). The model used for the analysis was $Y_{ijkl} = \mu+G_i+S_j+F_k+E_{ijkl}$, where, $Y_{ijkl} = l^{th}$ observation of the target trait, μ = overall mean, G_i = fixed effect of i^{th} genotype, $S_{j,}$ = fixed effect of j^{th} season of calving, F_k, = fixed effect of F^{th} sire and E_{ijkl} = random error. Here, season was classified into three groups i.e. summer (March to July), rainy (August to September) and winter (October to February). The additive genetic effects for different traits were calculated using DFREML animal model (Meyer, 1988). The BLAST search and other genetic analysis were made with "DNASIS MAX" software (Hitachi Genetic System, Miraibio Inc., USA).

RESULTS

Polymorphism

Hae III RFLP revealed three restriction patterns of which first pattern showed two bands of 316 and 162 bp and second pattern showed 283 and 162 bp. The third pattern depicted three bands of 316, 283 and 162 bp in the gel (Figure 1). But, the smaller fragments could not be resolved in the gel due to limitations of the agarose gel electrophoresis. However, the first pattern was assigned as genotype AA, second pattern as genotype BB and the third

TGGAGCTCTATGGAAATGGGTACTGGG
CTCTCACCCCACTGCGGACCCCTCTCC
CACTGGCTGGACCCCCCCGCCGGGTTG
GGGTCTTCCTTGACTATGAATCAGGAG
ACATCTTCTTCTACAACATGACTGATG
GATCCCATATCTATACTTTCTCCAAGG
CCTCTTTCTCTGGCCCCCTCCGGCCCT
TCTTCTGCTTGTGGTCCTGTGGTAAAA
AGCCCCTGACTATCTGCCCAGTCACTG
ATGGGCTTGAGGGAGTCATGGTAGTTG
CTGATGCCAAGGACATTTCAAAGGAGA
TCCCACTGTCCCCCATGGGGGAGGACT
CTGCCTCCGGGGATATAGAAACCCTCC
ATTCTAAACTAATCCCTCTACAGCCCA
GCCAAGGGGTGCCTTAAGAAATACTCC
AGCTCAGCTCTTCCCCTCTACTCTAAC
CCCCCTTCCACCACTCCCAGGGCTTCA
TCTGCCAGCTTTACTCAGCCCCTGTTT
CTTCCTGTTGGGTAG

(A)

ELYGNGYWALTPLRTPLPLA
GPPRRVGVFLDYESGDIFFYN
MTDGSHIYTFSKASFSGPLRP
FFCLWSCGKKPLTICPVTDGL
EGVMVVADAKDISKEIPLSPM
GEDSASGDIETLHSKLIPLQPS
QGVP

(B)

Figure 2. (A) Nucleotide sequence of butyrophilin gene. (B) Amino acid sequence of butyrophilin gene in crossbred cattle.

Table 1. Breeding values of different genotypes for milk quality traits. The values within parenthesis indicate actual concentration of different parameters in milk

Traits	Genotypes		
	AA	AB	BB
Total solid%	13.1	12.4	11.7
	(13.8)	(12.7)	(11.6)
SNF%	8.7	8.1	7.5
	(9.2)	(8.5)	(7.9)
Fat%	4.1	3.6	3.0
	(4.3)	(3.8)	(3.4)
Total protein%	4.0	3.9	3.6
	(4.3)	(4.0)	(3.9)

pattern as genotype AB. Thus, this locus revealed presence of two alleles namely, A and B in crossbred population.

Frequencies

The frequencies of three genotypes AA, BB and AB in crossbred cattle were found to be 0.78, 0.17 and 0.04, respectively. The frequencies of A and B alleles were estimated as 0.87 and 0.13, respectively.

Sequence variability

Two homozygotes namely, AA and BB were sequenced and the sequence of this gene was submitted to the EMBL genbank (Accession number:AY491470; Figure 2). The nucleotides substituted from allele A to B were observed as cytosine to guanine at 71st position of the fragment, cytosine to thiamine at 86th position, adenine to thiamine at 217th position, guanine to adenine at 258th position, adenine to cytosine at 371st position and cytosine to thiamine at 377th position of the fragment. From the BLAST search result, it has been found that there was a stop codon (T/UAA) at 395th position. The nucleotide position at 71st, 86th and 377th showed silent mutation where there was no change of amino acids at the corresponding locations. The nucleotide substitutions at 217th, 258th and 371st position from allele A to B revealed changes of amino acids from lysine to arginine, valine to isoleucine and leucine to proline at the respective position.

Association

The genotype was found to have significant effect ($p<0.05$) on total milk solid%, fat% and SNF%. AA genotype showed highest breeding value for total solid%, which was amounted to be 13.1 while BB homozygote had lowest breeding value for the trait amounting as 11.7. AB heterozygote produced medium range of breeding value, which was estimated to be 12.4. Both AA as well as AB homozygote gave higher breeding value for SNF%, which was 8.7 and 8.1, respectively. The highest breeding worth of fat% was observed in AA homozygote with an amount of 4.1. The lowest value for fat% was found for BB genotype and the breeding worth was estimated as 3.0 (Table 1). The medium range of breeding worth for this trait was found for AB heterozygote with an estimate of 3.6. However, the genotype was not found to be significantly associated with total milk protein%. But, there was a trend of increment of yield from substitution of B allele by A allele.

DISCUSSION

The size of the PCR product was 501 bp of which 395 bp was of coding region and rest was 3' UTR. Allelic variability was detected in translated region only but there was no such variability in UTR portion. In allele A, the

restriction sites were present in 163rd, 176th and 186th position of the gene while in allele B an additional restriction site was present in 217th position. Taylor et al. (1996) also reported the presence of two alleles in Holstein friesian cattle. However, the highest frequency was observed for AA genotype and A allele whereas lowest frequency was found for BB genotype and B allele. Such type of polymorphic information in other milk protein genes were also reviewed in cattle and Yak by Badola et al. (2004) and Mao et al. (2004), respectively. Thus, it may be inferred from these findings that milk component traits are highly variable and may be detected through molecular technique like RFLP. In the present study, firstly we conducted RFLP study to detect allelic pattern of the gene and consequently, we attempted to explore what kind of nucleotide changes being present in that gene other than RFLP variability. This information was generated by sequencing the alleles derived from RFLPs. Adoption of RFLP was performed for screening the herd and ultimately sequencing was carried out on small number of screened animals. Only two animals from each category of homozygous animals were involved for sequencing. It does not only reduce the workload but also reduce the economic involvement that might be arrived from sequencing.

Sequencing results confirmed the nucleotide changes between alleles. Changes in coding sequences have been observed between two alleles and consequently, amino acid substitution between alleles was also found. It does not only change the structure of polypeptides but also may change in post-translational modification generating changes in biological activity. Normally, secretion of fat globules in milk is happened when fat globules is processed with butyrophilin protein membrane. Without membrane there is no occurrence of secretion of fat in milk possible because fat droplets will not get the proper structure. Hence, it may be stated that butyrophilin may indirectly regulate the secretion of fat globules in milk. Although there is a pathway for biosynthesis of milk fat, at secretion level butyrophilin play a pivotal role. Hence, conclusively it may be stated that changes in structure or function of butyrophilin may play a role in causing secretion of differential level of fat in milk.

Milk quality traits like total solid%, fat% and SNF% were found to be significantly associated with genotypes while total protein% was not significantly affected by butyrophilin genotypes. For all the characters, AA genotypes yielded better than other genotypes. Physiologically, butyrophilin is directly involved in secretion of fat globules in mammary alveoli, which was revealed by Jack and Mather (1990). But, SNF% may have certain indirect influence from butyrophilin genotypes. As both the components were found to have significant impact from different genotypes, total milk solid as aggregates of both the components was influenced by butyrophilin genotypes. This sort of significant genotype-trait association may be used as genetic marker for selection of superior animals. But, before adopting these markers, the study may be conducted on very large size of samples. Incorporation of genetic markers in the selection experiment would ultimately enhance the rapid genetic progress. Use of genetic markers not only reduce the generation interval but also enhance the genetic gain over a period of time. Selection intensity with respect to certain trait is relatively higher through indirect selection like marker based approach (Bhattacharya and Gandhi, 1997) and thus, overall genetic progress can be improved for the desired trait at a considerably higher rate. The present study denotes the presence of polymorphism at butyrophilin gene and indicates the significant effect of genotype on some milk quality traits in crossbred cattle.

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