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Full Length Research Paper

DNA polymorphism of butyrophilin gene by PCR-RFLP technique

A. S. Sadr*, M. T. Beigi Nasiri, Kh. Alami-Saeid, J. Fayazi, H. Roshanfekr and M. Mohammadi

Ramin Agricultural and Natural Resources University, Mollasani, Ahwaz, Iran.

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Butyrophilin plays an important role in secretion of milk lipid. The present study describes polymorphism of butyrophilin gene. This is the first study of butyrophilin gene polymorphism in Najdi cattle of Iran. We used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique to screen for DNA polymorphism in 109 cattle. In all cattle, we amplified an 863 fragment consisting of part of exon 8. The amplified fragment digested with *Hae*III restriction endonuclease and subjected to electrophoretic separation in ethidium bromid-strained 3% agarose gel. Two alleles were observed, A and B. Five *Hae*III restriction site were found in the A allele as 371, 231, 185, 83 and 32 bp fragments. In the B allele, 371 bp were replaced with 338 bp. Three genotypes were observed, Frequencies were 73.4, 25.68 and 0.92% for AA, AB and BB, respectively. This gives frequencies of 0.86 and 0.14 for A and B alleles.

Key words: Butyrophilin gene, polymorphism, PCR-RFLP.

INTRODUCTION

Variation at DNA level contributes to the genetic characterization of livestock population and this may help to identify possible hybridization as well as past evolutionary events. Variation in exonic region of a gene may lead to change in amino acids which alter the expressed protein (Gelderman, 1996). Secretion of fat globule in milk is primarily based on getting proper shape of fat droplets with a group of membranous protein of which butyrophilin plays the important role during the process of lactogenesis (Bhattacharya et al., 2005). Butyrophilin (BTN1A1) is the most abundant protein in the milk fat globule membrane and it is specifically expressed in lactating mammary tissue. It is produced in the end of pregnancy and is maintained throughout lactation (Frank et al., 1981; Ogg et al., 2004). It constitutes more than 40% by weight of the total protein associated with the fat globule membrane of bovine milk (Mather and Lucinda., 1993). Butyrophilin is a type I transmembrane glycoprotein with the N-terminal domain facing the exoplasmic space containing two Tg-like folds, and the C-terminal domain facing the cytoplasmic containing a highly conserved B30.2 region (Banghart et al., 1998). This protein is normally placed between plasma membrane and surface of fat droplets and is synthesized as a peptide of 526 amino acids with an aminoterminal hydrophobic signal sequence of 26 amino acid, is cleaved before secretion (Mather and Lucinda., 1993). Butyrophilin is structurally related to proteins of the immune system, which suggest its possible immunologic function, unrelated to milk-fat secretion (Taylor et al., 1996a).

The BTN1A1 gene has been found in several mammalian species including cattle (Jack and Mather, 1990), human (Taylor et al., 1996b) and mice (Ishii et al., 1995). The bovine butyrophilin gene has been located to chromosome 23 (Ashwell et al., 1996). It consists of 8 exon and 7 intron (Figure 1). The knock-out of the BTN gene in mice causes the accumulation of large pools of lipid in the cytoplasm of milk-secreting cells and lipid droplets escape from the cells without an intact membrane (Ogg et al., 2004). BTN1A1 is likely QTL candidate that affects an economically important trait in dairy animal including milk yield and composition (Komisarek and Dorynek, 2003; Komisarek et al., 2006). The aim of present investigation was to study polymorphism in butyrophilin gene of Najdi cattle.

*Corresponding author. E-mail: ayehsadr@yahoo.com.

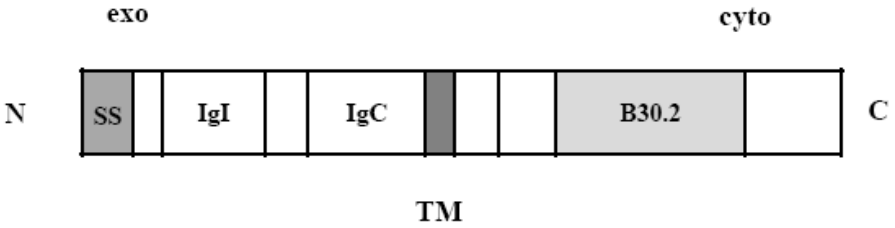

Figure 1. Structure of butyrophin (BTN). Proposed domain structure of BTN showing the signal sequence (SS) and the two proposed Ig folds (IgI and IgC1) in the exoplasmic (exo) domain, the transmembrane anchor (TM), and the conserved B30.2 domain (B30.2) in the cytoplasmic (cyto) tail.

Table 1. Primers used to amplify fragment of the butyrophilin gene (Taylor et al., 1996a; Zegeye, 2003).

Name	Sequence (5'-3')	PCR product size(bp)	RFLP endonuclease	RFLP site sequences
*Hae*III-RFLP	F: TCCCGAGAATGGGTTCTG R: ACTGCCTGAGTTCACCTCA	893	*Hae*III (6804-6807)	AGCC-GGCC

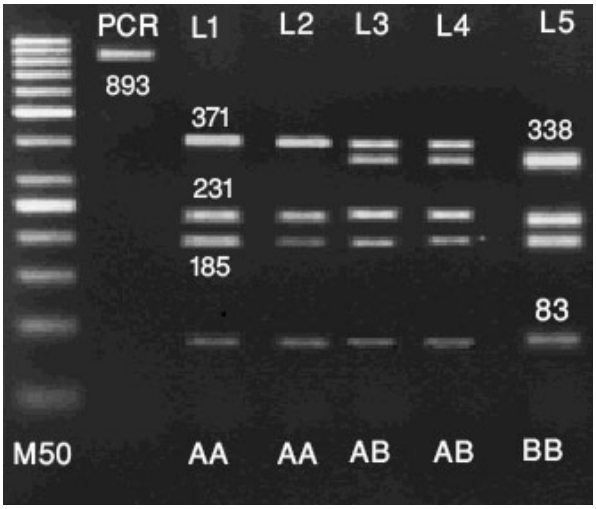

Figure 2. BTN genotyping by PCR-RFLP method. M: DNA marker. Lane 1: undigested amplification product; lanes 2 and 3: AA genotype; lanes 3 and 4: AB genotype; lane 5: BB genotype (32 bp has not seen on the gel).

MATERIAL AND METHODS

Our study was conducted on a total of 109 Najdi cattle from three different regions as follows: 47 cattle at the Najdi cattle Research Center, shoshtar; 47 cattle from Shadegan region and 15 from Mahshar (in Southwestern of Iran). From each animal, about 5 cc of blood was collected from the jugular vein with vaccum tubes coated with EDTA and transported in the laboratory and stored at 4°C until DNA extruction. Genomic DNA was isolated by using DNA Extraction Kit and was based on Boom et al. (1989) method. Spectrophotometer was used investigating quality and quantity; samples show an optical density (OD) ratio (260/280 nm) of between 1.6 and 1.8. From the purified genomic DNA, an 893 region of exon 8 of the butyrophilin gene was amplified by using primers (Table 1).

The PCR reaction volume of 25 µl contained approximately 33.3 ng of genomic DNA, 1.25 mM Taq DNA polymerase, 2.5 µl of 1 x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP and 10 pM of each primer. Amplification conditions included an initial denaturation at 95°C for 5 min, followed by 33 cycles at 95ºC for 30 s, 59ºC for 5 min, and 72ºC for 2 min, followed by a final extension at 72ºC for 7 min. The PCR products were separated by 1.2% (w/v) agaros gel electrophoresis.

The amplified fragment of butyrophilin was digested with 10 units of *Hae*III restriction enzyme and 15 µl of PCR product at 37°C overnight in a water bath. The digested PCR products were subjected to 3% (w/v) agarose gel electrophoreses and stained with ethidium bromide, the banding being visualized and documentation system. POPGENE software was used to estimate the gene and genotype frequencies, the heterozygosity and effective number of alleles (Yeh et al., 1999). Expected theoretical heterozygosity from Hardy-Weinberg assumption was calculated.

RESULTS AND DISCUSSION

The PCR reaction resulted in 893 bp long gene fragment. Five *Hae*III restriction site were found in the A allele as 371, 231, 185, 83 and 32 bp fregment. In B allele, 371 were replaced with 338 bp (Figure 2). Among the 109 Najdi cattles examined, there were 80 AA, 28 AB and 1 BB genotypes, which gave frequencies of 0.86 for A and 0.14 for B allele. Chi square (χ^2) test was used to evaluate Hardy-Weinberg equilibrium (HWE). The population followed Hardy-Weinberg equilibrium (χ^2=0.041). Low genetic diversity in this population was observed. The effective number of alleles was 1.32. The obtained results indicated that BB homozygote frequency was very

Table 2. Genotype and gene frequencies of the *Hae*III-RFLP in different regions cattle.

Region	Genotype frequencies			Gene frequencies	
	AA	AB	BB	A	B
Shoshtar	0.68	0.32	-	0.84	0.16
Shadegan	0.77	0.21	0.20	0.87	0.13
Mahshar	0.80	0.20	-	0.90	0.10

low. Our findings are the first report of butyrophilin gene polymorphism in Najdi cattle.

Genotype and gene frequencies of three regions were investigated separately, and the results presented in Table 2. Each population follows Hardy-Weinberg equilibrium. Average heterozygosity for three regions was estimated. The results indicated that average heterozygosity of Shoshtar was highest and Mahshar was the least. The BTN allele frequencies estimated in the present study (0.86 and 0.14) were approximately similar to the 0.875 and 0.125 (Taylor et al, 1996a), 0.85 and 0.15 (Husaini et al., 1999) and to the 0.88 and 0.12 (Komisarek and Dorynek, 2003) previously reported. The high frequency of the A allele suggests that this allele might have been favored by selection for dairy production. BTN1A1 is a likely QTL candidate that affects on economically important trait in dairy animal because its specifically expressed in lactating mammary tissue and the gene product BTN1A1 may function in secretion of milk lipid (Zegeye, 2003). The findings presented in this study indicate that the 893 bp butyropholin gene fragment is polymorphic in this population. The above conclusion should be confirmed in future investigation, taking into consideration all possible genotype at different loci and using other restriction enzymes for recognizing the variants.

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