Analysis of butyrophilin gene polymorphism in buffalo population in khouzestan province by pcr-rflp technique

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ABSTRACT: Butyrophilin gene is known as gene candidate for analysis the fatty milk and it has used as selective marker. Biochemical butyrophilin is acid glycoprotein that constituted more than 40% total protein fat globule membrane in cow. Study purpose was Analysis butyrophilin gene polymorphism in buffalo population in Khouzestan province. Blood sample were collected from 80 buffalos from Shadegan, Dezful, Shoshtar, Susangerd and Ahwaz station. DNA extraction was based on Boom et al method, and exons 8 of the butyrophilin gene were amplified to produce an 893 bp fragment. The amplified fragment were digested with *H*ae III restriction endonuclease, and then subjected to electrophoretic separation in ethidium bromide-stained 3% agarose gel. The results were revealed only allele A. All the samples studied indicating the monomorphic nature of the locus, showing AA genotype.

Key words: Polymorphism, Butyrophilin gene, PCR-RFLP, Buffalo

INTRODUCTION

Asian buffalo or Water buffalo is classified under the genus Bubalus, species bubalis. Composition of the buffaloes' milk was: protein: 4.18±0.07 percent, total solids: 17.71±0.35 percent, and fat: 8.11±0.20 percent. The fat-free solid content of buffalo milk was 11.91±0.17 percent. The water content of the milk was 82.29±0.35 percent (Borghese, 2005).The river buffalo is an economically important livestock species in many Asian and Mediterranean countries. Its genetic improvement, especially in reproductive performance and quantity of meat and milk production, ranks high among agricultural research needs of these countries (Othman, 2006). Genetic variation has the principle factor for the improvement of performance of animals and can be analyzed at phenotypic as well as genetic level (Bahattacharya et al., 2007). Gene maps and genetic polymorphism of genes related to the economic quantitative traits as tools for developing more efficient strategies have as targeted animal improvement by the genomic approach (Othman, 2006). Genotyping and DNA sequencing instead of amino acid sequencing can explore this kind of variability at polypeptide level. Normally amino acid sequencing is very costly and tedious. Thus, simple genotyping or DNA sequencing can be a valuable and cheaper tool to explore amino acid sequences of the polypeptide (Bahattacharya et al., 2007).

Secretion of fat globule in milk is primarily based on getting proper shape of fat droplet with a group of membranous proteins of which butyrophilin plays the pivotal role during the process of lactogenesis. Biochemically, butyrophilin is an acidic glycoprotein comprising more than 40% of total milk fat globule membrane protein in cow (Mather, 1980).

Butyrophilin is synthesized as a peptide of 526 amino acids with an amino terminal hydrophobic signal sequence of 26 amino acids, which is cleaved before secretion in association with the fat globule (Mather and Lucinda, 1993).

The molecular mechanism underlining milk fat globule secretion in mammary epithelial cells ostensibly involves the formation of complexes between plasma membranes butyrophilin and cytoplasmic xanthenes oxidoredoctase. These complexes bind adipophilin in the phospholipids monolayer of milk secretary granules, the precursors of milk fat globules, enveloping the nascent fat globules in a layer of plasma membranes and pinching them off the cell (Robenek, 2006). Two possibilities were proposed; either butyrophilin is an insoluble cytoskeleton component, specifically

associated with the cytoplasmic face of the apical plasma membrane and the protein coat of MFGM or it is a transmembrane protein with a large cytoplasmic domain which interacts with the surface of cytoplasmic lipid droplets (Lucinda, 1990). In the process, formation of milk fat globule membrane is assumed to stabilize milk fat droplets after synthesizing and doing storage and secretion from mammary gland. This protein is encoded by butyrophilin gene, which is located in the major histocompatibility complex class-1 region on 23rd chromosome of cattle, 6th chromosome in human and 13th chromosome in mouse (Ashwell et al., 1996), the results showed that BTN gene is assigned to the bi-armed buffalo chromosome 2 (Othman, 2006). The mammary specific expression of BTN revealed that it may function during late pregnancy and lactation either in the process of milk secretion or it may play substantial role in the immune system of mammary gland (Zegeye et al., 1990). Besides, butyrophilin provide passive immunity to the suckling neonates through milk (Bahattacharya et al., 2007).

Butyrophilin gene is constitutively secreted by surface budding associated with milk lipid production (Franke et al., 1981). Consequently, the potential involvement of the BTN gene on milk composition in dairy cattle is of interest (Taylor et al, 1996b). BTN gene is primarily responsible for size of fat globules by conferring major contribution towards the fat globule membrane, this gene product may be the crucial factor for determining the quantity as well as quality of milk fat production. However, biochemical studies in this respect may solve all the uncovered information about the role of BTN gene towards quality of milk fat synthesis (Bahattacharya et al., 2007). The BTN polymorphism analyzed in this study is located within the highly conserved region (know as B30.2 or rfp) of the C-domain that is probably involved in the protein-protein interactions (Jack and Mather, 1990; Ishii et al., 1995). The types of butyrophilin polypeptide across the species vary and thus, the level of activity of this protein is not similar in different species. Butyrophilin as membrane protein of fat globule regulates the size of fat globules. It is a fact that size of fat globules varies from species to species and this variation might be due to different types of butyrophilin polypeptide (Bahattacharya et al., 2007). The aim of this study was to investigate of butyrophilin gene (exon 8) polymorphism in buffalo population of Khuzestan-Iran.

MATERIALS AND METHODS

This study was conducted on a total of 80 buffalos from five different regions as follows: 20 buffalos from Shadegan, 20 buffalos from Shoshtar, 15 buffalos from Ahwaz, 14 buffalos from Dezful and 11 from Susangerd regions (in Southwestern of Iran). From each animal, about 3-5 cc of blood was collected from the jugular vein with vacuum tubes coated with EDTA and transported in the laboratory and stored at -22°C until DNA extraction Genomic DNA was isolated 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 primers F: 5'- T CCCGAGAATGGGTTCTG-3' and R: 5'-ACTGCCTGAGTTCACCTCA-3' (Taylor et al., 1996a and Zegeye, 2003).

The PCR reaction volume of 25 µl contained approximately 50ng of genomic DNA, 1.25 mM Taq DNA polymerase, 2.5 µl of 1x PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTP and 10 pM of each primer. Amplification conditions included an initial penetration at 90°C for 5 min, followed by 35 cycles at 95ºC for 30 s, 60ºC for 1min, and 72ºC for 2 min, followed by a final extension at 72ºC for 7 min. The PCR products were separated by 1.5% (w/v) agaros gel elctrophoresis the amplified fragments of butyrophilin was digested with 10 unit of *HaeIII* 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. Also the amplified fragment of butyrophilin was digested with 10 units of *RsaI* restriction enzyme and 10 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.

RESULTS AND DISCUSSION

In this study exon 8 butyrophilin gene is analyzed. The PCR reaction resulted in 893 bp long fragment. After digestion PCR product were found five *HaeIII* restriction sites at positions 371, 231, 185, 83, 23 in A allel (Figure 1). In B allele, 371 were replaced with 338 bp. But among 80 examined buffalo, B allele has not been observed. In this study only A allele was observed. Also, a PCR product of 893 bp fragment butyrophilin gene is digested using *Rsa1* restriction enzyme. *Rsa*I RFLP showed monomorphism at this locus. Besides, in this population, total animals have show AA genotype. Any polymorphism has been seen at between examined animals. Due to this research, all animal indicated monomorphism. In the recent study on investigation 8 exon butyrophilin gene the most livestock was shown most frequency A allele.

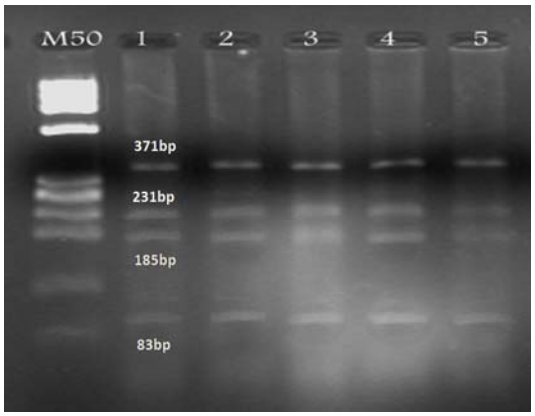

Figure 1. BTN genotyping by PCR-RFLP method. M50: DNA marker. Lan 1-5 (five sample Khozestan buffalo).

CONCLUSION

Findings presented in this study indicated that the 893 bp butyrophilin gene fragment was monomorphic in this population. This population not followed to Hardy-Weinberg Equilibrium.

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